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Assessment of genetic variation among 14 ecotypes of *Nyctanthes arbortristis* L. collected from western Odisha using cytological and DNA markers

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ABSTRACT

The genetic divergence among 14 ecotypes of *Nyctanthes arbortristis* (L.) were assessed using karyotype, meiotic studies and RAPD analysis. Mitotic and meiotic analysis confirmed 2n = 44 chromosomes. Somatic chromosome analysis revealed symmetric karyotype with prevalence of median and sub-median chromosomes with TF% ranging from 39.8 to 44.08. Meiotic analysis of 14 ecotypes also showed 22 bivalents in majority of PMCs, but in some PMCs bivalents (19.4±1.03 to 21.2±0.24), univalents (2.1±1.06 to 3.9±1.48) and quadrivalents (0.36±0.14 to 1.6±0.24) were also appeared in combination. The PMCs revealed high proportion of ring bivalents over rod bivalents with terminalised chiasmata, and terminalization coefficient ranged from 0.895 to 0.919. Although PMCs showed normal 22:22 segregation of chromosomes to two poles during Anaphase-I, univalents in the form of laggards were often observed. Pollen fertility and percentage of seed germination were low in all ecotypes which was indicative of partial genetic heterozygosity. RAPD marker profile generated by 10 RAPD primers showed moderate polymorphism among the ecotypes with Jaccards similarity indices ranging from 0.648 to 0.962 among the ecotypes of *N. arbortristis*. The clustering of ecotypes based on RAPD data also agreed with chromosome behaviour, pollen viability and germination of seeds. Moreover, these findings have many implications for future *in vitro* studies aimed at development of genotypes with higher content of metabolites (rengyolone, urosolic acid, arbortristosides and nyctanthic acid) of pharmaceutical importance.

1. Introduction

Nyctanthes arbortristis (L.), belongs to the family-Oleaceae and is native to India. It is an important medicinal plant commonly known as Parijatha (Sanskrit), Night Jasmine (English) and Gangasiuli (Odia). This plant is a large shrub or small tree, with flaky grey bark, stiff whitish hair on young branches and rough leaves (Agarwal and Pal, 2013). The flowers are fragrant having six white petals with an orange centre (Ratnasooriya *et al.*, 2005). It is also called as *Harsinghar* (sad tree) because its flowers open at dusk and fall down at dawn from the tree. Different plant parts of *N. arbortristis* are used as medicine for treatment of various ailments because it possesses anti-malarial (Kumari *et al.*, 2012; Nagendrappa *et al.*, 2013; Agarwal *et al.*, 2013; Godse *et al.*, 2016), antibacterial (Khatune *et al.*, 2001), anti-helminthic, anti-inflammatory (Saxena *et al*., 1984; Das *et al.*, 2008; Nirmal *et al.*, 2012), hepato-protective (Hukkeri *et al.*, 2006), immune-potential, antipyretic (Saxena *et al.*, 1987), antifungal (Gyanchandani *et al.*, 2000), immunomodulatory (Khan *et al.*, 1995; Bharshiv *et al.*, 2016), anti-leishmanial (Tandon *et al.*, 1991; Shukla *et al.*, 2011; Shukla *et al.*, 2012) properties Biologically important metabolites such as alkaloids, phytosterols, phenolics, tannins, flavonoids, glycosides and saponins were isolated and characterized from this plant (Priya and Ganjewala, 2007; Rahman *et al.,* 2011).

Genetic diversity assessment among natural populations of medicinal species is also quite important in the perspective of their phenotypic plasticity (Geng *et al.*, 2016) vis-a-vis their evolution, domestication and conservation. The ecotypes as well as the populations of *N. arbortristis* are quite heterogeneous in their respective natural habitat, and they are found to be distributed across the tropical and semiarid tropical regions either as ornamental plant or as

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wild tree. Therefore, estimation of genetic variation among the genotypes of this medicinal species is crucial prior to its conservation, domestication and implementation of genetic improvement strategies. Keeping this as requirement, various tools including morphological, cytological, biochemical and molecular markers have been used for genetic diversity studies in many medicinal species including *Osmanthus fragrans* (Duan *et al.*, 2013; Hu *et al.*, 2014), *Forsythia* species (Chung *et al.*, 2013), *Fraxinus* species (Sollars *et al.*, 2017) . In *N. arbortristis*, the cytological exploration mostly limited to somatic chromosome count and chromosome behavior pertaining to cytotaxonomical status. The somatic chromosome number of *N. arbortristis* has been reported from time to time as 2n = 44 (Bolkhorskikh *et al.*, 1969; George and Geethamma, 1984; George *et al.*, 1989) and as 2n = 46 (Kundu and De, 1968). During last couple of decades, DNA markers have been deployed as supplement for conventional biometric tools for genetic studies in many species (Sahu *et al.*, 2016). Recently, Rohilla *et al.* (2017) used 40 RAPD primers and assessed the genetic diversity among 16 ecotypes of *N. arbortristis* obtained from Northern-Central India.

Therefore, in this study an attempt has been made to assess the genetic diversity among the ecotypes of *N. arbortristis* collected from different parts of western Odisha using karyotype analysis, meiotic chromosome behavior and RAPD marker analysis.

2. Materials and methods

2.1 Plant materials

Fourteen ecotypes of *N. arbortristis* were collected from different parts of western Odisha (Table 1) and planted in the botanical garden of School of Life Sciences, Sambalpur University, Odisha, India. The roots of sprouted seedlings, flower buds and leaf samples were used for mitotic, meiotic and DNA marker analysis, respectively.

2.2 Mitotic analysis and 4C DNA estimation

Well developed roots (1-3 cm) obtained from the seedlings at 7.00-8.00 a.m. and pre-treated with saturated pre-chilled p-dichlorobenzene (PDB) solution for two hours at 20ºC, followed by fixation in 1:3 aceto-alcohol and kept overnight at room temperature. Subsequently, the root tips were transferred to 70% ethanol and stored at 4^0C. Hydrolysis of the root tips were carried out in preheated 1N HCl at 60^0C for 10 min followed by staining with the help of 1.5% aceto-orcein for one hour and squashed with 45% propionic acid. Suitable metaphase plates were observed under compound microscope (Unilab, India) and were documented using Nikon Coolpix-4500 camera. The images of metaphasic plates were analyzed manually as suggested by Fukui (1986) and idiogram was prepared. The chromosomes were classified as median (m), submedian (sm), subterminal (st) and terminal (t) according to the position of the centromere (Hirahar and Tatuno, 1967) and on the basis of chromosome length (long: e"3.0 µm, medium: e" 2.0 to < 3.0µm and Small: < 2.0µm). The chromosomes were morphologically grouped into following types- type A: Long median (A_M), type B: Medium median (B_M), type C: Medium submedian (C_{SM}), type D: Medium subterminal (D_{ST}), type E: Short median (E_M) and type F: Short submedian (F_{SM}). Total chromatin length, fraction of short arms in total chromatin length (TF%) and relative length of shortest chromosome compared to longest (S%) were also calculated.

Actively growing root tips of fourteen ecotypes of *N. arbortristis* and that of *Allium cepa* (as control) were excised and fixed and stored as explained above. These root tips were thoroughly washed and hydrolyzed for 10 min with 3N HCl at room temperature. After another wash the hydrolyzed tips were transferred to Feulgen stain (pH 3.2) for two hours at room temperature and squashed under a coverslip in glycerol. Four slides from each sample were made and readings in arbitrary units of 40 cells were obtained for each ecotype and control sample at 550 nm using 20/30 PV microspectrophotometer (Craic Technologies, USA). Only 4C nuclei at mid-prophase were measured and the arbitrary units were converted to picogram (pg) of DNA per nucleus by taking the mean of identical number readings of *A. cepa* root tips and its 4C nuclear DNA content (67.1 pg) as standard (Bennet *et al.*, 2000).

2.2 Meiotic analysis

The flower buds of appropriate size were fixed in 1:3 aceto-alcohols and kept for 24 h at 25 ± 2ºC and then it was transferred to 70% ethanol for and stored at 4ºC. The anthers of suitable size were squashed in a drop of 1.5% acetocarmine, and the meiotic behaviour of chromosomes, including chromosome association and segregation, were observed. An average of 25-30 Pollen Mother Cells (PMCs) of each ecotype were analyzed at diplotene/diakinesis/ metaphase-I stages. During anaphase-I, about 20 cells was analyzed and the segregation pattern of chromosomes was observed. The pollen viability was assessed by staining the pollen grains with 1:1 acetocarmine: glycerine.

2.3 RAPD marker analysis

Young apical leaves were obtained from 14 ecotypes and ground under liquid nitrogen to fine powder, and genomic DNA was isolated following the modified CTAB method and purified using RNAse and proteinase K treatment followed by 24 chloroform: 1 isoamyl alcohol washes

(Mishra *et al.*, 2013). The DNA was equilibrated to a concentration of 20ng/µl using $T_{10}E_1$ (10 mM Tris; 1 mM EDTA, pH 8.0) buffer. For RAPD analysis each amplification mixture of 20 µl contained 30 ng genomic DNA, 2.5µl of 10× assay buffer (100 mM Tris.HCl, pH 8.3; 0.5 M KCl; 0.1% Gelatin), 2 mM $MgCl_2$, 200 µM each of the dNTPs, 20 ng of RAPD primers and 1 U *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). Amplification was carried out in a thermal cycler (GENEAMP-9700; Applied Biosystems, Foster City, USA) and it comprise an initial denaturation at 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 60s, annealing at 37°C for 60s and an extension at 72 °C for 2 min, and final extension at 72 °C for 7 min. The amplified fragments were separated in 1.4 % agarose gel containing 0.5 ¼g ml^{-1} ethidium bromide in TAE buffer (40 mM Tris acetate, pH 8.0; 2 mM EDTA) at a constant 50 V for 60 to 80 min, and a tracking dye [20 % (w/v) sucrose; 0.1 M EDTA, 1.0 % (w/v) SDS; 0.25 % (w/v) bromo-phenol blue; 0.25 % (w/ v) xylene cyanol] was used to monitor the electrophoresis. The banding pattern was visualized under the gel documentation system (Geldoc XR system, Biorad, USA) and photographed. The sizes of fragments were calculated using 250 bp step up ladder (Bangalore Genei Pvt. Ltd.) as molecular weight marker, and TL120 software (Non-linear Dynamics, Total Lab Ltd., Newcastle Upon Tyne, UK).

Each amplified fragment was considered as unit character and was organized into 1-0 binary matrix, and Jaccards similarity coefficient was estimated. The pair wise similarity indices were used for the cluster analysis and generation of dendrogram using un-weighted pair group method with arithmetic mean (UPGMA), and also for principal coordinate analysis (PCoA; Mohammadi and Prasanna, 2003) using the NTSYSpc version 2.11s (Rohlf, 2008). The polymorphism information content (PIC) value for each locus was calculated using formula PICi = [2f_i (1" f_i)]/ n, where PICi is the polymorphic information content of the locus *i*, f_i is the frequency of the amplified fragments, (1" f_i) is the frequency of non-amplified fragments and 'n' represents total number of accessions (Roldan-Ruiz *et al.*, 2000). The PIC of each primer was calculated using the average PIC value from all loci of each primer. In addition average band informativeness (AvI_b) and resolving power (Rp) of RAPD primers were also estimated (Prevost and Wilkinson, 1999).

3. Results

3.1 Chromosome analysis and estimation of 4C DNA content

The root tip cells of *N. arbortristis* showed well condensed chromosomes at metaphase and somatic chromosome number was revealed to be 2n=44 (Fig. 1a). The 4C DNA content of all 14 ecotypes was quite homogeneous and varied from 18.94±0.82 to 21.46±1.88 pg (Table 1). The total chromosome length and TF% were also varied among the ecotypes from 98.7 to 102.7 µm and 39.8 to 44.08%, respectively (Table 1). Karyo-morphological studies revealed six (2n = 44: $2A_M + 26B_M + 8C_{SM} + 2D_{ST} + 4E_M + 2F_{SM}$) distinct chromosome types (Table 2, Fig. 1b). The karyotype of representative ecotype NAET-08 showed predominance of chromosomes with median (F% = 40.0% to 50.0%) and submedian (F% = 32.0% to 38.89%) centromere, though there were two sub terminal (F% = 19.04% to 22.72%) primary constrictions. Chromosome length was varied from 1.8 to 3.2 µm. Total chromatin length was 101.2 µm, and relative length of shortest chromosome arm compared to longest one (S%) was varied from 23.5 to 100.0% with average of 74.8%, and TF% was 102.09. Thus, the karyotype in *N. arbortristis* (NAET-08) was identified to be symmetric in nature and similar kinds of results were obtained for rest of the ecotypes tested here.

3.2 Meiotic analysis, pollen viability and germination of seeds

The pollen mother cells (PMCs) of 14 ecotypes revealed the gametic chromosome count is n = 22 (Fig 2a). Although in majority of PMCs across 14 ecotypes showed the existence of 22 normal bivalents (22II) as chromosome association at late diakinesis and metaphase-I, a few PMCs showed mixture of quadrivalents (IV), bivalents (II) and univalents (I) as chromosome associations (Table 3; Fig. 2b). Bivalents and univalents were observed in all ecotypes whereas quadrivalents were noticed in 10 ecotypes (Table 3). Mean value of quadrivalents, bivalents and univalent as chromosome association ranged between 0.36 to 1.6, 19.4 to 21.2 and 2.1 to 3.9, respectively (Table 3). Diplotene chromosomal configuration showed predominance of ring bivalents over rod bivalents with mean chiasmata of 23.95 in NAET02 ecotype to 24.43 in NAET 10 ecotype per cell (Range: 22-26). On average 22 chiasmata were terminalized with terminalization coefficient of 0.895 to 0.919 (Table 3). Majority of PMCs (80.1 to 88.6%) showed equal distribution (22II:22II) of chromosomes during anaphase-I (Fig, 2c), while rest showed unequal separation of chromosomes having complement 21II:2I:21II (10.2 to 16.4%) and 20II:4I:20II (1.3 to 5.4%) among the ecotypes assessed (Fig. 2d; Table 3).

In the present study, the pollen viability was ranged from 46.5% to 58.0% with an average of 52.39 among the studied ecotypes (Table 3). Pollens are spheroidal and lobate with smooth intine and unique exine having shrunken and tricolpate apertures which are appeared as granular pores. A germination assay of seeds of 14 ecotypes showed quite

low germination percentage as expected and was ranged between 42.33% in ecotype NAET 04 to 52.0 % in ecotype NAET 02.

3.2 Estimation of genetic variation among the ecotypes

Amplification with ten responding RAPD primers generated 60 unequivocal scorable DNA fragments, out of which 25 were polymorphic among the ecotypes tested (Fig 3). The range of amplified fragments varied from 365 to 3640 bp. A maximum of eight loci were amplified with primer OPA04 whereas, a minimum of four loci were amplified with the primer OPA05. These 10 primers were also exhibited a wide range of variation with regard to their PIC, AvIb and Rp (Table 4) with mean for the ten RAPD primers were 0.161, 1.673, and 10.327, respectively. The pairwise Jaccard's similarity coefficient values among the ecotypes were in the range of 0.648 to 0.962, which also evidenced low to moderate genetic variation among these ecotypes. NAET12 ecotype showed their closest affinity to NAET8 and NAET14 with similarity coefficient 0.962, whereas NAET4 and NAET5 ecotypes showed lowest similarity coefficient (0.648). The dendrogram generated by using the RAPD profile exhibited three major clusters in consonance with their geographical collection site baring one ecotype NAET10 (Fig. 4a). This clustering pattern was also affirmed by two dimensional principal coordinate analyses (PCoA; Fig. 4b), where two coordinates accounted for 57.05% of total genetic variation among the ecotypes of *N. arbortristis*.

4. Discussion

Medicinal plants possess unique identity in their 4C DNA content and somatic chromosome complements like other species, which could be evident in their size, shape and position of primary constrictions and also the secondary constriction and satellites, if any, in a few species. Studies on *in situ* nuclear DNA content along with karyotype analysis and behaviour of chromosomes during meiosis provide clues about the genome architecture in *N. arbortristis*.

In the present study, 14 ecotypes of *N. arbortristis* were assessed for somatic chromosome complement and 4C DNA content, and it has been observed that somatic chromosome count was 2n = 44 across all ecotypes with predominance of median and submedian chromosomes, thus symmetric karyotype. As expected, 4C DNA contents of all ecotypes reside in proximity of the mean values of 20.641pg. In addition to 2n= 44 (Bolkhorskikh *et al.*, 1969; George and Geethamma, 1984; George *et al.*, 1989), researchers have reported somatic chromosome numbers 2n = 46 (Kundu and De, 1968). This incongruence in somatic chromosome number might be attributed to the existence of different cytotypes in *N. arbortristis* in different geographical regimes as reported in *Abutilon indicum* (Bir and Sidhu, 1979; Krishnappa and Munirajappa, 1982; Rani *et al.*, 2012) and *Tribulus rajasthanensis* (Rawat *et al.*, 2006). The information generated from the present investigation, coupled with earlier published data, revealed that there are at least two somatic chromosome counts 2n = 2x= 44 and 2n= 2x=46 were adopted by the genotypes of *N. arbortristis* across different geographical regions of India including western Odisha. The 4C DNA content not varied significantly among the ecotypes tested in this study and this homogeneous distribution might be due to high G + C content of the genome and consistent occurrence of repetitive DNA sequences in the *N. arbortristis* genome as reported in other medicinal plants (Martel *et al.*, 1997; Behera *et al.*, 2010). The symmetric karyotype of *N. arbortristis* suggested towards its genetic stability across different habitats of a particular geographical regime (Schubert and Oud, 1997), and similar kind of reports were also made in *Cymbidium* (Sharma *et al.*, 2012), *Bacopa monnieri*, *Tylophora indica* and *Withania somnifera* (Samaddar *et al.*, 2012).

This somatic chromosome count was also reflected as chromosome associations in PMCs analysed at metaphase-I, where either 22 bivalents or a combination of quadrivalents, bivalents and univalents amounting into 44 chromosomes were noticed. Quadrivalents associations were occasionally noticed. The presence of quadrivalent indicate towards their evolutionary affinities among the chromosome(s) concerned and that they are formed by residual attractive forces acting between homeologous chromosomes as reported in *Brassica* sp. (Wills, 1966) and *Curcuma* sp. (Lamo and Rao, 2017). Even low frequency of quadrivalent associations as compared to bivalents has indicated towards allopolyploid lineage of *N. arbortristis* during the course of evolution and adaptation under different ecological niche (Feldman and Levy, 2005). The presence of univalents in the PMCs might be due to failure of synapsis, and the lowest mean value (2.1) of univalents was found in ecotype NAET12, while the maximum number of univalents per PMC (3.9) was recorded in ecotype NAET11. By and large, the univalents, wherever encountered, apparently behaved normally leading to an equal distribution of chromosomes at anaphase-I. However, in some cases these univalents were traced even at anaphase-I as laggards due to their non-disjunction towards respective poles. This kind of chromosomal behaviour might be due to desynapsis as reported in many tropical plants (Dawe, 1998; Rao and Kumar, 2003; Rawat *et al.*, 2006; Pradilo *et al.*, 2007; Behera *et al.*, 2010), and desynapsis among the bivalents might be attributed to influence of several environmental factors such as temperature, humidity and nutrition as reported in *Tribulus*

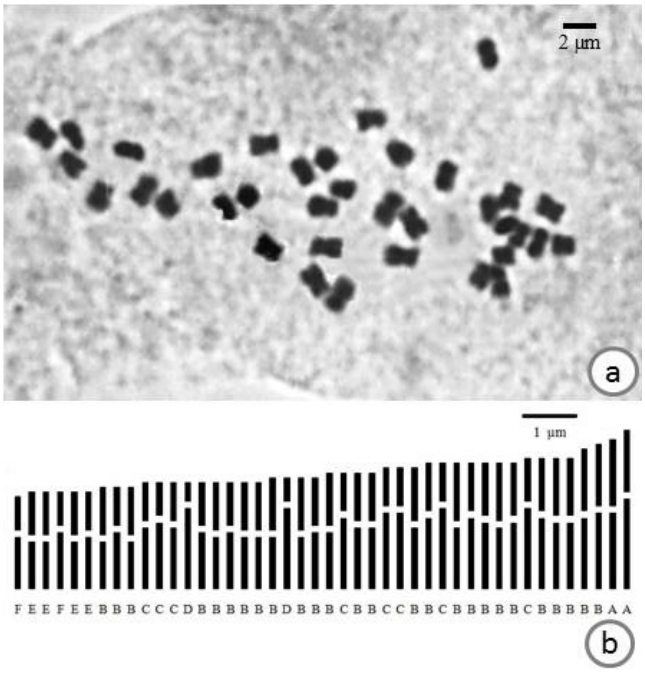

Fig.1: Mitotic analysis of *N. arbortristis*. (a) 2n=2x=44 chromosomes at early metaphase (Bar = 2.0 µM) and (b) Graphical representation of the symmetric karyotype of *N. arbortristis*, Ecotype NAET-08 (Bar = 1.0 µM).

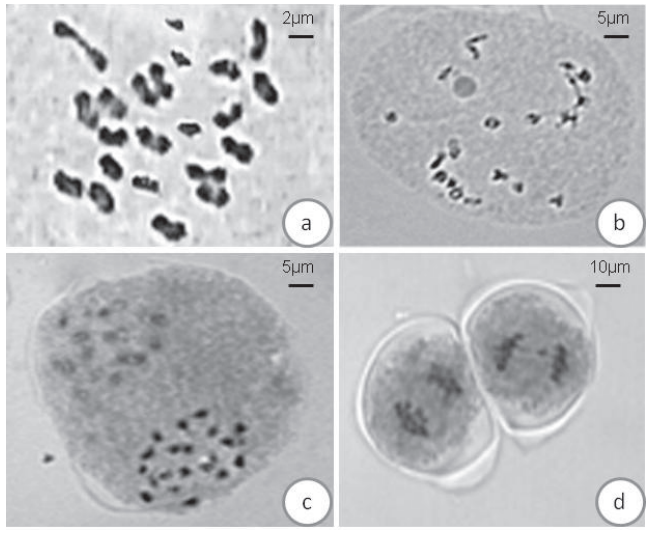

Fig.2: Meiotic behaviour of chromosomes in *N. arbortristis*. (a) Bivalents at late diakinesis metaphase; (b) Chromosome association (quadrivalents, bivalents and univalent) at metaphase-I; (c & d) Segregation of chromosome at anaphase-I with normal (c) and abnormal configurations showing laggards (d).

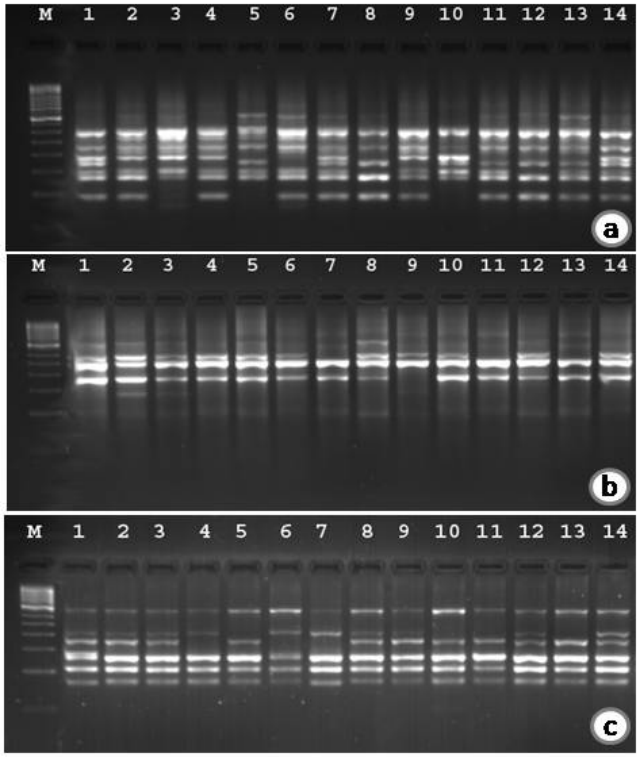

Fig.3: DNA banding pattern of 14 ecotypes of *N. arbortristis* generated by using RAPD primer OPA-04 (a), OPA-05 (b) and OPA-07 (c).

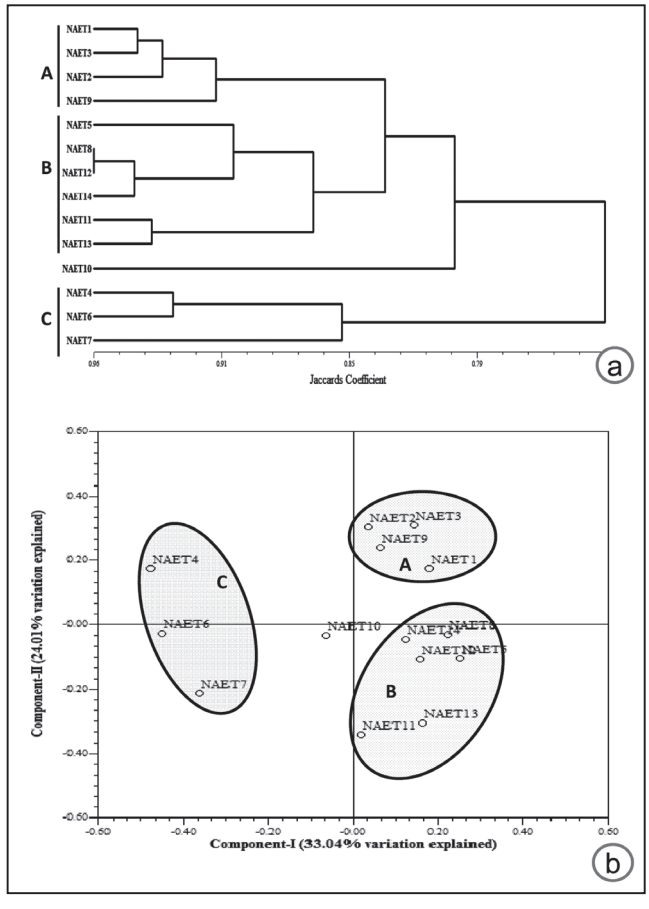

Fig.4: Genetic variation among 14 ecotypes of *N. arbortristis*. (a) Dendrogram (UPGMA) based on Jaccard's similarity coefficient and (b) Principal Coordinate analysis (PCoA) showing clustering of the ecotypes as depicted by RAPD marker analysis using 10 RAPD primers.

Table 1

Geographic distribution of 14 ecotypes collected from western Odisha, and their somatic chromosome count (2n), total chromosome length, TF% and 4C DNA content

Ecotype No.	Collection Site	Altitude (M)	Latitude (N)	Longitude (E)	2n	Total Chr. Length (μM)	TF%	4C DNA content (pg)
NAET1	Jharsuguda	218.0	21.8554°	84.0062°	44	99.90	44.01	20.48±2.48
NAET2	Jharsuguda	218.0	21.8554°	84.0062°	44	99.30	43.65	19.96±2.23
NAET3	Jharsuguda	218.0	21.8554°	84.0062°	44	100.60	43.01	20.86±2.11
NAET4	Sambalpur	135.0	21.4669°	83.9812°	44	100.20	42.65	20.90±2.22
NAET5	Bargarh	171.0	21.2550°	83.5070°	44	99.70	43.45	20.58±2.24
NAET6	Sambalpur	135.0	21.4669°	83.9812°	44	98.70	44.62	18.94±0.82
NAET7	Sambalpur	135.0	21.4669°	83.9812°	44	101.40	39.96	21.04±1.69
NAET8	Burla	173.0	21.4888°	83.8844°	44	101.20	42.09	21.02±1.68
NAET9	Jharsuguda	218.0	21.8554°	84.0062°	44	102.70	41.5	21.46±1.88
NAET10	Sambalpur	120.0	21.4669°	83.9812°	44	100.80	41.92	20.82±1.90
NAET11	Bargarh	171.0	21.2550°	83.5070°	44	101.00	40.8	20.94±1.82
NAET12	Burla	173.0	21.4888°	83.8844°	44	100.60	39.8	20.64±1.99
NAET13	Bargarh	171.0	21.2550°	83.5070°	44	100.50	44.08	20.50±2.22
NAET14	Burla	173.0	21.4888°	83.8844°	44	102.50	43.06	20.84±1.92
Mean					**44**	**100.65±1.07**	**42.47±1.47**	**20.641±1.94**

M: Meter; N: North; E: East; pg: picogram

Table 2

Karyotype details of *N. arbortristis* (Ecotype NAET-08) as revealed by its metaphase

Chromosome Type#	Number of chromosomes	Length* (μm)	Centromeric Index (F%)	Short Arm to long arm ratio (S%)	Centromeric position
A_M	02	3.0 - 3.2	40.625 – 46.667	0.684 – 0.875	Median
B_M	26	2.0 – 2.9	40.000 – 50.000	0.667 – 1.000	Median
C_{SM}	08	2.1 – 2.6	32.000 – 38.095	0.471 – 0.615	Sub-Median
D_{ST}	02	2.1 – 2.2	19.048 – 22.727	0.235 – 0.294	Sub-Terminal
E_M	04	1.9	42.105 – 47.368	0.727 – 0.900	Median
F_{SM}	02	1.8 – 1.9	36.842 – 38.889	0.583 – 0.636	Sub-Median
	44	1.8 – 3.2	19.048 – 50.000	0.235 – 1.000	—

*# A_M- Long median, B_M– Medium median, C_{SM}– Medium sub-median, D_{ST}– Medium sub-terminal, E_M– Shortmedian, and F_{SM}– Short sub median; * Length wise grouping: Short- < 2.0 μm; Medium- e"2.0 μm and < 3.0 μm; Long- e" 3.0 μm*

Table 3

Meiotic analysis in pollen mother cells (PMCs) of *N. arbor-tristis* at diplotene, diakinesis, metaphase-I and anaphase-I

Ecotype	Chromosome Association						Chiasmata properties				Terminalization coefficient	Anaphase-I Distribution (%)			Pollen viability (%age)	Seed germination Percentage (Mean ± SD)
	Quadrivalent (IV)		Bivalent (II)		Univalent (I)											
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	No. of Chiasmata Mean ± SD	Range	Unterminal-ised Chiasmata Mean ± SD	Terminalised Chiasmata Mean ± SD		22II:22II	21II:2I:21II	20II:4I:20II		
NAET01	0.45 ± 0.14	0-2	21.0±0.24	18-22	2.3 ±0.36	0-4	24.04±0.97	22-26	2.16±0.85	21.88±0.92	0.91	83.3	12.5	4.1	46.5	51.67±1.53
NAET02	0.85 ± 0.36	0-2	20.4±1.04	18-22	2.1±1.08	0-4	23.95±0.74	23-26	2.43±0.59	21.57±0.74	0.901	87.5	10.2	1.3	50.6	52.00±2.00
NAET03	0.75 ± 0.49	0-2	20.5±0.74	18-22	2.2±1.49	0-6	24.13±0.85	23-26	2.37±0.71	21.71±1.08	0.9	86.9	11.8	1.3	51.4	47.33±2.52
NAET04	—	—	20.8±0.65	19-22	3.4±1.36	0-6	24.11±0.87	23-25	2.47±0.69	21.58±0.83	0.895	80.2	14.5	5.4	53.4	42.33±1.53
NAET05	1.6 ± 0.24	0-2	19.5±0.93	18-22	2.8±1.92	0-4	24.13±0.87	23-26	2.21±0.73	21.87±0.69	0.906	80.1	16.2	3.7	51.2	47.67±0.58
NAET06	0.8 ± 0.64	0-2	19.4±1.03	18-22	3.2±2.06	0-4	23.95±0.80	23-25	2.33±0.79	21.57±0.97	0.901	89.5	10.2	0.3	58.0	52.00±1.00
NAET07	—	—	21.2±0.24	20-22	2.7±2.12	0-4	24.15±0.93	23-26	2.25±0.78	21.85±0.93	0.905	85.0	12.8	2.2	51.8	49.33±2.52
NAET08	—	—	20.3±1.03	18-22	3.8±2.06	0-6	24.1±0.85	23-25	2.20±0.76	21.85±0.83	0.907	81.8	16.4	1.7	50.8	45.67±0.58
NAET09	0.36 ± 0.14	0-2	20.5±0.93	19-22	2.7±1.86	0-4	24.0±0.97	23-25	2.05±0.78	22.0±0.66	0.915	85.0	14.2	0.8	54.2	50.33±1.53
NAET10	0.65 ± 0.36	0-2	19.5±0.93	18-22	2.8±1.87	0-4	24.43±0.81	23-26	2.43±0.59	21.95±0.74	0.899	84.2	13.2	2.6	53.6	51.00±1.00
NAET11	—	—	20.1±0.74	19-22	3.9±1.48	0-6	24.2±0.86	23-26	2.13±0.64	21.93±0.88	0.906	86.6	12.1	1.3	50.8	50.33±2.52
NAET12	0.75 ± 0.36	0-2	19.9±1.03	18-22	2.1±1.06	0-6	23.95±1.25	23-25	2.15±0.76	22.0±1.15	0.919	84.3	12.8	2.9	53.4	53.67±1.53
NAET13	1.6 ± 0.36	0-2	19.8±0.83	19-21	2.3±1.68	0-4	24.05±0.84	23-25	2.43±0.69	21.58±1.17	0.897	88.6	10.2	1.2	54.5	51.33±1.53
NAET14	1.1 ± 0.49	0-2	19.6±1.01	18-21	2.8±2.03	0-6	24.04±0.97	23-26	2.16±0.85	21.88±0.92	0.91	85.0	13.1	1.9	53.2	48.00±1.00

Table 4

Information about RAPD primers used for estimation of genetic diversity among 14 ecotypes of *N. arbortristis* showing polymorphic information content (PIC), average band informativeness (AvIb) and resolving power (Rp)

Primer	Primer sequence	No. of Loci amplified	Polymorphism (%)	Size amplified fragments (bp)	Polymorphic information content PIC	Average band Inform-ativeness (Av Ib)	Resolving power (Rp)
OPA 02	5'-TGCCGAGCTG-3'	5	40.00	585 - 1225	0.149	1.800	9.0
OPA 03	5'-AGTCAGCCAC-3'	6	50.00	480 - 1600	0.189	1.643	9.86
OPA 04	5'-AATCGGGCTG-3'	8	62.50	750 - 2165	0.253	1.607	12.86
OPA 05	5'-AGGGGTCTTG-3'	4	75.00	1190 - 2050	0.232	1.536	6.143
OPA 07	5'-GAAACGGGTG-3'	6	33.33	665 - 1905	0.124	1.833	11.0
OPA 08	5'-GTGACGTAGG-3'	9	44.44	365 - 2500	0.188	1.714	15.43
OPA 09	5'-GGGTAACGCC-3'	6	33.33	560 - 1925	0.145	1.786	10.71
OPA 13	5'-CAGCACCCAC-3'	5	20.00	455 - 1090	0.098	1.524	9.14
OPB 01	5'-GTTTCGCTCC-3'	5	20.00	580 - 3640	0.082	1.523	9.14
OPB 03	5'-CATCCCCCTG-3'	6	33.33	420 - 2595	0.153	1.764	9.99
	Total/ Average	60	41.66	365 - 3640	0.161	1.673	10.327

rajasthanensis (Rawat *et al.*, 2006). In majority of PMCs, the chiasmata were distally localized and were found to be terminalized by late diakinesis/early metaphase-I. This might be due to existence of short conserved segment of chromosomes and their involvement during the event of recombination, and such observations were commonly reported in species where the chromosome size is relatively small and morphological identical chromosomes are found (Dawe, 1998; Behera *et al.*, 2010). Presence of either distal or proximal chiasmata has been reported in several plant species (Gottschalk and Kaul, 1980; Kumar and Rao, 2002, 2003; Rawat *et al.*, 2006; Iqbal and Datta, 2007), and this has been attributed to chromosome pairing and/ or interference pattern coupled by availability of short segments for genetic crossing over in *N. arbortristis*. Majority of PMCs showed 22II:22II segregation of chromosomes, but in few PMCs laggards were observed during anaphasic separation. The presence of univalents and their subsequent failure in orientation and disjunction at anaphase might be associated with laggard formation (Gupta, 1995; Kumar and Rao, 2002; 2003; Rawat *et al.*, 2006; Iqbal and Datta, 2007; Sharma *et al.*, 2010).

The percentage of pollen viability was quite low, and it ranges from 46.5% to 58.0% across the ecotypes of *N. arbortristis* assessed, and it might be attributed to meiotic irregularities (Pagliarini, 2000) and environmental stress prevailed by relative humidity and temperature (Aronne, 1999). Similarly, the seeds showed very low germination rate (Rout *et al.*, 2008), which might be due to either abnormal pollen biology leading to unsuccessful fertilization event coupled with immature seed development or leaching out of phenolic compounds present in the pericarp and seed coat of *N. arbortristis* (Data not shown) as reported in other members of Oleaceae such as in *Abeliophylum* (Sahu *et al.*, 2012; Ghimire *et al.*, 2015).

Genetic polymorphisms among species and even between the genotypes of same species have manifold implications during the evolution and conservation of plant species across diverse ecological niche. Thus, depiction of genetic variation has long been based on morphological traits in general and even on phytochemical attributes in case of medicinal species. Both morphological and phytochemical attributes are subjected to penetrance and expressivity under the influence of environmental factors. Thus, diverse kinds of DNA markers have been used in recent time to assess the genetic variability as complimentary tools to conventional approaches in genetic resource management (Panigrahi *et al.*, 2015; Sahu *et al.*, 2016). However, the determinants of this variation have been poorly understood in *N. arbortristis*. Rohilla *et al.* (2017) used RAPD markers and reported on the existence of moderate genetic diversity among 16 accessions of Northern India. In the present study, 60 RAPD markers generated by ten responding primers substantiated the moderate genetic diversity among the 14 ecotypes *N. arbortristis* of western Odisha. In this study RAPD primers showed average PIC,

AvIb and Rp and estimation of moderate genetic divergence, and these findings were in good agreement with earlier report (Rohilla *et al.*, 2017). Dendrogram and PCoA based on Jaccard's similarity indices among these ecotypes of *N. arbortristis* formed three major clusters in consonance with their collection site. This might be due to similar kind of evolutionary forces at respective geographic regimes during the course of adaptation under different ecological niche. The similarity coefficient (0.648 to 0.962) was an indicator of moderate degree of genetic variation between *N. arbortristis* ecotypes used in this study. The ecotypes also showed their close genetic affinity among themselves and this might be due to their pedigree as reported in other medicinal species.

In summary, this study presented the suitability of RAPD markers as complimentary tool along with conventional cytological analysis for genetic divergence study in *N. arbortristis*. Use of advanced generation DNA markers such as simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) could also be used as genomic tools for elucidation of genetic relationships and domestication pathway of this medicinal species, and genetic augmentation of *N. arbortritis* through molecular breeding aiming at superior therapeutic potential.

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Aquatic angiosperms of Bonai Forest Division, Sundargarh district, Odisha

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ABSTRACT

The Bonai Forest Division of Sundargarh district is situated between 21º39¹-22º8¹ N and 84º30¹-85º23¹ E towards the North-western boundary of the state of Odisha. The forest division spreads over an area of 2934.21 sq. km. The edaphic and climatic conditions of Bonai provide ideal habitats for a variety of aquatic life forms. During the floristic survey of Bonai forest division, a total of 125 aquatic, semi-aquatic and wetland species belonging to 77 genera under 33 families were recorded. Cyperaceae and Poaceae were the dominant families with 34 and 14 aquatic species respectively in the study area. Present investigation revealed that forests of Bonai in Sundargarh district harbour rich wetland plant diversity and documentation and conservation of these resources is the need of the hour.

1. Introduction

Aquatic plants play vital role in the primary productivity of aquatic ecosystems. These plants have been used for various purposes mainly for food, fodder, fibre and medicine since historical times. The major habitats for the aquatic plants of Bonai forest division are ponds, pools, seasonal puddles, rivers and streams. Besides, several low-lying rice fields and ditches along roads are scattered throughout the forest division which form good habitats for aquatic plants. Currently, aquatic habitats face tremendous anthropogenic pressures such as large scale change in land use pattern and improper use of wetlands, which in turn greatly influence the aquatic biodiversity (Prasad *et al*., 2002). Therefore, there is an urgent need to record and assess the diversity of aquatic plants and their habitats in Bonai forest division before they vanish for ever.

2. Study Area

The Bonai forest division is situated between 21º39¹-22º8¹N and 84º30¹-85º23¹ E towards the North-western boundary of the state of Odisha. The forest division spreads over an area of 2934.21 sq. km of Sundargarh district. It is bounded on the North by Jharkhand State and Rourkela forest division. On the east, it is bounded by Keonjhar forest division and Deogarh forest division. On the west and south it is surrounded by Bamra forest division and Deogarh forest

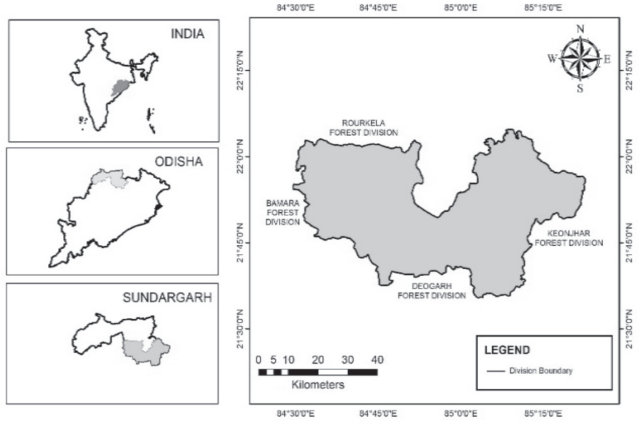

Fig. 1: Location map of Bonai Forest Division, Odisha

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division. There are seven forest ranges in this division namely Bonai, Kuliposh, Tamra, Jarda, Sole, Barsuan and Koira. The forest division is part of the Chotanagpur plateau of Deccan Peninsular Biogeographic zone (Rodgers and Panwar, 1988).

3. Botanical Exploration: Past & Present

Haines (1921-25), the pioneer plant explorer of the state provided very scanty information about the flora of Sundargarh district as well as forests of Bonai. Later, Mooney (1950), Saxena & Brahmam (1994-96), Misra (2004) have contributed significantly to the flora of the region. Taxonomy and ecology of aquatic plants of India are well documented (Agarkar, 1923; Biswas & Calder, 1937; Subramanyam, 1974; Gupta, 1979; Lavania *et al.,* 1990), whereas studies on wetland plants of Odisha have been carried out by several workers (Choudhury & Patnaik, 1985; Choudhuy & Choudhury, 1996; Pal & Nimse, 2006; Misra *et al.,* 2012; Subhadarsini *et al.*, 2016). However, some workers (Acharya *et al*., 2010; Mallick *et al.,* 2015) have carried out floristic studies of Rourkela of Sundargarh district. Realizing the meagerness of the floristic work done for the district and considering the phyto-geographical and ethnobotanical importance of plant resources of this region, an inventory of flora of Sundargarh district has been undertaken since 2015 and the present work on aquatic angiosperms of Bonai Forest Division is a part of the study, which documents the rich aquatic plant biodiversity of a forest division of Sundargarh district.

4. Materials and methods

The present work was the outcome of the extensive survey of flora in and around rivers, rivulets, streams, waterfalls, ponds, pools, seasonal puddles, rice fields and other wetlands of Bonai forest division during the period December, 2015 to November, 2017. The entire forest division was covered in different months of the year to record the seasonal variation in plant diversity. All the species of aquatic plants were collected and detailed field notes were recorded in the field, which includes field number, date of collection, locality, habitat, phenology, local name, uses and the macroscopic characters which cannot be revealed from the dried specimens. Four samples of a species were carefully collected so as to include all possible types of variations. For the preservation of the specimens, standard guidelines were followed with little modification, wherever required. The specimens were identified with the help of relevant floras (Haines, 1921-25; Mooney, 1950; Saxena & Brahmam, 1994-96). Some doubtful specimens were taken to Central National Herbarium (CNH), Howrah and were matched with authentic materials available there. Up-to-date nomenclature of plants was determined in consultation with different floras, monographs, revisions, The Plant List and IPNI databases. All the specimens were deposited in the Herbarium of the P.G. Department of Botany, North Orissa University, Baripada.

In the present study aquatic plants were classified depending upon their habitat, relation with water, soil, air and light.

Free floating: Commonly seen in stagnant water bodies, slow flowing water and are in conduct with only water, air and light. Such species typically float on water surface with extensive root system. Very often these species occur in pure communities and completely cover up the water surface where favourable condition exists.

Submerged: Generally, in such species the foliage is entirely submerged, conduct with soil or rock but their reproductive parts are raised slightly above the water level.

Fixed floating: These types of plants are in contact with soil, water and air. Some of the plants occur on soft wet muddy substratum or root in water surface and are in contact with soil, water and air, even after the substratum is considerably dried up.

Amphibious: Commonly occur on exposed or submerged soils where the water table is beneath the soil surface. These plants are adopted to sustain in both aquatic and terrestrial modes of life. The aerial parts of these amphibious hydrophytes are with mesophytic characters and submerged part shows true hydrophytic characters. Many of these thrive well even after the substratum is considerably dried up.

Marshy: These are also known as border line plants and the soil is usually saturated with water at least in the early part of the plant life. They are frequently observed with in wet rice fields, bank of water bodies, wet areas near human habitation, along hill swamps and streams forests.

5. Results and discussion

As a result of intensive survey, we have collected 125 species of aquatic and semi-aquatic plants belonging to 77 genera under 33 families. Out of these monocots were represented by 68 species under 38 genera and 10 families, while 57 species under 39 genera and 23 families constituted the dicot flora. Besides, there were 78 species of marshy plants belonging to 41 genera and 16 families. Twenty six (26) species were amphibious in nature. Apart from these, fixed floating, submerged and free floating aquatics were represented by 8, 7 and 6 species respectively.

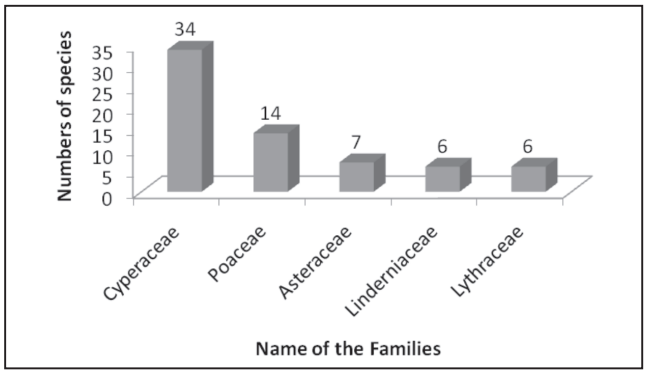

Fig. 2: Five dominant families of aquatic plants of Bonai Forest Division in terms of species content

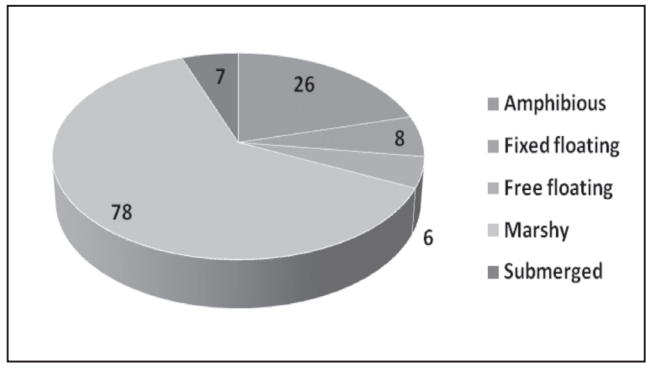

Fig. 3: Habitat wise distribution of aquatic plants in Bonai Forest Division

Cyperaceae with 34 species was the most speciose family followed by Poaceae with 14 species, Asteraceae with 7 species and Linderniaceae and Lythraceae with 6 species each. The five dominant families in terms of species content are shown in Fig.-2. The habitat -wise distribution of species is represented in Fig.-3. The enumeration of aquatic plant species with up-to-date nomenclature, family name and habitat types are given in Table-1 and statistical analysis of flora of Bonai Forest Division is provided in Table-2.

Table 1

List of aquatic plants of Bonai Forest Division with family name and habitat

Sl.No	Botanical Name	Family	Habitat
1	*Acmella paniculata* (Wall. ex DC.) R.K.Jansen	Asteraceae	Marshy
2	*Actinoscirpus grossus* (L.f.) Goetgh. & D.A.Simpson	Cyperaceae	Amphibious
3	*Aeschynomene aspera* L.	Leguminosae	Amphibious
4	*Aeschynomene indica* L.	Leguminosae	Amphibious
5	*Alloteropsis cimicina* (L.) Stapf	Poaceae	Amphibious
6	*Alternanthera sessilis* (L.) R.Br. ex DC.	Amaranthaceae	Amphibious
7	*Ammannia baccifera* L.	Lythraceae	Marshy
8	*Ammannia multiflora* Roxb.	Lythraceae	Marshy
9	*Aponogeton natans* (L.) Engl. & K.Krause	Aponogetonaceae	Submerged
10	*Bacopa monnieri* (L.) Wettst.	Plantaginaceae	Marshy
11	*Blyxa echinosperma* (C.B.Clarke) Hook.f.	Hydrocharitaceae	Submerged
12	*Brachiaria distachya* (L.) Stapf	Poaceae	Marshy
13	*Centella asiatica* (L.) Urb.	Apiaceae	Marshy
14	*Centranthera indica* (L.) Gamble	Orobanchaceae	Marshy
15	*Ceratophyllum demersum* L.	Ceratophyllaceae	Submerged
16	*Chrysopogon zizanioides* (L.) Roberty	Poaceae	Marshy
17	*Colocasia esculenta* (L.) Schott	Araceae	Amphibious
18	*Commelina benghalensis* L.	Commelinaceae	Marshy
19	*Commelina erecta* L.	Commelinaceae	Marshy
20	*Commelina paludosa* Blume	Commelinaceae	Marshy
21	*Cyathocline purpurea* (Buch.-Ham. ex D. Don) Kuntze	Asteraceae	Amphibious
22	*Cyperus cephalotes* Vahl	Cyperaceae	Submerged

Sl.No	Botanical Name	Family	Habitat
23	*Cyperus compressus* L.	Cyperaceae	Marshy
24	*Cyperus difformis* L.	Cyperaceae	Marshy
25	*Cyperus diffusus* Vahl.	Cyperaceae	Marshy
26	*Cyperus distans* L.f.	Cyperaceae	Marshy
27	*Cyperus imbricatus* Retz.	Cyperaceae	Marshy
28	*Cyperus involucratus* Rottb.	Cyperaceae	Marshy
29	*Cyperus iria* L.	Cyperaceae	Marshy
30	*Cyperus nutans* Vahl	Cyperaceae	Marshy
31	*Cyperus paniceus* (Rottb.) Boeckeler	Cyperaceae	Marshy
32	*Cyperus pilosus* Vahl	Cyperaceae	Marshy
33	*Cyperus platystylis* R.Br.	Cyperaceae	Marshy
34	*Cyperus procerus* Rottb.	Cyperaceae	Marshy
35	*Cyperus rotundus* L.	Cyperaceae	Marshy
36	*Cyperus squarrosus* L.	Cyperaceae	Marshy
37	*Cyperus tenuispica* Steud.	Cyperaceae	Marshy
38	*Dentella repens* (L.) J.R.Forst. & G.Forst.	Rubiaceae	Marshy
39	*Dopatrium junceum* (Roxb.) Buch.-Ham. ex Benth.	Plantaginaceae	Marshy
40	*Echinochloa colona* (L.) Link	Poaceae	Marshy
41	*Eclipta prostrata* (L.) L.	Asteraceae	Marshy
42	*Eichhornia crassipes* (Mart.) Solms	Pontederiaceae	Free floating
43	*Eleocharis congesta* D.Don	Cyperaceae	Marshy
44	*Eleocharis dulcis* (Burm.f.) Trin.ex Hensch.	Cyperaceae	Marshy
45	*Emilia sonchifolia* (L.) DC. ex DC.	Asteraceae	Marshy
46	*Enydra fluctuans* DC.	Asteraceae	Amphibious
47	*Eragrostis ciliaris* (L.) R.Br.	Poaceae	Marshy
48	*Eragrostis pilosa* (L.) P.Beauv.	Poaceae	Marshy
49	*Eragrostis unioloides* (Retz.) Nees ex Steud.	Poaceae	Marshy
50	*Eriocaulon quinquangulare* L.	Eriocaulaceae	Amphibious
51	*Fimbristylis aestivalis* Vahl	Cyperaceae	Marshy
52	*Fimbristylis dichotoma* (L.) Vahl	Cyperaceae	Marshy
53	*Fimbristylis littoralis* Gaudich.	Cyperaceae	Marshy
54	*Fimbristylis quinquangularis* (Vahl) Kunth	Cyperaceae	Marshy
55	*Floscopa scandens* Lour.	Commelinaceae	Marshy
56	*Fuirena ciliaris* (L.) Roxb.	Cyperaceae	Marshy
57	*Glinus oppositifolius* (L.) Aug.DC.	Molluginaceae	Marshy
58	*Gnaphalium polycaulon* Pers.	Asteraceae	Marshy
59	*Homonoia riparia* Lour.	Euphorbiaceae	Amphibious
60	*Hydrilla verticillata* (L.f.) Royle	Hydrocharitaceae	Submerged
61	*Hydrocotyle sibthorpioides* Lam.	Araliaceae	Marshy
62	*Hydrolea zeylanica* (L.) Vahl	Hydroleaceae	Amphibious
63	*Hygrophila auriculata* (Schumach.) Heine	Acanthaceae	Amphibious

Sl.No	Botanical Name	Family	Habitat
64	*Ipomoea aquatica* Forssk.	Convolvulaceae	Fixed floating
65	*Ipomoea carnea* Jacq.	Convolvulaceae	Marshy
66	*Ipomoea pes-caprae* (L.) R. Br.	Convolvulaceae	Marshy
67	*Isachne globosa* (Thunb.) Kuntze	Poaceae	Marshy
68	*Kyllinga brevifolia* Rottb.	Cyperaceae	Marshy
69	*Lasia spinosa* (L.) Thwaites	Araceae	Marshy
70	*Leersia hexandra* Sw.	Poaceae	Marshy
71	*Limnophila aquatica* Alston	Plantaginaceae	Amphibious
72	*Limnophila indica* (L.) Druce	Plantaginaceae	Amphibious
73	*Lindernia anagallis* (Burm.f.) Pennell	Linderniaceae	Marshy
74	*Lindernia antipoda* (L.) Alston	Linderniaceae	Marshy
75	*Lindernia ciliata* (Colsm.) Pennell	Linderniaceae	Marshy
76	*Lindernia crustacea* (L.) F.Muell.	Linderniaceae	Marshy
77	*Lindernia procumbens* (Krock.) Philcox	Linderniaceae	Marshy
78	*Lindernia rotundifolia* (L.) Alston	Linderniaceae	Marshy
79	*Lipocarpha chinensis* (Osbeck) J.Kern	Cyperaceae	Marshy
80	*Lipocarpha gracilis* (Rich. ex Pers.) Nees	Cyperaceae	Marshy
81	*Lippia javanica* (Burm.f.) Spreng.	Verbenaceae	Marshy
82	*Ludwigia adscendens* (L.) H.Hara	Onagraceae	Fixed floating
83	*Ludwigia octovalvis* (Jacq.) P.H.Raven	Onagraceae	Fixed floating
84	*Monochoria hastata* (L.) Solms	Pontederiaceae	Amphibious
85	*Monochoria vaginalis* (Burm.f.) C.Presl	Pontederiaceae	Amphibious
86	*Nelumbo nucifera* Gaertn.	Nymphaeaceae	Fixed floating
87	*Nymphaea nouchali* Burm.f.	Nymphaeaceae	Fixed floating
88	*Nymphaea pubescens* Willd.	Nymphaeaceae	Fixed floating
89	*Nymphoides hydrophylla* (Lour.) Kuntze	Menyanthaceae	Fixed floating
90	*Nymphoides indica* (L.) Kuntze	Menyanthaceae	Fixed floating
91	*Oldenlandia corymbosa* L.	Rubiaceae	Marshy
92	*Oryza rufipogon* Griff.	Poaceae	Marshy
93	*Ottelia alismoides* (L.) Pers.	Hydrocharitaceae	Submerged
94	*Panicum repens* L.	Poaceae	Marshy
95	*Paspalidium flavidum* (Retz.) A.Camus	Poaceae	Marshy
96	*Persicaria barbata* (L.) H.Hara	Polygonaceae	Amphibious
97	*Persicaria glabra* (Willd.) M.Gómez	Polygonaceae	Amphibious
98	*Persicaria hydropiper* (L.) Delarbre	Polygonaceae	Amphibious
99	*Phyla nodiflora* (L.) Greene	Verbenaceae	Marshy
100	*Pistia stratiotes* L.	Araceae	Free floating
101	*Polygonum plebeium* R.Br.	Polygonaceae	Amphibious
102	*Pycreus flavidus* (Retz.) T.Koyama	Cyperaceae	Marshy
103	*Pycreus polystachyos* (Rottb.) P.Beauv.	Cyperaceae	Marshy
104	*Pycreus pumilus* (L.) Nees	Cyperaceae	Marshy

Sl.No	Botanical Name	Family	Habitat
105	*Pycreus puncticulatus* (Vahl) Nees	Cyperaceae	Marshy
106	*Pycreus sanguinolentus* (Vahl) Nees	Cyperaceae	Marshy
107	*Rotala indica* (Willd.) Koehne	Lythraceae	Marshy
108	*Rotala mexicana* Schltdl. & Cham.	Lythraceae	Marshy
109	*Rotala rotundifolia* (Buch.-Ham. ex Roxb.) Koehne	Lythraceae	Marshy
110	*Rotula aquatica* Lour.	Boraginaceae	Marshy
111	*Sacciolepis indica* (L.) Chase	Poaceae	Marshy
112	*Sacciolepis interrupta* (Willd.) Stapf	Poaceae	Marshy
113	*Sagittaria guayanensis* Kunth	Alismataceae	Amphibious
114	*Schoenoplectiella articulata* (L.) Lye	Cyperaceae	Amphibious
115	*Scleria terrestris* (L.) Fassett	Cyperaceae	Marshy
116	*Sesbania bispinosa* (Jacq.) W.Wight	Leguminosae	Amphibious
117	*Smithia conferta* Sm.	Leguminosae	Amphibious
118	*Smithia sensitiva* Aiton	Leguminosae	Amphibious
119	*Spirodela polyrrhiza* (L.) Schleid.	Araceae	Free floating
120	*Synedrella nodiflora* (L.) Gaertn.	Asteraceae	Marshy
121	*Trapa natans* L.	Lythraceae	Free floating
122	*Typha domingensis* Pers.	Typhaceae	Amphibious
123	*Utricularia aurea* Lour.	Lentibulariaceae	Free floating
124	*Vallisneria natans* (Lour.) H. Hara	Hydrocharitaceae	Submerged
125	*Wolffia arrhiza* (L.) Horkel ex Wimm.	Araceae	Free floating

Table 2
Statistical analysis of aquatic flora of Bonai Forest Division.

Groups	Dicots		Monocots		Total
	Number	Percent	Number	Percent	
Families	23	69.6%	10	30.3%	33
Genera	39	50.6%	38	49.3%	77
Species	57	45.6%	68	54.4%	125

The ecological classification of aquatic plants presented here is, to some extent, arbitrary and has been followed only for convenience and to depict the habit and habitat of the taxa. The hydrophytes sometimes show plasticity in their phenotypes and adaptability to a wide range of habitats. During rainy season, when water is plenty the species like *Ipomoea aquatica*, *Enydra fluctuans, Ipomoea aquatica* form floating mats but when the water bodies dry up, these species establish themselves in the wet soil and continue to grow as terrestrial plants. At times, some species such as *Eichhornia crassipes* and *Monochoria hastata* are found as emergent amphibious plants and *Hydrilla verticillata* as suspended submerged hydrophytes. Thus, several species change their survival strategy to adapt to available ecological conditions.

However, majority of the species reported in this work fall under the category of marshy and amphibious types, indicating that the margin of the water bodies and river banks form ideal habitats for luxuriant growth of amphibious species. The true hydrophytes are considerably less in number.

6. Conclusion

The Bonai Forest Division eas found to be a rich habitat for aquatic angiosperms and have a number of local use

values such as medicine, fodder and leafy vegetables. Further, they help in maintaining the ecosystem by providing habitats for a wide variety of water birds and wild animals. The present study provides first-hand field level information on aquatic angiosperms of Bonai, which will be helpful in compilation of biodiversity database of this forest division and identify species needing conservation action.

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Effect of climatic and physical factors on yield and quality of essential oil of *Pandanus odorifer* (Forssk.) Kuntze

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ABSTRACT

Pandanus odorifer (Forssk.) Kuntze., commonly known as Kewda, is one of the major bioresources of Ganjam district, Odisha used in small scale perfumery industry for aromatic compound extracted from the male inflorescences. In order to establish the effect of climatic and physical factors on perfume yield and quality, oil samples of Kewda flowers collected from the coastline of Ganjam, Odisha, India were analyzed. Gas chromatography (GC) analysis revealed the presence of Phenyl ethyl methyl ether or PEME (75-85%) and Terpinen-4-ol (4-10%) as major constituents of Kewda oil. The climatic factors were found to significantly influence the yield and quality of Kewda oil. The yield percentage of major constituents was found to be low in summer, followed by rainy season and winter season. The physical factors like quality of flowers, time of plucking and process of distillation also had major effect on the yield and quality of the oil.

1. Introduction

The genus *Pandanus* (Pandanaceae) is comprised of about 500 species, which are distributed in the tropical parts of the world extending from Western Africa in the west to the Polynesia, Pacific island in the east and in Northern Australia. Some 36 are reported to occur in India (Padhy *et al*., 2016). Of these, *Pandanus odorifer* (Forssk.) Kuntze. (Syn. *Pandanus odoratissimus* Linn) has widespread distribution and has several vernacular names such as Ketakee, Gandhapushpa, Sthiragandha, Indu Kalika, Jambala (Sanskrit); Kewra, Keora, Kewda, Gagandhul (Hindi); Kedgi, Kevda, Keora (Marathi); Kewoda (Gujarati); Kiya, Ketakee (Odia); Keya, Kedki, Keori (Bengali); Thazhai, Thalay, Thazhampoo (Tamil); Mogali, Gajangi (Telugu); and Kaitha, Kaida, Thala (Malayalam). In India, the plant grows widely along the entire coast of Indian peninsula and the Andamans. The species also occurs in the coastal regions of Iran, Malaysia, Mauritius, Myanmar, Java, China, Taiwan and southern islands of Japan (Panda *et al*., 2010). Though the plant is distributed all along the coast of Odisha from Ganjam in south-west to Baleswar in the North-east, it is fairly abundant in the coastal belt of Ganjam district. Especially, a stretch of about 4 km along the coast between the two rivers Bahuda and Rushikulya supports luxuriant growth of several populations of Kewda. Traditionally, the plants are used as vegetative fence around agricultural fields and planted along the coast as a wind breaker and soil binder (Panda *et al*., 2009; 2010). Besides, the leaves and roots are processed and used in local cottage industry for making mats, bags, baskets etc. and flowers in folklore and traditional medicine (Dutta *et al*., 1987). The plant is dioecious and the inflorescences of male plants are the source of essential oil or '*Rooh*' (2 Phenyl ethyl methyl ether, terpinen-4-ol, phenyl ethyl alcohol etc.) and perfume or '*Ittar*' (Panda *et al*., 2009). Generally, the female plants do not bear floral bouquets and develop into fruits (Rout *et al*., 2005). Essential oil of the Kewda has various commercial applications as food additives, aromatherapy, ayurvedic medicines, hair oils, agarbattis (incense sticks), lotions, cosmetics, soaps and perfume and significantly supports the local economy in

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Ganjam district (Panda *et al*., 2012). The populations growing in this belt are considered superior in terms of the quantity and quality of essential oil like higher yield and essence value of the flowers (Fig. 1).

Fig. 1: *Pandanus odorifer* (Forssk.) Kuntze plant with flower

The small-scale perfumery industries located along the coast of Ganjam, Odisha are thriving due to abundance of Kewda plants in the region (Panda *et al*., 2009). The post-process wastes of distilled flowers are also being used in production of low grade cardboards by the paper mills and as manure.

The chemistry of the essential oil of kewda suggests that its major constituent, Phenyl ethyl methyl ether (PEME) contents varies between 75 to 85 percent among different populations and geographical regions (Sahu & Mishra, 2007; Misra *et al*., 2000; Mahalingam *et al*., 2012; Raina *et al*., 2004). Significant variation in major constituents of kewda oil has been attributed to edaphic and climatic factors. The present study was undertaken to establish whether climatic and physical factors play any role with regard to variation in major constituents of Kewda essential oils among the germplasms of different regions of Ganjam district of Odisha. The essential oils from flowers of seven male populations of *Pandanus odorifer* (KW-I to KW-VII) growing naturally along the coastline of Ganjam were analysed to suggest suitable physical conditions of flowers and climatic requirement to obtain quality essential oil with higher oil yield.

2. Materials and methods

2.1. Collection and processing of flowers

A survey was carried out along the coastline of Ganjam district of Odisha covering a distance of 80 km from Biswanathpur to Rushikulya River bank region for sample collection. For determining the effect of weather conditions on the contents of major constituents of the Kewda essential oil, male flowers were collected from seven populations from three zones (Table 1). The flowers were collected in the early morning between 8.0-9.0 AM in three prominent seasons (summer, rainy and winter). The collected flowers were hydro-distilled within an hour of collection in laboratory using Clevenger's apparatus at FFDC Extension Unit, Berhampur. Traditional method of distillation practiced in the area was also used. In order to assess the effect of flower condition on the yield and quality of the essential oil, the decayed flowers, semi-bloomed flowers and buds were also collected and distilled as per the process described earlier.

Table 1

Geographical locations of collection of populations of *P. odorifer* from Ganjam district, Odisha

Populations	Regions	Lattiude	Longitude
KW-I	Rushikulya River Belt	$19^0$23'11.77"N	$85^0$1'36.53"E
KW-II	Chamakhandi	$19^0$10'15.27"N	$84^0$56'0.33"E
KW-III	Kalipalli	$19^0$17'48.97"N	$84^0$55'37.37"E
KW-IV	Keluapalli	$19^0$13'3.7"N	$84^0$48'29.84"E
KW-V	Indrakhi	$19^0$11'44.59"N	$84^0$49'20.5"E
KW-VI	Markandi	$19^0$11'35.02"N	$84^0$48'32.79"E
KW-VII	Mantridi	$19^0$11'14.94"N	$84^0$46'0.69"E

2.2. Distillation

Freshly plucked flowers collected from aforesaid locations were chopped to smaller pieces of about 1-2 inches length after the green parts of the flowers are removed as they contain no oil. After that the chopped flowers were added to the round bottom flask of 5 L capacity with water in the ratio of 1:3. It was connected to the Clevenger's apparatus and kept on the heating mantle for hydro-distillation for 3 hours at a stock temperature of 60-70^{0}C. The first distillate came in a vaporized form at a temperature of 100-110^0 C after one and half hour and about 80% of the essential oil could be recovered during this phase. The remaining oil was extracted after completion of 3 hours of distillation.

2.3. Traditional distillation (Deg-Bhapka) method

The fresh Kewda flowers harvested from the fields in the early morning (6 AM) were graded and dressed up by removing the green leaf materials in order to maintain the aroma of the oil. The selected flowers were kept in the traditional copper stills (*Deg*) which could accommodate 400-500 flowers in a batch for distillation (Sahu *et al*., 2007). In each still, depending upon the number of flowers added, 80-110 L of redistilled water was added. The process of heating was done using firewood. At a temperature of 60 - 70^{0}C, the vaporization process started. The same is collected in the container locally known as "*Bhapka"*. In each batch, approximately 12-15 L of hydrosol (mixture of water and essential oil) was collected. The process of DEG-BHAPKA

(traditional distillation method) took about 2-3 hrs (Sahu *et al*., 2007). After completion of the distillation process of a day, the collected hydrosols of all the batches were redistilled to recover the essential oil of Kewda and oil was separated from water using separating funnel.

2.4. Collection and storage of essential oil

After completion of hydro-distillation, the extracted essential oil of Kewda was collected and stored in air tight glass vials for further analysis. The percent oil yield was measured using standard methods.

2.5. Gas Chromatography (GC) analysis

Gas Chromatography (GC) analysis was done with nitrogen as carrier gas flowing at a rate of 1.0 ml/min. Flame Ionizing Detector (FID) supplied with air and hydrogen flowing at a rate of 350 ml/min and 35 ml/min respectively was used. The essential oil sample injected volume was 0.02µl. Initial oven temperature was 50^0C and hold time was 5 mins. Constituents of Kewda oil were analyzed through GC/GC-MS and compounds identified by comparing their mass spectral data with those in NIST library (Stein, 1990). Further, the major constituents of Kewda oil were analyzed employing Gas Chromatography system (GC, Agilent Technologies 6890 System, USA).

3. Results and discussion

3.1. Variation in constituents of essential oil in different seasons

The major constituents of essential oil showed significant variation in response to climatic variables and among different individuals and populations occurring in a range of habitats. The PEME (2 phenyl ethyl methyl ether) and terpinen-4-ol constitute about 90-95 % of the essential oil of Kewda. Results of the present study revealed that the contents of PEME and terpinen-4-ol are inversely proportional. While the PEME value decreased in summer, terpinen-4-ol increased during this period. In rainy season, which is the peak flowering period for Kewda, an increase in the PEME value and decrease in terpinen-4-ol content could be observed and this trend continued till flower harvest in winter. The PEME and terpinen-4-ol contents varied between 65.42-77.19% and 9.08-16.50 % during summer respectively. In rainy season, variation in PEME content was in the range of 70.38-81.5% and terpinen-4-ol between 7.95-15.53%, whereas during winter harvest the PEME values ranged between 80.05-83.62% and terpinen-4-ol from 4.68% to 9.97%.

3.2. Annual average variation in major constituents among different populations

The annual average variation in major constituents among different populations is shown in Fig. 2. It revealed

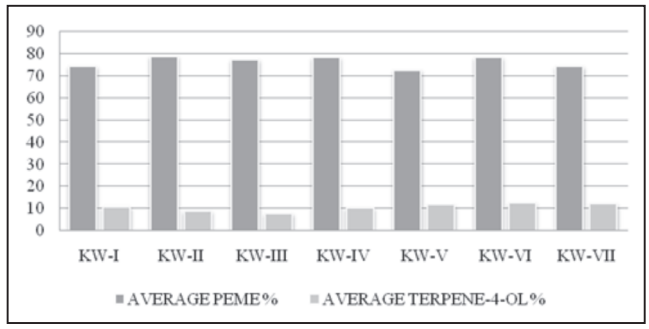

Fig. 2: Annual average variation in major constituents (PEME and Terpinen-4-ol) among different populations of Kewda

that the percentage of PEME varied from 72.57-78.85 % and terpinen-4-ol from 7.71-12.41 % among the populations studied.

3.3 Annual average variation in major constituents in the species

Comparative analysis of cumulative PEME and terpinen-4-ol percentage in the essential oil of Kewda showed significantly higher average PEME (81.71 %) in winter compared to summer (71.64 %) and rainy season (75.52 %), while terpinen-4-ol contents were found to be the lowest (7.54 %) in winter compared to 12.61 % in summer and 11.49 % during rainy season (Fig. 3).

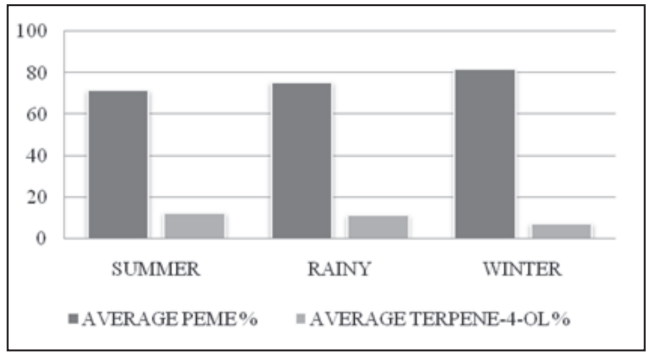

Fig. 3: Annual average variation in major constituents (PEME and Terpinen-4-ol) in the species (*P. odorifer*)

3.4. Effect of seasons on the essential oil yield

The essential oil yield of Kewda was significantly influenced by the seasonal variations in climatic parameters. In most of the populations, the oil yield was significantly higher during the winter season (0.34 %), while it decreased to 0.17% during the summer months (Fig. 4). Climate play a big role in plant secondary metabolite production as reported by several workers (Sandeep *et al*., 2016; Hassiotis *et al*., 2014; Løvdal *et al*., 2010;Mølmann *et al*., 2015;Tayade *et al*., 2013; Payyavula *et al*., 2012). In the present investigation, the essential oil yield was found to be high in winter followed by rainy and summer season and is in agreement with the earlier findings.

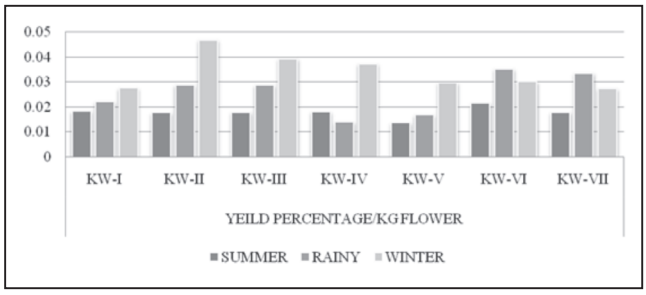

Fig. 4: Yield percentage of essential oil of Kewda per kg fresh weight of flower in different seasons

3.5. Impact of flower conditions on contents of major constituents of essential oil

The PEME and terpinen-4-ol contents in the essential oil varied due to physical conditions of the flowers. Fresh flowers had more PEME (81.70%) and less terpinen-4-ol (12.54 %) contents, where as in preserved and decayed flowers as low as 76.23 % of PEME and high terpinen-4-ol (14.66 %) values were determined. Interestingly, highest PEME (87.55 %) and lowest terpinen-4-ol (7.46%) contents were detected in flower buds of Kewda (Fig. 5).

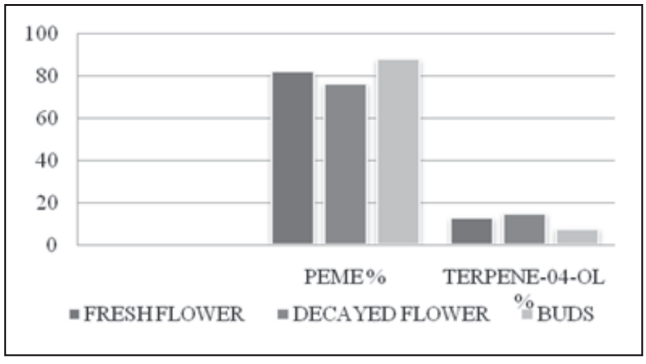

Fig. 5 Variation in major constituents (PEME and Terpinen-4-ol) of essential oil of Kewda at different stages of flower development

3.6. Impact of flower conditions on the yield of essential oil

The data obtained from the present study showed that the fresh flowers give better oil yield compared to decayed and buds. The fresh flower has a percent yield of 0.037% per Kg fresh weight whereas decayed and buds had an average yield of 0.013 and 0.010% per Kg fresh weight of flowers (Fig. 6).

Flower buds were found to contain higher quantity of good quality essential oil. The essential oil of Kewda is a very useful raw material for many value added products in Ayurvedic medicines, perfumery and other pharmaceutical products. Due to high demand and cost of Kewda oil, adulteration does take place and quality control becomes a key issue for authentication of the product. Therefore, the current study on variation in quality and quantity of essential oil in Kewda and how environment and genotypes modify the oil yield will provide baseline data for quality control of the oil. Further, the present work could be helpful for selection of sites for cultivation, provide clues for deciding correct time of harvesting and right stage of flower collection to ascertain better oil yield.

Fig. 6: Variation in essential oil yield percentage at different stages of flower development

Table 2

Variation in major constituents of essential oil of Kewda in different seasons and among populations of Ganjam district, Odisha

Locations/ Populations	Seasons	Average	
		Peme %	Terpinen-04-ol %
KW-I	Summer	67.4306083	13.92807667
	Rainy	75.447848	10.593688
	Winter	80.0502129	7.766814286
KW-II	Summer	71.7048225	12.0545675
	Rainy	81.8398467	8.751156667
	Winter	83.0345233	5.933706667
KW-III	Summer	75.1007125	9.08480125
	Rainy	76.2730275	7.954645
	Winter	80.8480667	6.111936667
KW-IV	Summer	77.193084	11.77467
	Rainy	77.4609925	9.189055
	Winter	80.2827733	9.972891667
KW-V	Summer	65.4275725	16.505385
	Rainy	70.388554	13.947032
	Winter	81.90102	4.682375
KW-VI	Summer	75.609705	12.45288
	Rainy	75.694915	15.5301625
	Winter	83.62995	9.263813333
KW-VII	Summer	69.0460967	12.50985667
	Rainy	71.54673	14.4955525
	Winter	82.281206	9.089582

4. Conclusion

The results demonstrated that significant variation do exist in major constituents of Kewda essential oil due to climatic variables and stages of flower development. Considerable changes in percentage of major constituents such as PEME and terpinen-4-ol were detected in samples collected in different seasons and at different stages of flower development. The analysis of quality parameters revealed that winter crop gives better quality oil compared to summer and rains. Similarly, in terms of yield too, winter crop was the best. The PEME and terpinen-4-ol percentage was recorded high in buds and fresh flowers, whereas decayed flowers had lower percentage of these constituents.

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Carbon stocks of trees outside forests in Anantapuramu District, Andhra Pradesh

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ABSTRACT

In the present study, carbon stocks of trees outside forest in Anantapuramu district of Andhra Pradesh was estimated through sampling of 255 hectare area in 655 plots. A total of 97 species belonging to 78 genera of 36 families were recorded in the sampled plots. The total mean biomass and carbon stocks were calculated as 1078.217 tons and 512.448 tons respectively and 0.890 Mt of biomass and 0.422 Mt of carbon stocks were extrapolated to the study area. The carbon sequestration potential of the trees outside forests of Anantapuramu district is estimated at 1.544 Mt CO_2.

1. Introduction

Climate change is one of the most important global environmental challenges affecting all natural ecosystems (Bujarbarus and Baruah, 2009), because climate and natural ecosystems are closely related and depend on each other (Bharali and Khan, 2011). Unabated burning of fossil fuels over the past one century has increased the concentration of greenhouse gases in the atmosphere especially CO_2 consequently resulting in enhanced greenhouse effect, the global warming. During 2005-2014, 91% of the total emissions were caused by fossil fuels and industry and 9% by land-use change (Le Quere *et al.*, 2015). Carbon dioxide levels which were under 300 ppm during the past 6,50, 000 years are now touching 407 ppm, average for May, 2016 (Tans and Keeling, 2016). Due to these increased levels of CO_2 concentration, rise of atmospheric temperature by 0.5°C is recorded over the past hundred years and it is projected to rise by 0.6 to 5°C in the next 100 years according to latest report of Intergovernmental Panel on Climate Change (IPCC, 2014).

Atmospheric CO_2 removed from atmosphere by capturing and securing it during photosynthesis and subsequently to dead organic matter is called as 'carbon sequestration. Apart from forest ecosystems, trees outside forests also have great potential in sequestration of atmospheric carbon (Dhyani *et al.,* 2009). Trees Outside Forests (from now onwards, abbreviated as TOF) refers to trees found on lands not defined as 'Forest' and 'Other Wooded Land' irrespective of their patch size (FSI, 2009; FAO, 2010). TOF include agricultural land (including meadows and pastures), built-on land (including settlements and infrastructure) and barren land (including sand dunes and rocky outcroppings), orchards and plantations. In spatial terms they may be scattered on farmland and pasture, or growing continuously in line-plantings along roads, canals and watercourses, around lakes, in towns, or in small aggregates with a spatial continuum such as clumps of trees, sacred woods, urban parks (Alexandrov *et al.*, 1999). In recent decades, TOF has begun to attract more and more attention with growing acknowledgements of their potential

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economic importance and political interest in their environmental services (de Foresta *et al.*, 2013). A major challenge to for a better evaluation of trees and their services globally is to improve our understanding of the status and dynamics of all tree resources including trees outside forests (de Foresta *et al.*, 2013; FAO, 2001).

There are five carbon pools of terrestrial ecosystem: above ground biomass, below ground biomass, dead mass of litter, woody debris and soil organic matter (Eggleston *et al*., 2006). Indian Space Research Organization (ISRO) has initiated National Carbon Project under the auspices of Indian Geosphere Biosphere Programme (IGBP) (Singh and Dadhwal, 2008). The objective of the project is to collect data for biomass and carbon pool assessment following uniform methodology for a specific time frame. The major carbon pools in India are estimated based on very coarse resolution data and extrapolation because the primary data for the many regions of the country are non-existed or over-estimated (Dadhwal and Nayak, 1993). Due to the lack of reliable data on standing biomass and rates of forest degradation, the net carbon emission estimates for India are highly variable (Ravindranath *et al.*, 1997). Precise information on TOF at micro level is lacking and this has become a major hindrance in estimating TOF potential in carbon sequestration.

The present study is oriented with this background, to estimate the carbon stocks of TOF of Anantapuramu district, Andhra Pradesh following as a comprehensive format design of Vegetation Carbon pool Assessment (VCP) National Carbon Project (Singh and Dadhwal, 2008). Accordingly, TOF are classified into 3 categories: linear, scattered and block; under linear structures, roads, canals, river bunds, rail tracks are included; scattered structures are agricultural fields (field bunds), wastelands, settlements (towns/villages); and block structures are: orchards/government and private plantations.

2. Study area

Anantapuramu district is located between 76^{r} 50Œ§ to78^{o} 30Œ§ E longitudes and 13^{o}40Œ§ to 15^{o}15Œ§ N Latitudes and has geographical area of 19,130 km$^{2.}$ The district has 3.58% of forest cover to its total geographical area and remaining is under different land use systems. The average elevation varies 280 to 1018 m above MSL. The annual average temperature range from 14^{o} C to 41^{o} C and average annual rainfall is about 553 mm. The major soil types are red and in some Mandals red and black soils are in equal proportion. Rivers of major importance are Pennar, Jayamangala, Chitravathi, Vedavathi and Thungabhadra Project Higher Level Canal. The remaining geographical area after deducting forest area is considered to be the area outside forest. It represents 18,443.3 Sq. km (18.443 lakh hectares) of the study area. This area is mainly used for many activities i.e. laying roads, canals, railway tracks, cultivating crops, establishment of industries, settlements and for many other purposes. There is 10832.12 km road found all over the district, of which 181.52 km National highway, 791.75km State highway, 1489.59km Major district road and 1502.11km other district road are under the maintenance of Roads and Buildings Department 349.18km railway track is occurred in the district 257.52km length of canal found, of which 83.520km is under the name of Tungabhadra Project Higher Level Canal, 112km is under Pennar (Anon., 2011) and remaining area is under Chitravathi and Hagari. The total cropped area in the district 11.79 lakh hectares (Anon., 2011). Cultivated orchards comprise 84,965 hectares of land (Personal comm. with Horticultural Officer)**.**

3. Materials and methods

In the present study, a non-destructive approach of above ground biomass estimation was done. A comprehensive format design of Vegetation Carbon pool Assessment (VCP) of Indian Institute of Remote Sensing (IIRS) (Singh and Dadhwal, 2008) was adopted for ground data collection. Sampling plots were identified randomly and located with the help of google earth software and Survey of India toposheets. The geographical co-ordinates for each plot were identified with the help of Global positioning system (GIS). All taxa inventoried in the sampled plots were identified following regional and local floras. For the present study, field data collected from outside forest area in Anantapuramu district. A total of 655 sampled plots were laid across the study area. Among them, 344 plots in linear category of 0.1 ha (100×10 m) size, 211 sample plots in scattered category with 100x100 m (i.e., 1 ha) dimensions and 100 sample plots of size 31.6 x31.6 m (i.e., 0.1 ha) were laid in block category, across the study area. A total area sampled in the present study is 255 hectare which is equivalent to 0.01% of the total geographical area of TOF of the Anantapuramu district 18,443.3 km^{2} Girth of all enumerated trees was measured at breast height (gbh at 1.37m) by measuring tape and height was measured using Opti-logic meter. On slopes, the observations have been taken from elevated site.

3.1. Biomass Estimation

In the present study, non-destructive approach of above ground biomass estimation was used. Basal area, volume and specific gravity for trees have been estimated as follows.

(i) Basal area: Basal area of each tree was calculated by using following standard formula: Basal Area (m^2 ha^{-1}) = ðr^2 × area (ha)

(ii) Growing Stock (Volume) Estimation: Volume of each tree was estimated using the selected species specific volumetric equation developed and compiled by Forest Survey of India (FSI, 1996).

(iii) Specific Gravity: Specific gravity values of different species were selected from literature (Reyes *et al.,* 1992; FRI, 1996; Mani and Parthasarathy, 2007). For stems with unknown specific gravity, the arithmetic mean of all known species was substituted and used in particular sample plot following Brown *et al.,* (1989).

3.2. Estimation of above ground biomass

(i) Bole biomass ≥10cm diameter

The estimated volume was converted into biomass by multiplying with specific gravity (Rajput *et al*., 1996; Limaye and Sen 1956). Biomass of all the trees was summed to obtain biomass for 1 ha.

Biomass (tons) = Volume (m^3) × Specific gravity

(ii) Bole biomass <10 cm diameter

Volume equations for trees <10cm diameter are not available, hence a methodology for trees of this class developed following Singh and Dadhwal (2008) and Patil *et al*. (2011) by relating basal area and biomass. The model developed was Y=3.6808*X+0.264 and used for assessing the AGB of trees <10cm diameter; where, Y= biomass, X= basal area of trees (>10cm diameter and <10cm diameter) and 3.6808 and 0.264 are coefficients

The biomass of trees having≥10cm diameter and <10cm diameter in each plot were added together to get biomass of 1 ha plot.

3.3. Estimation of below ground biomass

In the present study, 26% of the total above ground biomass was considered as root biomass following Houghton *et al*. (2001) and Ramankutty *et al*. (2007).

3.4. Total biomass

Total biomass for each 1 ha plot was obtained by the addition of total above-ground biomass and below ground biomass. Further the mean was calculated and extrapolated for the whole study area.

3.5. Extrapolation of biomass of TOF area

Based on the mean biomass estimation of sampled plots, total carbon stock of TOF of Anantapuramu district was estimated by extrapolating the same for the whole district area. For this, tree covered area under each TOF sub category and sub-sub category was determined on 2011 official statistics of Anantapuramu district (Anon., 2011) as follows and the same has been used for the estimation of biomass and carbon stocks of respective category.

3.6. Category-wise tree covered area

(a) Linear category (Sampled unit: 100 × 10 m = 0.1 ha)

$$\text{Estimated area (ha)} = \frac{\text{Length of linear category (m)} \times 2 \text{ (both sides)} \times 10 \text{ m (transect width)}}{10{,}000}$$

Tree covered area (ha) =Estimated area × percentage of mean basal area of sampled plots

Tree covered area of linear category was calculated using above formulae in sub category wise. The total tree covered area is 1356.21 ha of which 1238.4 ha, 75.35 ha, 42.46 ha under roads, along canals and rail track respectively.

(b) Scattered category (Sampled unit: 100×100 m =1 ha)

(i) Settlement-Village

Settlement area= 10 % of non-agricultural land of the district after deduction of town and Linear category area

Tree covered area (ha) = Settlement area×percentage of mean basal area of sampled plots

Tree covered area of settlement village category was calculated using above formulae. The projected tree covered area of this category is 485.020 ha.

(ii) Settlement-Town

Settlement area for TOF is considered as 50% of a town area

Tree covered area (ha) = Settlement area×percentage of mean basal area of sampled plots

Tree covered area of settlement town category was calculated using above formulae. The projected tree covered area of this category is 754.586 ha.

(iii) Agriculture- field bund

Field bund area of one hectare = 1m width of perimeter of a hectare =199 m^2

$$\text{Total field bund area (ha)} = \frac{\text{Field bund area of a hectare} \times \text{Total cropped area of the district}}{10{,}000}$$

Tree covered area (ha) = Total field bund area × percentage mean basal area of sampled plots

Tree covered area of agriculture field bund category

was calculated using above formulae. The projected tree covered area of this category is 701.516 ha.

(iv) Settlement-Wasteland

Settlement area for TOF is considered as 10% of a waste land area

Tree covered area in ha = Settlement area x percentage of mean basal area of sampled plots

Tree covered area of waste land category was calculated using above formulae. The projected tree covered area of this category is 1112.265 ha.

(c) Block category (Sampled unit: 31.62 × 31.62 m)

(i) Orchard

Tree covered area (ha) = Orchard area × percentage of mean basal area of sampled plots

Orchards area was estimated based on the information taken from Horticulture department of Anantapuramu district (pers. comm. with Horticultural Officer). A total of 84965 ha orchard area is available in the district and the projected area of tree cover under this category is 8505 ha

(ii) Government and Private Plantation

Tree covered area (ha) = Settlement area × percentage of mean basal area of sampled plots

Government and Private Plantations area information was taken from Revenue department of Ananatapuramu district (Personal comm. with Anantapuramu Revenue Officer). A total of 1958 ha plantation area is available in the district and the projected area of tree cover under this category is 647.12 ha.

3.7. Extrapolation of biomass

Extrapolated biomass was estimated by multiplying the tree covered area with mean biomass of sampled plots of respective category.

3.8. Carbon stocks of TOF of Anantapuramu district

Carbon stock was estimated by multiplying the extrapolated biomass with IPCC default carbon fraction (0.475) of each category and finally, all the three TOF categories are added to get the carbon stocks of TOF of the Anantapuramu district.

3.9. Carbon sequestration potential

Carbon sequestration potential of trees was calculated following Eneji *et al.* (2014) and Chavan and Rasal (2012) through the ratio of CO_2 to C, i.e. multiplying carbon content with 3.666.

4. Results

4.1. Species diversity

In the present study, a total of 97 species belonging to 78 genera and 36 families were recorded in 655 sampled plots (Table-1). In Linear category, 66 species were belonging to 53 genera and 26 families; in scattered category, 89 species belonging to 71 genera and 35 families and in block category, 13 species belonging to 13 genera and 8 families were recorded. In total 17,720 tree individuals were inventoried in 655 sampling plots in Anantapuramu district.

Table1

Trees inventoried in the TOF sampled plots

Sl. No.	Name of the Species	Family (As per APG-IV, 2016)	Linear	Scattered	Block
1	*Acacia auriculiformis* Benth.	Fabaceae - Mimosoideae	-	+	-
2	*Acacia holosericea* G.Don	Fabaceae - Mimosoideae	-	+	-
3	*Acacia leucophloea* (Roxb.)Willd.	Fabaceae - Mimosoideae	-	+	-
4	*Acacia nilotica* (L.) Delile	Fabaceae - Mimosoideae	+	+	+
5	*Aegle marmelos* (L.) Corrêa	Rutaceae	+	+	-
6	*Ailanthus excelsa* Roxb.	Simaroubaceae	+	+	-
7	*Alangium salvifolium* (L.f.) Wangerin	Cornaceae	-	+	-
8	*Albizia amara* (Roxb.) B.Boivin	Fabaceae - Mimosoideae	+	+	-
9	*Albizia lebbeck* (L.) Benth.	Fabaceae - Mimosoideae	+	+	-
10	*Albizia saman* (Jacq.) Merr.	Fabaceae - Mimosoideae	+	+	-
11	*Anacardium occidentale* L.	Anacardiaceae	-	-	+
12	*Annona reticulata* L.	Annonaceae	+	+	-
13	*Annona squamosa* L.	Annonaceae	+	+	-

Sl. No.	Name of the Species	Family (As per APG-IV, 2016)	Linear	Scattered	Block
14	*Areca catechu* L.	Arecaceae	-	+	+
15	*Artocarpus heterophyllus* Lam.	Moraceae	+	+	-
16	*Araucaria araucana* (Molina) K.Koch	Araucariaceae	-	+	-
17	*Azadirachta indica* A.Juss.	Meliaceae	+	+	-
18	*Balanites aegyptiaca* (L.) Delile	Zygophyllaceae	+	+	-
19	*Bauhinia purpurea* L.	Fabaceae - Caesalpinioideae	+	+	-
20	*Borassus flabellifer* L.	Arecaceae	+	+	-
21	*Callistemon citrinus* (Curtis) Skeels	Myrtaceae	-	+	-
22	*Cascabela thevetia* (L.) Lippold	Apocynaceae	+	+	-
23	*Cassia fistula* L.	Fabaceae - Caesalpinioideae	+	+	-
24	*Cassia roxburghii* DC.	Fabaceae - Caesalpinioideae	+	-	-
25	*Casuarina equisetifolia* L.	Casuarinaceae	-	+	-
26	*Ceiba pentandra* (L.) Gaertn.	Bombacaceae	-	+	-
27	*Citrus aurantiifolia* (Christm.) Swingle	Rutaceae	-	+	-
28	*Citrus sinensis* (L.) Osbeck	Rutaceae	+	-	+
29	*Cocos nucifera* L.	Arecaceae	+	+	+
30	*Cordia dichotoma* G.Forst.	Boraginaceae	+	+	-
31	*Cycas revoluta* Thunb.	Cycadaceae	-	+	-
32	*Dalbergia latifolia* Roxb.	Fabaceae - Faboideae	+	+	-
33	*Dalbergia sissoo* DC.	Fabaceae - Faboideae	+	+	-
34	*Delonix elata* (L.) Gamble	Fabaceae - Caesalpinioideae	+	+	-
35	*Delonix regia* (Hook.) Raf.	Fabaceae - Caesalpinioideae	+	+	-
36	*Dendrocalamus strictus* (Roxb.) Nees	Poaceae	-	+	-
37	*Dypsis lutescens* (H.Wendl.) Beentje & J.Dransf.	Arecaceae	-	+	-
38	*Erythrina variegata* L.	Fabaceae - Faboideae	-	+	-
39	*Eucalyptus camaldulensis* Dehnh.	Myrtaceae	+	+	+
40	*Euphorbia tirucalli* L.	Euphorbiaceae	+	+	-
41	*Ficus amplissima* Sm	Moraceae	+	+	-
42	*Ficus benghalensis* L.	Moraceae	+	+	-
43	*Ficus benjamina* L.	Moraceae	+	+	-
44	*Ficus drupacea* Thunb.	Moraceae	+	-	-
45	*Ficus racemosa* L.	Moraceae	+	+	-
46	*Ficus religiosa* L.	Moraceae	+	+	-
47	*Gliricidia sepium* (Jacq.) Walp.	Fabaceae - Faboideae	+	+	-
48	*Grevillea robusta* A.Cunn. ex R.Br.	Proteaceae	-	+	-
49	*Hardwickia binata* Roxb.	Fabaceae - Caesalpinioideae	+	+	+
50	*Holoptelea integrifolia* Planch.	Ulmaceae	-	+	-
52	*Jacaranda mimosifolia* D.Don	Bignoniaceae	+	-	-
51	*Kigelia africana* (Lam.) Benth.	Bignoniaceae	+	+	-
53	*Lannea coromandelica* (Houtt.) Merr.	Anacardiaceae	+	+	-
54	*Lawsonia inermis* L.	Lythraceae	+	+	-
55	*Leucaena leucocephala* (Lam.) de Wit	Fabaceae - Mimosoideae	+	+	-

Sl. No.	Name of the Species	Family (As per APG-IV, 2016)	Linear	Scattered	Block
56	*Limonia acidissima* Groff	Rutaceae	+	-	-
57	*Madhuca longifolia* var. *latifolia* (Roxb.) A.Chev.	Sapotaceae	+	+	+
58	*Magnolia champaca* (L.) Baill. ex Pierre	Magnoliaceae	-	+	-
59	*Mangifera indica* L.	Anacardiaceae	+	+	+
60	*Manilkara zapota* (L.) P.Royen	Sapotaceae	+	+	+
61	*Melia azedarach* L.	Meliaceae	+	+	-
62	*Millingtonia hortensis* L.f.	Bignoniaceae	+	+	-
63	*Mimusops elengi* L.	Sapotaceae	-	+	-
64	*Moringa oleifera* Lam.	Moringaceae	+	+	-
65	*Muntingia calabura* L.	Muntingiaceae	+	-	-
66	*Murraya koenigi*i (L.) Spreng.	Rutaceae	-	+	-
67	*Parkinsonia aculeata* L.	Fabaceae - Caesalpinioideae	-	+	-
68	*Peltophorum pterocarpum* (DC.) K.Heyne	Fabaceae - Caesalpinioideae	+	+	-
69	*Phoenix dactylifera* L.	Arecaceae	+	+	-
70	*Phoenix sylvestris* (L.) Roxb.	Arecaceae	-	+	-
71	*Phyllanthus acidus* (L.) Skeels	Euphorbiaceae	-	+	-
72	*Phyllanthus emblica* L.	Euphorbiaceae	-	+	-
73	*Pithecellobium dulce* (Roxb.) Benth.	Fabaceae - Mimosoideae	+	+	-
74	*Plumeria alba* L.	Apocynaceae	-	+	-
75	*Plumeria rubra* L.	Apocynaceae	-	+	-
76	*Polyalthia longifolia* (Sonn.) Thwaites	Annonaceae	+	+	-
77	*Pongamia pinnata* (L.) Pierre	Fabaceae - Faboideae	+	+	-
78	*Prosopis cineraria* (L.) Druce	Fabaceae - Mimosoideae	+	+	-
79	*Prosopis juliflora* (Sw.) DC.	Fabaceae - Mimosoideae	+	+	-
80	*Psidium guajava* L.	Myrtaceae	+	+	+
81	*Punica granatum* L.	Lythraceae	-	+	-
82	*Roystonea oleracea* (Jacq.) O.F.Cook	Arecaceae	-	+	-
83	*Roystonea regia* (Kunth) O.F. Cook	Arecaceae	-	+	-
84	*Santalum album* L.	Santalaceae	+	+	-
85	*Sapindus emarginatus* Vahl	Sapindaceae	-	+	-
86	*Senna siamea* (Lam.) Irwin & Barneby	Fabaceae-Caesalpinioideae	+	+	-
87	*Sesbania grandiflora* (L.)Pers.	Fabaceae-Faboideae	+	-	-
88	*Sterculia foetida* L.	Malvaceae	+	+	-
89	*Syzygium cumini* (L.) Skeels	Myrtaceae	+	+	-
90	*Tamarindus indica* L.	Fabaceae-Caesalpinioideae	+	+	+
91	*Tectona grandis* L.f.	Verbenaceae	+	+	+
92	*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.	Combretaceae	+	+	-
93	*Terminalia catappa* L.	Combretaceae	+	+	-
94	*Thespesia populnea* (L.) Sol. ex Correa	Malvaceae	-	+	-
95	*Vitex negundo* L.	Lamiaceae	+	+	-
96	*Wrightia tinctoria* R.Br.	Apocynaceae	+	+	-
97	*Ziziphus jujuba* Mill.	Rhamnaceae	+	+	-

Table 2

TOF Sub-category-wise mean values per hectare for Basal Area, Volume, Biomass and Carbon Stock of Linear, Scattered and Block Categories

Sl.No.	TOF type	Sub category	Sub-sub category	TNI/ha	Basal area (m^2 ha^{-1})	Volume (m^3 ha^{-1})	Above ground biomass (t ha^{-1})	Below ground biomass (t ha^{-1})	Total tree biomass (t ha^{-1})	Carbon (t ha^{-1})
1	Linear	Road	National High way	10.83	11.472	9.048	65.449	17.019	82.478	39.177
			State High way	11.96	22.338	18.451	132.258	34.388	166.648	79.157
			Major District road	12.84	15.622	12.346	88.654	23.050	111.710	53.416
			Approach road	13.40	12.585	7.688	97.317	25.303	122.619	58.188
		Canal	–	20.60	14.630	14.917	137.866	35.845	173.711	82.512
		Rail track	–	7.140	6.083	5.341	44.502	11.571	56.072	26.634
2	Scattered	Settlements	Village	63.731	4.977	23.972	23.972	6.227	30.176	14.334
			Town	58.526	3.400	21.267	21.267	5.512	26.714	12.689
		Agriculture	Field Bund	32.250	2.990	13.510	13.510	3.480	16.517	7.845
			Waste land	25.50	2.637	12.983	12.983	3.375	16.358	7.770
3	Block	Orchard	–	54.506	10.012	8.909	47.676	12.396	60.072	28.534
		Govt./private plantation	–	31.684	33.052	22.924	170.748	44.394	215.142	102.192

Table 3

Biomass and Carbon Stock of TOF of Anantapuramu District

Sl.No.	TOF type	Structure Sub category	Sub-sub category	Tree covered area (ha)	Mean biomass (tons ha-1)	Extrapolated Biomass (tons)	Carbon stock (tons)
1	Linear	Road	National High way	41.54	82.478	3426.136	1627.414
			State High way	353.59	166.648	58925.066	27989.406
			Major District roads	465.34	111.710	51983.131	24691.987
			Approach road	377.93	122.619	46337.997	22010.540
		Canal	–	75.35	173.711	13089.123	6217.333
		Railway track	–	42.46	56.072	2380.732	1130.888
					Sub total	176142.27	82819.558
2	Scattered	Settlements					
			Village	485.496	31.300	15181.126	7211.034
			Town	754.586	25.018	18878.232	8967.160
		Agriculture	Field bund	701.516	16.517	11586.939	5503.796
			Waste land	1112.265	16.358	18194.430	8642.354
					Sub total	63840.727	30324.344
3	Block	Orchard	–	8505	60.072	510912.36	242683.371
		Govt./private plantation	–	647.12	215.142	139222.691	66130.778
					Sub total	650135.051	3,08,814.149
					Total	890118.648	422806.069
						0.890 (Mt)	0.422 (Mt)

4.2. Basal area

Sub- sub category wise mean basal area was calculated in linear plots. The mean basal area of national high way is 11.472 m^2 ha^{-1}, 22.338 m^2 ha^{-1}in state high way, 15.622m^2 ha^{-1}in major district road, approach roads is 12.585 m^2 ha^{-1}, 14.63 m^2 ha^{-1} along canals and 6.083 m^2 ha^{-1} in rail track. Sub-sub category wise mean basal area was calculated in scattered plots. The mean basal area of villages was 4.977m^2 ha^{-1}, 3.400 m^2 ha^{-1} in towns and 2.990 m^2 ha^{-1} in field bunds and waste lands was 2.637 m^2 ha^{-1}. Sub category wise mean basal area was calculated in block plots. The mean basal area in orchards is 10.012 m^2 ha^{-1} and 33.052 m^2 ha^{-1} in plantations (Table-2).

4.3. Growing stock

The mean growing stock of trees with ≥10 cm diameter in linear plots was 13.82 m^3 ha^{-1}, 23.66 m^3 ha^{-1} in scattered plots and 11.09 m^3 ha^{-1} in block plots. In linear category, the correlation between basal area and biomass of trees with e”10 cm diameter revealed the determination of coefficient of R^2 is 0.805 (Fig.1), in scattered category, it is 0.751 (Fig.2) and in block category, it is 0.955 (Fig.3).

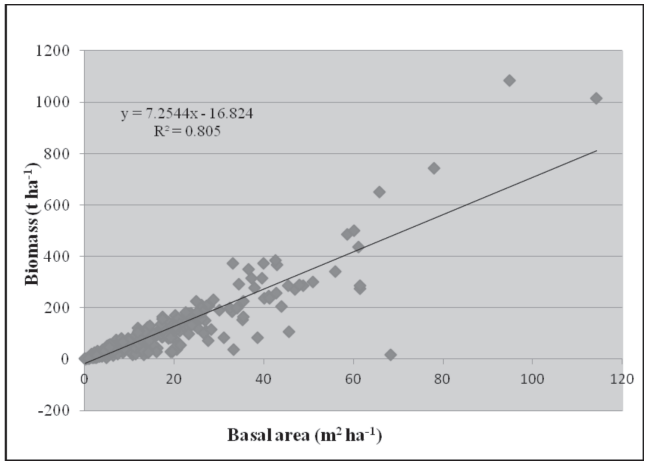

Fig. 1: Correlation between basal area and biomass of ≥10 cm diameter of trees sampled in linear plots

4.4. Biomass

The total mean biomass was 1078.22 tons ha^{-1} In all plots, sub- sub category wise biomass was calculated. In linear plots mean biomass of national high ways was 82.478 tons ha^{-1}, 166.648 tons ha^{-1}in state high ways, 111.710 tons ha^{-1}in major district roads, 122.619 tons ha^{-1}in approach roads 173.711tons ha^{-1} along canals and 56.072 tons ha^{-1}in rail track. In Scattered plots the mean biomass of villages was 30.176 tons ha^{-1}, 26.714 tons ha^{-1} in towns and 16.517 tons ha^{-1}in field bunds, 16.358 tons ha^{-1} in wastelands. In block plots the mean biomass in orchards is 60.072 tons ha^{-1} and 215.142 tons ha^{-1} in plantations (Table-2).

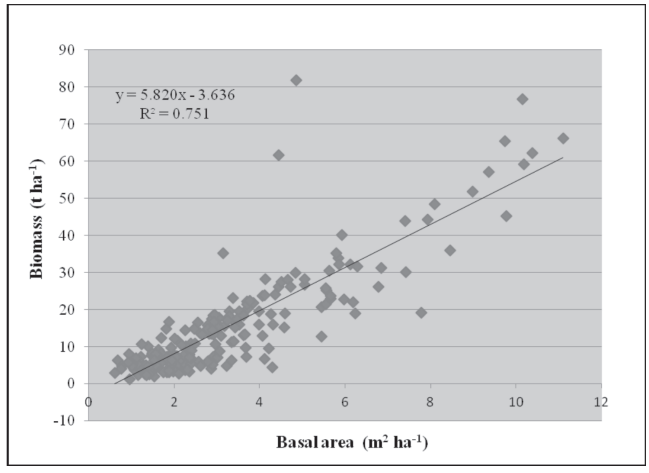

Fig. 2: Correlation between basal area and biomass of ≥10 cm diameter of trees sampled in scattered plots

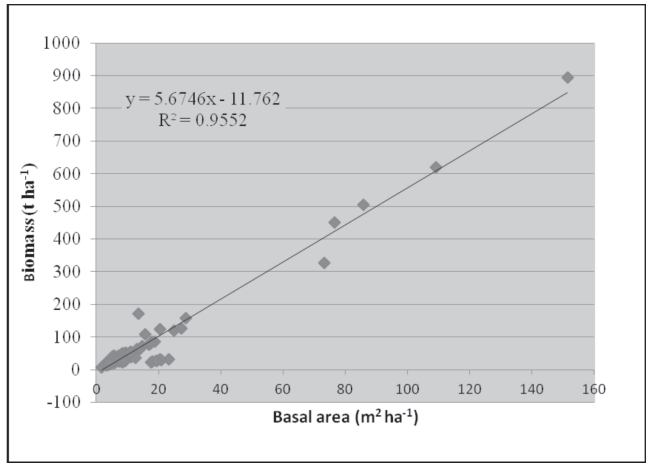

Fig. 3: Correlation between basal area and biomass of ≥10 cm diameter of trees sampled in block plots

4.5. Carbon Stocks

The total mean carbon stocks was 512.448 tons ha^{-1} of which in linear plots mean carbon stocks of national highways was 39.177 t ha^{-1}, 79.157 t ha^{-1} in state highways, 53.416 t ha^{-1} in major district roads, 58.188 t ha-^{1} approach roads 82.512 t ha^{-1} along canals and 26.634 t ha^{-1}in rail track. In Scattered plots the mean carbon stocks was 14.334 t ha^{-1} in villages, 12.689 t ha^{-1} in towns,7.845 t ha^{-1} in field bunds and 7.770 t ha^{-1}. In block plots the mean carbon stocks in orchards was 28.534 t ha^{-1} and 1021.19 t ha^{-1} in plantations (Table-2).

4.6. Extrapolated biomass of different categories

Extrapolated biomass was estimated by multiplying the tree covered area with mean biomass of sampled plots of respective category. All sub categories in a category were summed. Total biomass of TOF area of Anantapuramu district

was 0.890 Mt, of which 0.1761Mt under linear category, 0.0638 under scattered category and 0.6501 Mt under block category (Table-3).

4.7. Carbon stocks of TOF of Anantapuramu district:

The extrapolated carbon stocks TOF of Anantapuramu district is 0.422 Mt, of which 0.0828 Mt is under linear category; 0.0303 Mt under scattered category and 0.308 Mt under block category (Table-3). Anantapuramu district carbon stock is almost less by 0.6 Mt, when compared to Kurnool district TOF carbon stock (1.012 Mt) as estimated by Ramesh (2015). Further 0.422 Mt carbon stocks of TOF of Anantapuramu district represent the ability of sequestering 1.544 Mt CO_2.

5. Discussion

In the present study, a total of 97 species belonging to 78 genera and 36 families were recorded in 655 sampled plots and a total of 17,720 tree individuals were inventoried in 655 sampling plots in Anantapuramu district. In linear, scattered and block categories, the R^2 values are very high as reported by Haripriya (2000), Brown *et al*. (1989) and Cannell (1984). Anantapuramu district carbon stock is almost less by 0.6 Mt, when compared to Kurnool district TOF carbon stock (1.012 Mt) as estimated by Ramesh (2015). A critical analysis revealed that out of 63 mandals, 40 mandals have less than 300 tree individuals in the sampled plots and these are mostly distributed in northern parts of the district and hence low carbon stocks for the district with respect to TOF. Settur mandal recorded highest number of individuals (971) followed by Dharamvaram (808).

6. Conclusion

Evaluation of trees biomass potential in TOF area of Anantapuramu district highlight the importance of trees outside forests in maintaining recognisable amounts of carbon stocks and their ability in sequestering carbon dioxide. The present work may be considered as a model and the present study is the first of its kind especially in arid zone of Anantapuramu district of Andhra Pradesh to understand the potential of TOF in any area. TOF carbon stocks of Anantapuramu is less when compared to Kurnool district due to better climatic conditions, diversified water resources and high density of trees in Kurnool. Further this work advocates planting more broad leaved trees, outside the forests.

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Endophytic microbial diversity and population dynamics in wild and cultivated rice genotypes

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ABSTRACT

Diversity and dynamics of endophytic heterotrophic (HB), spore forming (SFB), spore-crystal forming (SCB), nitrifying (NB), denitrifying (DNB), phosphate solubilizing (PSB) bacteria, actinomycetes (ACT) and fungi (FUN) in leaf, stem and root of leaf folder tolerant (PTB-12 and Nivara) and susceptible (Naveen and Tapaswini) rice (*Oryza* spp.) genotypes were analyzed which are unattained to date. Tapaswini roots produced all 8 types endophytes but that of Nivara, Naveen and PTB12 produced 6, 5 and 4 types of communities but stems and leaves had lower diversity. The HB, SFB and SCB were universal but NB, DNB, PSB, ACT and FUN had discrete occurrence. The SCB (*Bacillus thuringiensis*) was recorded first time from the cultivated rice genotypes. Quantum ($x10^2$ cfu/g dr. wt.) of endophytic microbes in different parts of the rices were 0.05-53.14; higher population of HB was in *O. nivara* stem (4.23) and root (53.14), Naveen leaf (10.40), and SF (44.11) or SCF (41.91) in Tapaswini root. At least one part of each plant had endophytic FUN (0.05-0.88 $x10^2$ cfu/g) but ACT (0.33-1.09 $x10^2$ cfu/g) was present in root of most rices, DNB (0.05-1.33 x 10^2 cfu/g) was less pronounced, NB (0.05-4.10 x 10^2 cfu/g) was undetectable in leaf, and PSB (1.00 x 10^2 cfu/g) was present in Tapaswini root only. Broadly, the endophytic microbes were 2-4 exponent lower than the native soil microbial pool. Wide dynamics and diversity of beneficial endo-microbial communities would variously help growth and development of the rice genotypes, and the *B. thuringiensis* would intrinsically suppress rice pests and diseases.

1. Introduction

Alike *ex-planta* plant growth promoting microbes (PGPM), endophytes have been reported from almost all plant groups including monocots and woody plants (Bent and Chanway 1998, Shishido *et al*., 1999). The beneficial endophytic microbes are universally associated with plants (Azevedo *et al*., 2009) which have superioreffect than superficial microbiome (Ladha *et al*., 1998). Rice (*Oryza sativa* L.) genotypes are also natural reservoirsof hundreds of endo-microbes (Barraquio *et al*., 1997) and each plant harbours more than two types of organisms (Strobel *et al*., 2004; Senthilkumar *et al*., 2011). The plant growth promoting (PGP) and biocidal microbes viz. *Aeromonas, Berkholderia, Bacillus, Pseudomonas, Methylobacterium, Curtobacterium, Flavobacterium, Herbaspirillum, Pantoea, Klebsiella, Azospirillum, Enterobacter, Streptomyces, Penicillium, Fusarium* spp. etc. have been recorded as natural phytonic (rhizo-/phyllo-/endo-/ectospheric) residents of various wild and cultivar rice genotypes (Mano and Morisakai, 2008, Francis *et al*., 2010, del Castillo *et al*., 2015). Besides nutrient supplement (Mano and Morisakai, 2008; Francis *et al*., 2010; Bashan *et al*., 2014), they metabolize various growth regulators which support growth and development of plants (Duran *et al*., 2014), as well as, combat biotic and abiotic stresses (Azevedo *et al*., 2000; Sturz *et al*., 2000; Compant *et al*., 2005; Francis *et al*., 2010; Mitter *et al*., 2013).

Endophytic population (10^2 to 10^9 cells/g) has been assessed from several plant species, which would vary with genotype or environmental conditions (Lamb *et al*.,1996,

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Hallmann *et al*., 1997, Overbeek *et al*., 2008, Chi *et al*., 2005), as well as, the endophytic diversity decreases from root upwards *i.e.* stem and leaves (Lamb *et al*., 1996). Numerical abundance of endospheric microbes (10^3-10^6cfu/g) recorded lower than ectosphericones (10^6-10^8cfu/g) in rice, cotton, corn etc. (Lindow and Brandl, 2003; Bashan *et al*., 2014) and the polyvalent plant growth promoting bacteria (PGPB) (8.91 x 10^1 to 7.24 x 10^6 cells/g) of cultivated (Sabita, Swarna, Swarna *Sub1*) and wild (*Oryza eichingeri*) rices also differed widely (Banik *et al*., 2016, 2017). However, except for N-fixing endo-PGPB (Mano and Morisaki, 2008; Francis *et al*., 2010; Banik *et al*., 2015, 2016), quantum of endospheric microbial dynamics and diversity of cultivated and wild rices have been unsystematically investigated (van der Lelie, 2012). Considering poor knowledge on diversity and dynamics of rice (cultivar and wild) endophytes and significance of their functionalities on overall rice improvement, the endo-microbial guilds and pool sizes in some wild and cultivar rice genotypes were investigated to understand their significance in relation to the host rice genotypes.

2. Materials and methods

2.1. Experimental site and soil physico-chemical characters at transplantation

The experimental rice (*Oryza sativa* L. and *O. nivara* Sharma & Shastry) genotypes were grown in the rice field of ICAR-National Rice Research Institute, Cuttack, Odisha located at 20°312 232 2 N and 85°472 172 2 E. The experimental field soil is deltaic sediment, sandy (52.5%)-clayey (25.9%)-loam (21.6%) with pH 6.28, electrical conductivity (EC) 0.53dS/m, total C 5.01 g/kg, 0.52g/kg total N, 18.52 mg/kg Olsen Pand 120.49 mg/kg available K. The field was prepared following standard agronomic practices supplemented with 5t/ha farm yard manure.

2.2. Collection of pre-transplantation soil samples for microbial analysis

Five subsurface (1-5 cm depth) soil samples (25 g each) from 5 locations at 5 m apart were collected from the pre-transplanted field before subplot preparation. The soilsamples were mixed and the composite soil wasused to assess the microbial guilds.

2.3. Cultivation, selection and collection of plant genotypes

Leaf folder (LF) tolerant genotype i.e. *O. sativa* cv. PTB-12 and *O. nivara* (wild species), and susceptible genotypes *O. sativa* cv. Naveen and *O. sativa* cv. Tapaswini were selected for analysis of endo-microbial dynamics and diversity.The cultivars viz. *O. sativa* var. PTB-12, Naveen and Tapaswini were grown fertilized with NPK @ 60:40:40 kg/ha. The wild sp. *O. nivara* was grown with periodic weeding only. Healthy plants were uprooted at panicle initiation (PI) stage, stored in polythene bags, brought to the laboratory and processed immediately or stored at -80°C for isolation of endophytic bacteria.

2.4. Microbial diversity and dynamics of rice endophytes and in soil of experimental field

Healthy tillers were thoroughly washed under running tap water to remove adhered soil particles, thereafter individually washed in sterile (autoclaved at 1.1 kg/sq. cm pressure, 121°C) distilled water and surface sterilized with 0.1% (w/v) $HgCl_2$or 1% chloramine T for assessment of the endophytes (Barraquio *et al.,* 1997). For endophyte isolation from leaf, stem and root, each part was cut into 1cm pieces andsix segments were used for endophyteisolation while another six parts were used for gravimetric assessment of dry wt. Under a laminar air flow, eachtype of plant pieces were sterilized washed twice in 90% ethanol, followed by sterile distilled water followed by surface sterilized with 0.1% $HgCl_2$ for 2 min and washed with sterile distilled water. The samples weremacerated in 1 ml sterile distilled water, removed the fibrous materials and the macerates were used for enumeration of eight different microbial guilds (Collee and Miles, 1989, Collins *et al*., 2004).

The composite soil of the experimental field prior to transplantation was optimally dried within sterile (mentioned elsewhere) blotting papers, 1 g blotted soil was suspended in 10 ml sterile distilled water and diluted to 10^{-3} level for microbial analysis following standard methods (Collee and Miles, 1989, Collins *et al*., 2004). Soil dry weight was estimated gravimetrically.

2.5. Viable count of different endophytic and soil microorganisms

The macerates (200µl) of the plant parts and soil suspensions (100 ìl, 10^{-3} dilution) were individually mixed with different media, poured into 5 plates and incubated at 28±0.1°C for 3-5 days or more (if required) to assess the microbiome (Collee and Miles, 1989; Collins *et al*., 2004). Total population was counted and expressed as colony forming units (cfu)/g dr. wt. To estimate heterotrophic bacterial guild, 100 mlnutrient agar (NA) medium (g/l: peptone 5.0, beef extract 3.0, NaCl 3.0, pH 7.0, agar 20) was mixed with macerate/soil suspension and colonies were counted after 72h incubation. For spore, the macerate/soil suspension were heated at 60 ± 0.1°C for 1h, mixed separately with 100 ml NA, plated and colonies were counted after 3d as spore (depicting reflecting structure under phase objective) producing bacteria. For spore-crystal producers (tentative *Bacillus thuringiensis*), heated macerate/soil

suspension was plated in NA containing 0.25M Na-acetate (Das and Dangar, 2008), after 3d the colonies were observed under 100x phase objective and those produced crystal inclusion bodies (bright structures) along with spore were counted as spore-crystal former.

The nitrifying (NH_4^+oxidizer) and denitrifying (NO_3^- reducer) bacteria were determined using the macerate/soil suspension mixed with 100 ml Winogradsky medium (g/l: K_2HPO_4 1, NaCl 2, $MgSO_4.7H_2O$ 0.5, $FeSO_4.7H_2O$ trace, $CaCl_2.2H_2O$ 0.02, pH 8.5, agar 18) containing separately filter (0.22ìm) sterilized 1g/l $(NH_4)_2SO_4$ and KNO_3 or $NaNO_3$, respectively and incubated for 25-30d and 7d, respectively. To determine the nitrifying/denitrifying bacterial population, sulfanillic acid reagent (eqi-volume mixture of 0.8% sulphanillic acid and 0.5% á-naphthyl amine, both in 5M acetic acid) was poured into plates and the pink colonies were counted.

Glucose asparagine (GA) medium (g/l: glucose 10, asparagine 0.5, K_2HP0_4 0.5, pH 7.0, agar 15) was used for actinomycetes enumeration. To 100 ml of the medium, macerate/soil suspension were mixed separately, plated and the dry chalky colonies were counted after incubation for 10d or more.

The macerate/soil suspension were mixed with calcium phosphate agar medium (g/l: $Ca_3(PO_4)_2$ 10, $MgSO_4.7H_2O$ 0.2, $FeSO_4.7H_2O$ 0.01, $Ca(NO_3)_2.4H_2O$ 0.01, glucose 10, $(NH_4)_2SO_4$ 0.1, agar 18) and the colonies encircled with halo zones were noted as P-solubilizers.

The fungal population was estimated in mycological agar (MBA) medium (g/l: peptic digest of soybean meal 10, dextrose 40, pH 7.0, agar 18) plated with the macerate/soil suspension and the wooly colonies were counted after 3-5d growth.

Population of the microbial guilds were estimated following: Population (cfu/g. dr. wt.) = (Average population count x dilution factor x 1000)/Dr. wt. (mg) of tissue or soil.

2.6. Phenotyping of spore-crystal forming bacteria

The spore-crystal forming bacteria were characterized by cultural, morphological, staining, physiological and biochemical characters following standard techniques (Collee and Miles, 1989; Collins *et al*., 2004). The vegetative cells, spores and crystals, and motility were recorded under a phase contrast microscope (100X) from 6-96h old shake cultures. Gram staining of 6-8 h old vegetative cells, malachite green staining of spores and amido black 10B staining of crystals from 72-96 h old cultures were observed under 100X light microscope.The organisms were identified using the diagnostic phenotypic characters (Logan and de Vos, 2009).

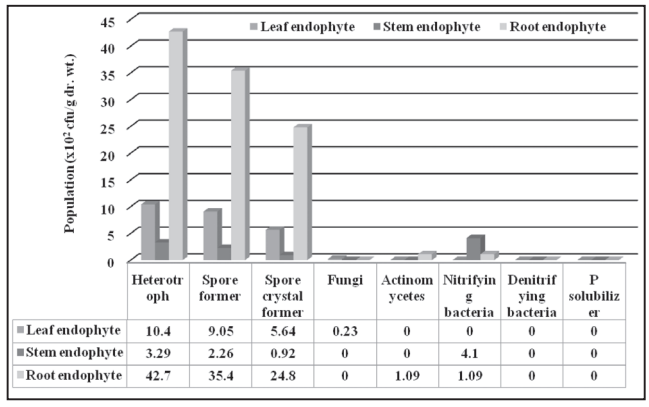
3. Results and discussion

Diversity of endophytic microbial groups (types) viz. heterotrophic (HB), spore forming (SFB), spore-crystal forming (SCB), nitrifying (NB), denitrifying (DNB), phosphate solubilizing (PSB) bacteria, actinomycetes (ACT) and fungi (FUN) in root, stem and leaf of LF tolerant (PTB-12 and Nivara) and susceptible (Naveen and Tapaswini) rice genotypes are presented in Figs. 1-4. Endo-microbe diversity revealed that the roots of Tapaswini accommodated optimum i.e. 8 diverse types of microbes (Fig. 3) followed by Nivara (6 types), Naveen (5 types) and PTB12 (4 types) (Figs. 1, 2, 4). Relative to roots, the stems and leaves of the rice genotypes possessed fewer viz.3 (Nivara) to 5 types of bacterial communities (Figs. 1-4). However, HB, SFB and SCB resided in the endospheric niches of all rices but other groups i.e. NB, DNB, PSB, ACT and FUN discretely inhabited in different parts of the rice genotypes and microbe community types had no systematic relation either with plant parts or genotype (Figs. 1-4) but the reasons thereof could not be explained form the present study. Nevertheless, presence of SCB (tentatively *Bacillus thuringiensis i.e*. Bt) in all plants suggested that Bt would be universal endophyte of rice which, however, been recorded from cultivated rices for the first time but known in wild rice *O. brachyantha* (Acharya *et al*., 2017). Quantum of endophyte diversity was greater (5-8 types) in various parts of Tapaswini compared to those in the counterparts of the remainder rices (Figs. 1-4). Presence of various microbe communities in all genotypes suggested that like environmental and ectospheric microbes, endogenous microbes also regulate diverse biogeochemical processes within the rice varieties and supports occurrence of different microbial guilds in various plants (rice, cotton, corn etc.) including cultivated and wild rices (Sabita, Swarna, Swarna *Sub1* and *O. eichingeri*) (Hallman *et al*., 1997; Bashan *et al*., 2014; Banik *et al*., 2016, 2017; Acharya *et al*., 2017). The results also proved that endogenous microbial diversity was more in roots of all varieties than either in leaves or stems (Figs.1-4).Generally the endophytic microbes colonize from soil mainly through rootsystem which would result in more microbial communities in root than upper plant parts of both wild and cultivated rices (Reinhold-Hurek and Hurek, 1998; Banik *et al*., 2015, 2016, 2017; Acharya *et al*., 2017). Nevertheless, to conclude the reason of presence of all microbial guilds in different parts of Tapaswini, indifferent relations of the microbiomes with different genotype and plant parts needs to be studied thoroughly targeting specific plant-microbe interactions.

Plant part wise, the root, stem and leaf of Tapaswini had numerically ($x10^2$cfu/g dr. wt.) more HB (6.13 - 47.42) (but Nivara stem/root), SFB (5.75 – 44.10) and SCB (5.42

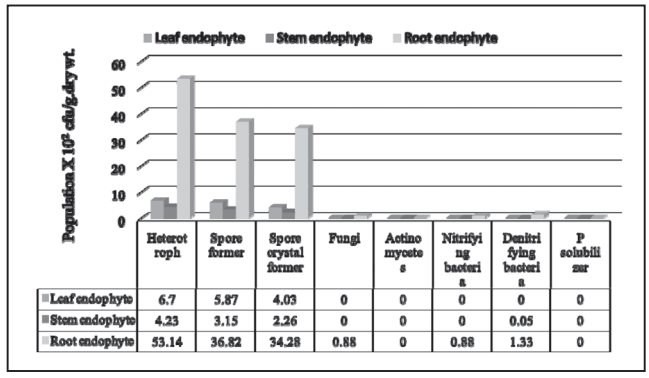
– 41.91) pool than the corresponding populations viz. 2.30-53.14, 2.05-36.82 and 0.92-34.28 of other genotypes (Figs. 1-4). All parts of different genotypes did not harbor NB, DNB, PSB, ACT and FUN, and their spatial occurrence also did not follow any general trend (Figs. 1-4). Although Tapaswini root possessed all microbe communities with pool dynamics range 0.50-1.53 x10^2cfu/g dr. wt. (Fig. 3) but leaf, leaf/stem and stem did not accommodate FUN, NF/PSB and DNB, respectively (Fig. 3). Other than roots, NB, DNB, PSB, ACT and FUN could not be obtained from different parts of other rice genotypesalso (Fig. 1, 2, 4). Microbial guild wise, endophytic HB population (x10^2 cfu/g dr. wt.) was more in stem (4.23) and root (53.14) of wild variety *O. nivara*, Naveen had maximum HB count in leaf (10.40), Tapaswini had more SF (44.11) and SCF (41.91) abundance in root (Figs. 1-4). Endophytic fungi (x10^2 cfu/g dr. wt.) occurred in the leaf of Naveen (0.23) and PTB12 (0.05); root of Nivara (0.88) and Tapaswini (0.50), and stem of Tapaswini (0.06) but actinomycetes (0.33 in PTB12 to 1.09 in Naveen) were detected in root of most rice genotypes (Figs. 1-4). Nevertheless, nitrifying bacterial population density (0.05-4.10 x 10^2 cfu/g dr. wt.) was limited to stem and root zones while undetectable in leaf, denitrifying bacteria (0.05-1.33 x 10^2 cfu/g dr. wt.) were quite low and phosphate solubilizing bacteria (1.00 x 10^2 cfu/g dr. wt.) were obtained in Tapaswini root only (Figs. 1-4). Unlikely, different authors could culture various microbial communities from all parts of different cultivated (*e.g*. Sabita, Swarna, Swarna *Sub1* etc.) and wild (e.g. *O. eichingeri*) rice genotypes (Elbeltagy *et al*., 2000; Banik *et al*., 2017). Numerical abundance (0.05-53.14x 10^2 cfu/g dr. wt.) of endogenous microbes of the rice varieties of the present study was lower than those (10^2-10^9cfu/g) of various other plants (Hallman *et al*., 1997; Bashan *et al*., 2014), as well as, rice cultivars Sabita, Swarna *Sub1*, Swarna and wild *O. eichingeri* (8.91 x 10^1 to 7.24 x 10^6cfu/g) (Banik *et al*., 2017). However, in the cultivars/wild rices of the present investigation,absence of some microbial guilds in some aerial plant parts proved that endospheric colonization of microbes would not be universal i.e. all parts of each rice variety or any one part of all genotypes might be microbe free which favoured the report of differential colonization of microbial guilds in different parts of the wild rice *O. brachyantha* (Acharya *et al*., 2017), as well as, gradual declining trend of endogenous microbes supported the proposition of Lamb *et al*. (1996).

In soil of the pre-transplanted field, numerical abundance (x10^6cfu/g dr. soil) of HB (1.42), SFB (0.99), SCB (0.45), NB (0.89), DNB (1.12), PSB (0.17), ACT (0.19) and FUN (0.84) were recorded. The results corroborated with the population dynamics (0.15-1.70 x10^6cfu/g dr. soil)



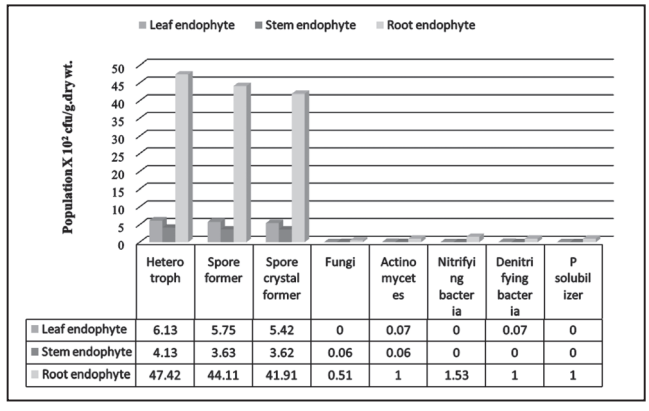
	Heterotroph	Spore former	Spore crystal former	Fungi	Actinomycetes	Nitrifying bacteria	Denitrifying bacteria	P solubilizer
Leaf endophyte	10.4	9.05	5.64	0.23	0	0	0	0
Stem endophyte	3.29	2.26	0.92	0	0	4.1	0	0
Root endophyte	42.7	35.4	24.8	0	1.09	1.09	0	0

Fig. 1: Population dynamics of *Oryza sativa* cv. Naveen



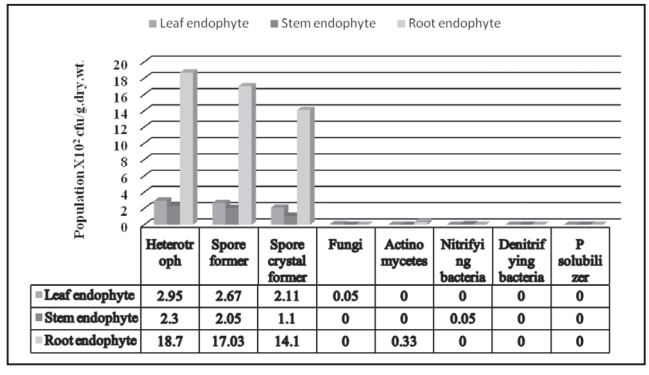
	Heterotroph	Spore former	Spore crystal former	Fungi	Actinomycetes	Nitrifying bacteria	Denitrifying bacteria	P solubilizer
Leaf endophyte	6.7	5.87	4.03	0	0	0	0	0
Stem endophyte	4.23	3.15	2.26	0	0	0	0.05	0
Root endophyte	53.14	36.82	34.28	0.88	0	0.88	1.33	0

Fig. 2: Population of endophytic microbes in *Oryza nivara*



	Heterotroph	Spore former	Spore crystal former	Fungi	Actinomycetes	Nitrifying bacteria	Denitrifying bacteria	P solubilizer
Leaf endophyte	6.13	5.75	5.42	0	0.07	0	0.07	0
Stem endophyte	4.13	3.63	3.62	0.06	0.06	0	0	0
Root endophyte	47.42	44.11	41.91	0.51	1	1.53	1	1

Fig. 3: Population of endophytic microbes in *Oryza sativa* var. Tapaswini



	Heterotroph	Spore former	Spore crystal former	Fungi	Actino mycetes	Nitrifying bacteria	Denitrifying bacteria	P solubilizer
Leaf endophyte	2.95	2.67	2.11	0.05	0	0	0	0
Stem endophyte	2.3	2.05	1.1	0	0	0.05	0	0
Root endophyte	18.7	17.03	14.1	0	0.33	0	0	0

Fig. 4: Population of endophytic microbes in *Oryza sativa* var. PTB-12

of other rice fields of experimental site (Das *et al*., 2013). Comparative results of native soil microbes and endophytic microbes of different plants revealed that the latter populations were about 2-4 exponent lower than the former ones. As endophytic microbes colonize mainly through the roots (Reinhold-Hurek and Hurek, 1998), observations of significantly lesser endophytes in the cultivar and wild rices suggest that all soil-inhabiting microbes wouldnot be rice endo-colonizers, otherwise, interaction for migration of microbes in plants is highly restricted.However, the observations supported the proposition that endophytic diversity is highly depended on host genotype, and soil and environmental conditions (Pillay and Nowak, 1997; Tan *et al*., 2003).

Phenotypic characters showed that the vegetative cells of the SCB were motile, rod shaped, gram positive; spores were elliptical formed within non-swollen sporangium, spore stained with malachite green; along with spores inclusion crystals were produced which were amido black 10B stain positive; the organisms produced protease, catalase, oxidase and nitrate reductase but none was strict anaerobe. The diagnostic phenotypic characters identified the SCB as *Bacillus thuringiensis* (Logan and de Vos, 2009). Thus the results proved that all cultivars and wild rices investigated during the study endophytically colonized Bt which conformed with the record of endo-Bt in wild rice *O. brachyantha* (Acharya *et al*., 2017).

4. Conclusion

Leaf folder tolerant (wild Nivara and cv. PTB-12) and susceptible (cv. Naveen and Tapaswini) rices have wide endomicrobial diversity, predominantly in the roots. Dynamics and diversity of various endophytic microbial communities of diverse biogeochemical groups would modulate the functionalities inside the plant like the ecto-/ environmental microbiome and support growth and development rice. Besides, the SCB (Bt) would be intrinsic biocides against pests and pathogens of rice. Exploitation of theeffective endophyticPGPB would supplement nutrition and Bt would suppress pests and diseases.

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Phytoaccumulation of trace elements by *Grevillea pteridifolia* Knight grown on iron ore tailings: Implications for phytoremediation

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ABSTRACT

Pot experiments were conducted to investigate the effect of iron ore tailings (IOT) both individually as well as in combination with soil (at different proportions) on growth, photosynthetic pigments, antioxidant enzymes and accumulation heavy metals (Fe, Cu, Zn, Ni, Cr, and Pb) from Iron ore tailings by *Grevillea pteridifolia*. Results suggested that the plants grown on tailings showed an increased growth, chlorophyll content, as well as metal accumulation with increasing proportion of tailings in the soil. Further, an increase in antioxidant activities in plants grown on tailings as compared to control suggests plant efficiency to overcome stress generated due to excess accumulation of heavy metals. The order of accumulation of various heavy metals in the plant parts was observed to be Fe> Zn>Cr> Cu> Pb> Ni. Overall, *Grevillea pteridifolia* was found to be well adapted in iron ore tailings and it may be recommended for phytoremediation of most of the studied metals.

1. Introduction

Mining operations generate considerable amount of waste materials and tailings, which are either deposited on the surface as mine spoil dumps or stored in large size ponds called tailing ponds. Removal of fertile topsoil, formation of unstable slopes prone to sliding and erosion, and siltation of water bodies due to wash off of mineral overburden dumps are some other negative effects of mining. The metals released from mining, smelting, forging, and other sources would accumulate in the soil, altering its chemistry (Khan *et al*., 2009; Kumar, 2013). Mine contaminated soils represent a very harsh environment for crop production with low pH, nutrients and limited topsoil availability. Thus, reclamation of mine dumps and abandoned mine lands is a complex multi-step process involving improvement of physical and chemical nature of the site (ameliorative) and careful selection of species, cultivars, or ecotypes (adaptive), both to be used in juxtaposition with one another (Johnson *et al.,* 1994).

In recognition of the role of trees to improve soil fertility (Nair *et al.,* 2010), agro-forestry systems (growing trees and crops in an integrated manner) are believed to have a great potential to reclaim the mine contaminated sites. This conjecture is based on the notion that tree incorporation would result in greater export of pollutants, improve site fertility, and render the sites productive (Kumar, 2013). The present study aimed at studying the phytoremediation potential of *Grevillea pteridifolia* Knight, which is widely used in afforestration owing to its fast growing nature and pleasant appearance. Because of thick and fleshy leaves with petioles flexible and capacity to withstand vibration *Grevillea pteridifolia* is also used for noise control at the industry sites (Kumar *et al*, 2013). In spite of its wide spread use in agro-forestry programmes and multiple uses, phytoremediation potential of *Grevillea pteridifolia* was not explored yet. Thus, the objectives of the study were to examine the (1) growth of *Grevillea pteridifolia* on iron ore tailings and (2) accumulation and translocation of various heavy metals within the plant body under different treatments.

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2. Materials and methods

2.1 Tailings substrata analysis

pH, EC and WHC (%) of IOT as well as soil samples were determined according to Chaturvedi *et al*. (2013). Exchangeable Ca, Na, K and Mg were extracted by 1(N) ammonium acetate solution (Gupta, 2000). Organic carbon content was determined by rapid dichromate oxidation technique (Walkey and Black, 1934) and CEC by 1(N) ammonium acetate extraction method (Jackson, 1973). Diethylene triamine penta acetic acid (DTPA) extractable (plant available) metals were determined using 0.005 M DTPA solution (Lopez- Sanchez *et al.*, 2000) while Available N and P by alkaline permanganate (Subbiah and Asija, 1956) and ammonium fluoride extraction (Bray and Kurtz, 1945) methods, respectively.

2.2. Biochemical parameters

2.2.1. Photosynthetic pigments

A photosynthetic pigment like chlorophyll a, b and total was quantified spectrophotometrically following the method of Porra *et al*. (1989).

2.2.2. Antioxidant enzyme assay

Activities of CAT, POD and SOD were measured following the method of Chance and Maehly (1955), Singh *et al*. (2006) and Misra and Fridovich (1972) respectively. The activity of these enzymes was expressed as specific activity (U^{-1} mg protein).

2.2.3. Heavy metal analysis from soil and plant samples

Rhizospheric soil samples were obtained following Yanai *et al.* (2003). Oven dried (60° C) soil tailing and plant samples were ground using a mortar and pestle and digested in aquaregia (HNO3/ HCl, 1:3), and thereby, concentration of heavy metals were determined using the AA-6300 SHIMADZU Atomic Absorption Spectrophotometer after adjustment of required dilution factor. All the reagents and reference standards were of analytical grade from Merks (Darmstandt, Germany) and Suprapure hydrochloric and nitric acids (Merks, Darmstandt, Germany) were used for sample digestion and preparation of standards.

3. Results and discussion

3.1. Physico-chemical parameters

The physico-chemical characteristics of soil and tailings samples are presented in Table 1. The chemical analysis of the iron ore tailings revealed about 59.70% Fe_2O_3, 18.1% Al_2O_3 and 1.77%, SiO_2 and 9.7% LOI. Tailings were comparatively acidic than the garden soil with pH 5.5 for tailings and 6.2 for garden soil. The percentage of WHC was found maximum for the garden soil (38.7%) and minimum for IOT (22.7%). Scanning Electron images revealing the morphology of soil and tailing samples has been presented in Fig 1.

The soil and tailing samples showed large differences between their nitrogen content, but little differences were observed between the potassium and phosphorus content of the two (Table 2). The concentration of environmentally and plant available metals increased or decreased proportionately with the increasing or decreasing proportion of tailings in the soil for most of the metals (Fig 2).

3.2. Growth and photosynthetic pigments

A significant increase in plant height was observed in all the treatments in *Grevillea pteridifolia* (Fig 3). Significant positive correlations ($p < 0.01$) between root length, shoot length, root fresh weight, shoot fresh weight, root dry weight

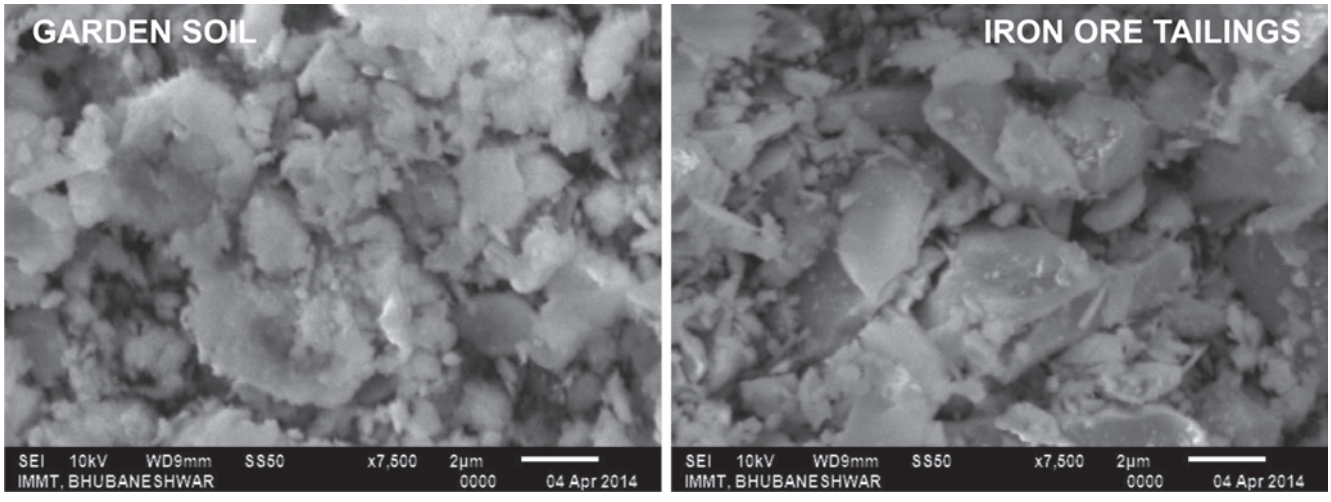

Fig. 1: Scanning electron image of garden soil and iron ore tailings (IOT)

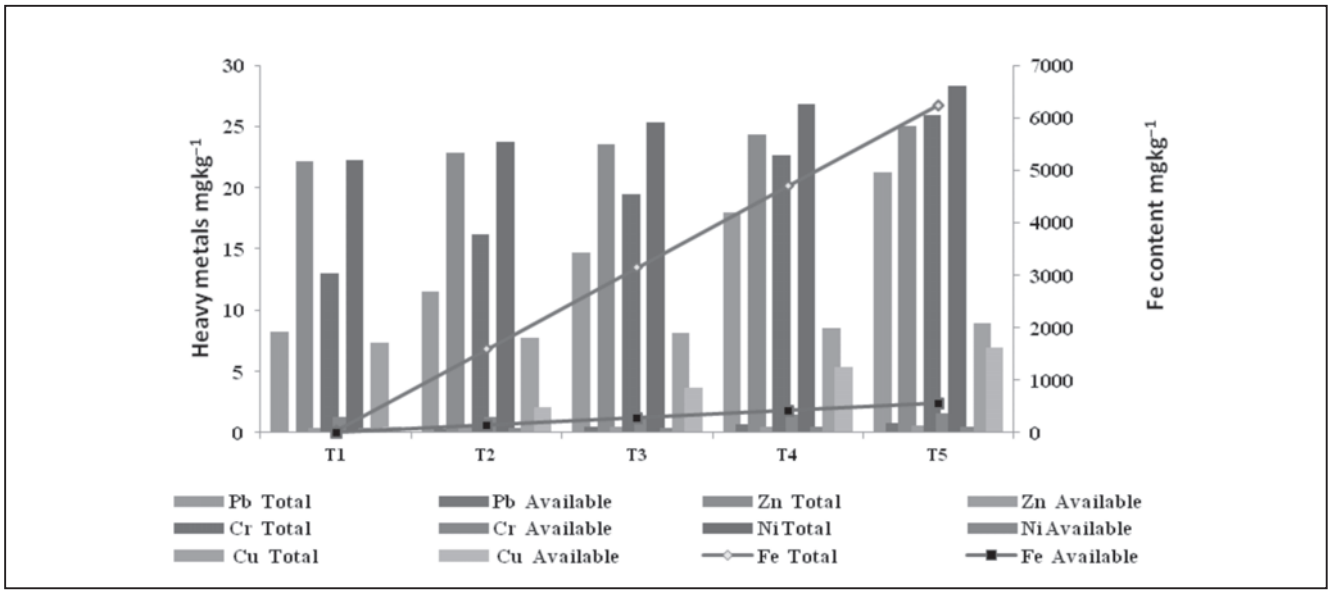

Fig. 2: Environmentally and plant available metal contents ($mgkg^{-1}$) in control and various treatments

Table 1

Physico-chemical characteristics of soil and tailing samples (n=4, Mean ± SE).

Treatments	pH	EC (µS/cm)	OC (%)	CEC c mol (+) kg^{-1}	WHC (%)
T0	6.2 ± 0.025*	103 ± 0.017*	3.3 ± 0.007*	0.464 ± 0.033*	38.7 ± 0.009*
T1	6.1 ± 0.26*	108 ± 0.021*	2.4 ± 0.024*	0.533 ± 0.043*	33.4 ± 0.012*
T2	5. 9± 0.026*	112 ± 0.015*	1.4 ± 0.009*	0.599 ± 0.025*	29.8 ± 0.021*
T3	5.7 ± 0.017*	115 ± 0.022*	0.98 ± 0.013*	0.654 ± 0.042*	25.9 ± 0.020*
T4	5.5 ± 0.014*	119 ± 0.029*	0 ± 0.002*	0.731 ± 0.030*	22.7 ± 0.015*

Table 2

Nitrogen, phosphorus and exchangeable cations of soil and tailing samples (n=4, Mean ± SE).

Treatments	Available N (mg/kg)	Available P(mg/kg)	Exchangeable Cations [c (+) mol/kg]			
			Ca	Na	K	Mg
T0	159 ± 0.003*	7.6 ± 0.035*	0.34 ± 0.015*	1.36 ± 0.013*	0.33 ± 0.009*	2.8714 ± 0.013*
T1	116.9 ± 0. 019*	6.1 ± 0.203*	2.94 ± 0.002*	1.97 ± 0.003*	0.27 ± 0.012*	2.5876 ± 0.005*
T2	86.12 ± 0.120*	4.2 ± 0.045*	3.83 ± 0.009*	1.22 ± 0.005*	0.20 ± 0.035*	1.8192 ± 0.017*
T3	44.67 ± 0.035*	2.3 ± 0.111*	5.18 ± 0.016*	0.86 ± 0.029*	0.14 ± 0.014*	1.1856 ± 0.003*
T4	28.00 ± 0.009*	1.2 ± 0.169*	8.13 ± 0.005*	0.58 ± 0.111*	0.10 ± 0.005*	0.3142 ± 0.009*

as well as shoot dry weight and the concentration of various heavy metals (Fe, Cu, Pb, Zn, Cr and Ni) in root and shoot parts of *Grevillea pteridifolia* further confirms synergistic effect of these metals/ IOT on growth of the plant Regarding the effect of heavy metals on photosynthetic pigments significant positive correlations between Zn, Ni and chlorophyll content was observed which suggests excellent tolerance mechanism of *G. pteridifolia* towards these toxic metals. Moreover, Chl b synthesis was much lower than Chl a in all the treatments as well as in control. This change in the ratio of Chl a/b suggests differential effect of metals on light-harvesting complexes like LHC2 of PS2, (Aravind and Prasad, 2004).

Furthermore Carotenoids, which are regarded as non-enzymatic antioxidants, serve as an accessory pigment for photosynthesis and protect the chlorophyll pigment under stress conditions by quenching the photodynamic reactions, replacing peroxidation and collapsing membrane in chloroplasts (Kenneth *et al*., 2000). An increase in the concentration of carotenoids with increase in metal uptake confirms the same (Prakash *et al*., 2007).

3.3. Antioxidant activity

Exposure of plants to tailings (both with and without additives) led to an increase in the activities of CAT, POD and SOD (Fig 4). The activity of enzymes increased with increase in doses and duration of exposure of tailings. Significant positive correlations ($p < 0.01$) were observed between the shoot heavy metals (Fe, Cu, Zn, Ni, Cr and Pb) and activity of CAT, POD and SOD in both control and the treatments. The higher oxidative enzymes activity is possibly a result of gradual shift of reductive metabolism to oxidative metabolism. These results suggest that heavy metals (Fe, Cu, Zn, Ni, Cd and Pb) present in the tailings induced oxidative stress in the plants and that elevated activity of antioxidant enzymes could play an important role in mitigating oxidative injury.

3.4. Metal accumulation pattern

The comparative accumulation of different heavy metals by *Grevillea pteridifolia* subjected to various treatments is shown in Fig 5. The metal concentrations in IOT was found to be significantly ($p <0.01$) higher than the control. The mean metal concentration in the plants increased with increasing IOT (and hence metal) conc. in the soil. Also, the maximum and minimum values of each metal were found to be comparatively higher in treated plants than control. Accumulation of Pb, Cr, Ni and Zn was maximum in the root while Fe and Cu in the shoot. The overall order of accumulation of various heavy metals by *Grevillea pteridifolia* was Fe> Zn>Cr> Cu> Pb> Ni. Though there is severe dearth of literature on accumulation of heavy metals by *Grevillea pteridifolia,* a different species of *Grevillea* namely *Grevillea exul* has been reported to accumulate substantial amount of manganese content in the epidermal tissues. Similarly, Léon *et al*. (*2005*) and Rabier *et al*. (*2008*) reported accumulation of *Ni* in different parts of *Grevillea exul* like seed coat and phloem of basal stem and roots respectively (Rabier *et al*., *2008*). Rabier *et al*., (*2008*) reported accumulation of *Ni* in different parts of *Grevillea exul.* like seed coat and phloem of basal stem and roots respectively.

4. Conclusion

Thus, the present study which is to the best of our knowledge is the first detailed report on assessment of heavy metal accumulation potential of *Grevillea pteridifolia* on iron ore tailings clearly suggests that, the plant has not only the potential to survive on metallic wastes like Iron ore tailings but can also accumulate substantial amount of heavy metals. Hence, it can be used as a potential tool for remediation of industrial wastes. Considering its multipurpose uses and beautiful appearance *Grevillea pteridifolia* must be given a serious try in remediation and re-vegetation of industrial wastes and mining zones.

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Phytochemical and cytogenetic studies of medicinally important *Oxalis* species occurring in India: A critical review

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ABSTRACT

The paper is a comprehensive review of the work done on cytogenetic and phytochemical evaluation of three medicinally important species of *Oxalis* of India. Compared to the other two, *Oxalis corniculata* is a well-studied species with regard to its phyto-constituents, antioxidant and antimicrobial properties. While *O. corniculata* and *O. debilis* are used in traditional system of medicine, *Oxalis triangularis* is grown as an ornamental plant in gardens. In view of the folk medicinal uses, *O. debilis* is attracting attention of workers in recent times and publications on phyto-constituents, pharmaceutical, toxicological, anti-microbial and nutraceutical properties have been brought out. The current review highlights the medicinal importance of *O. debilis* and *O. triangularis* and emphasizes the need for future research in the field of phytochemistry, pharmaceutical evaluation, bioactivity studies, cytogenetic and molecular studies for optimization of drug yield and genetic improvement of these two species of *Oxalis*.

1. Introduction

Medicinally active compounds are the natural products of plant metabolites and discovered since thousands of years ago. Traditional medicine plays an important role in human health and is used by over 80% of the world's population for primary healthcare (Fainsworth, 1985) as they are cost effective and have not much side effects (Agarwal *et al.*, 2011). Ayurvedic and Unani medicines are also make use the benefits of herbal drugs. About 1000 B.C., Charaka Samhita has documented the use of over 2000 herbs of medicinal value, which have provided us some important lifesaving drugs (Goyal *et al.*, 2007). Herbal drugs are safer to the human body compared to synthetic drugs. Hence, different laboratories are engaged in screening of plants for biologically active therapeutics and potential compounds. Thus, investigations on chemical constituents of the plants are important in developing medicinally important novel molecules of high therapeutic values. Importance of dietary items as nutraceutical elements has been realized and their significance in the treatment of chronic diseases has been highlighted (Williamson *et al.,* 1996).

The genus *Oxalis* of the family Oxalidaceae is comprised of about 570 species and is distributed in America, Africa, Asia and Europe (Christenhusz & Byng, 2016; Loutieg, 2000). This genus contributes to about 89% of the total species diversity of the family. The members of *Oxalis* are morphologically variable, which includes shrubs, herbs, stem succulents, annuals and geophytes, with a cosmopolitan distribution. In India, the genus *Oxalis* L. is represented by 10 species (Manna, 1997) with larger species concentration in Kashmir (Muzafar *et al.,* 2015). Three species of *Oxalis* namely, *Oxalis corniculata*, *O. debilis* and *O. triangularis* are widely distributed in Indian sub continent (Figs. 1a-1c). *Oxalis debilis* Kunth is a cosmopolitan, gregarious and perennial herb having long petiole and is believed to be native to South America. At present, it has spread and naturalized in several tropical countries including Hawaii, Fiji, New Caledonia, Australia and the Galapagos Islands (Lourteig, 2000). The present review is a comprehensive account of the past work done on cytogenetic and

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Fig. 1: Habit and flowering plants of (A) *Oxalis corniculata* (B) *O. debilis* and (C) *O. trianguaris*

phytochemical evaluation of three medicinally important species of *Oxalis (O. corniculata*, *O. debilis* and *O. triangularis*) occurring in India.

Oxalis corniculata L., commonly known as "Creeping Wood Sorrel" is wide spread in India with several traditional medicinal uses. The plant is a delicate and low-growing herb characteristic of shady and moist localities, lawns, roadsides and forest floors in warmer part of country. *Oxalis debilis*, commonly known as "Large-flowered Pink Sorrel" or "Pink Wood Sorrel" is native to South America (Denton, 1973; Lourteig, 2000) but has become a very cosmopolitan species in all continents except Antarctica. It has got naturalized in different subtropical and tropical parts of India especially in the Brahmaputra Valley region of the North Eastern part of India (Junejo *et al*., 2016) and Kashmir (Muzafar *et al.,* 2015). *Oxalis debilis* has uniform distribution in Assam including Dibrugarh district and is locally called as Bor-tenggshi (Assamese) and is an important wild edible plant which is often incorporated in various traditional cuisines like sour fish and bottle gourd dishes (Patiri and Borah, 2012). The species has traditional medicinal use in various major health complications like in treatment of diarrhea, diabetes, piles and scurvy besides its use as an antidote for toxicity (Singh and Dubey, 2012) in different parts of the India. This perennial herb flowers from March to September and being a tristylous species without seeds, it mainly propagates through bulbils. The tiny bulbils have a prodigious ability to persist for several years in soil, which can germinate on attaining favorable conditions (Luo *et al.*, 2006). It is used for treatment of various ailments such as diarrhea by local people (Kirtikar and Basu, 1988), but there was no report on the antioxidant activity and nutraceutical properties of *O. debilis* until the publication by Sarma *et al.* (2015) was brought out. Two taxonomic varieties such as *O. debilis* var. *debilis* and *O. debilis* var. *corymbosa* are recognized within the species. The basic difference between the two varieties of *O. debilis* is the manner in which the clusters of oxalate crystals are arranged; whether they are along the leaf margin or spaced evenly throughout the lamina. In South America, the range of distribution of the two varieties overlap and both set seeds. Besides, the two varieties are also frequently cultivated as ornamental plants and have become naturalized throughout the world (Lourteig, 2000).

Oxalis triangularis A. St-Hil., known as "False Shamrock", is endemic to Brazil and has attracted worldwide due to its fish tail like leaf shape and beautiful pinkish-purple colour. This woodsorrel is a perennial, typically grown as a houseplant but can be grown outside, preferably in light shade. The plant is edible and mostly propagates through their bulbils (Taha *et al.*, 2013). The current review deals with work done on the medicinal properties, phytochemical diversity and cytogenetics of three species of *Oxalis* viz. *Oxalis corniculata*, *O. debilis* and *O. triangularis*, growing in the wild or as cultivated plants in India.

2. Phytochemical characteristics

There are several published reports on the phytochemical constituents of *O. corniculata*. In contrast, very scanty information is available of the phytoconstituents of *O. debilis* and *O. triangularis*. Our work on TLC and HPTLC profiling of methanolic extracts of *O. debilis* has shown promising result that revealed the presence of a number of phytochemicals. TLC analysis of methanolic extract of four ecotypes of *O. debilis* in one solvent system indicated the presence of diverse type of phytochemicals in this plant. In methanolic extracts of *O. debilis,* HPTLC fingerprint established the qualitative and quantitative presence of phytoconstituents (Figs. 2 and 3A-D). HPTLC chromatograms of different ecotypes of *O. corniculata* and *O. debilis* showed qualitative and quantitative variation in phytocompounds. The reported phytochemical constituents in three species of *Oxalis* under review in presented in Table 1. Among ecotypes of each plant samples, methanolic extracts of *O. debilis* possessed phytocompounds with higher

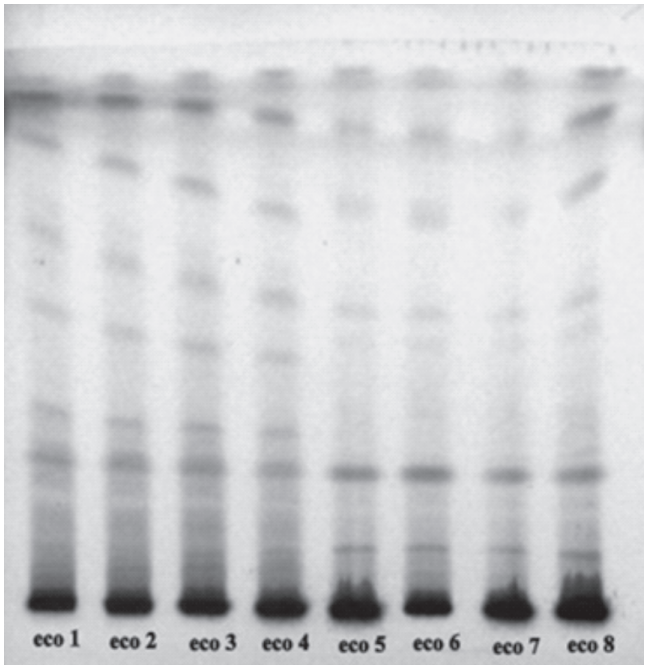


Fig. 2: TLC plate showing various bands in methanolic extracts of ecotypes of *O. corniculata* (eco-1, eco-2, eco-3, and eco-4) and *O. debilis* (eco-5, eco-6, eco-7, eco-8) under UV light 254 nm

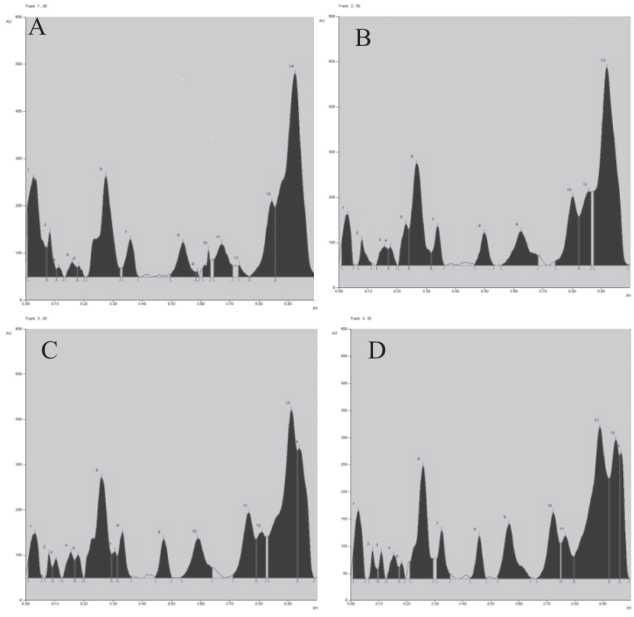


Fig. 3: HPTLC chromatograms of different ecotypes of methanolic extracts of *O. cornicuata* (A=eco-1, B=eco-2, C=eco-3,D=eco-4 showing various peaks.

number of peaks. HPTLC chromatograms of each ecotype showed little variation in Rf values (Figs. 2-4). Area occupied by the phytocompounds and their peaks also differed considerably as earlier reported by us (Panda *et al.*, 2016).

3. Nutraceutical properties

The leaves of *O. corniculata* are tangy in taste, rich in moisture, crude carbohydrate, protein and fibre and therefore,

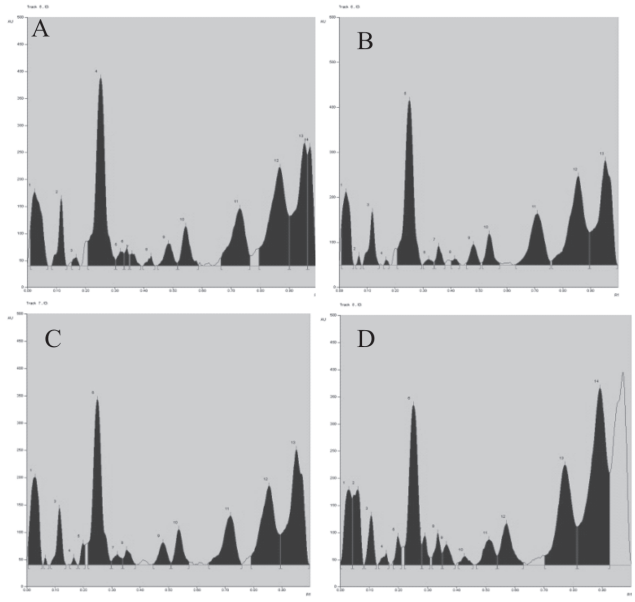


Fig. 4: HPTLC chromatograms of different ecotypes of methanolic extracts of *O. debilis* (A=eco-1, B=eco-2, C=eco-3, D=eco-4 showing various peaks.

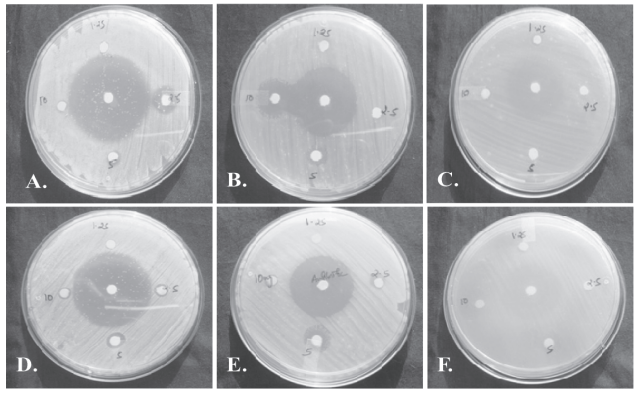
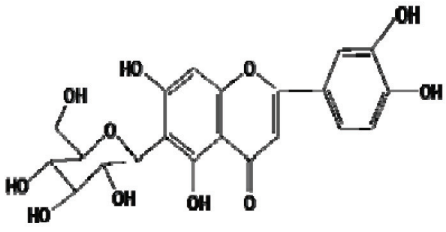
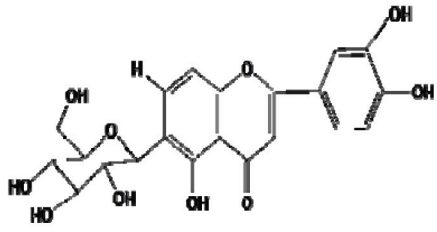
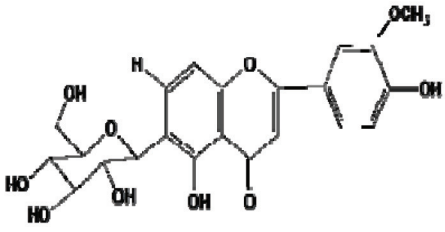
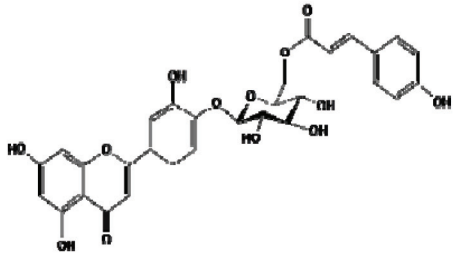
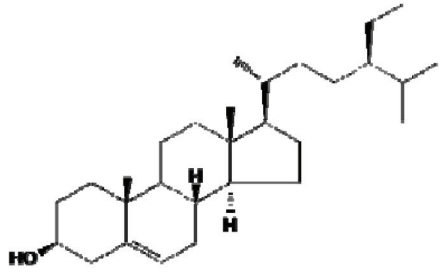
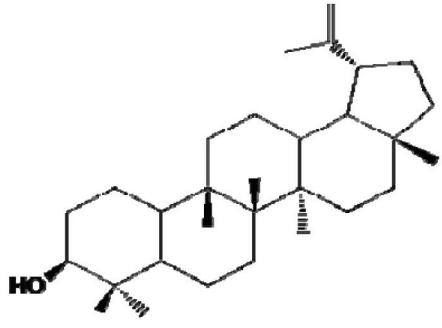


Fig. 5: (A-C): Antibacterial activity by methanolic extract of *O. corniculata* against (A) *Bacillus subtilis* (B) *Streptomyces epidermis* and (C) *Pseudomonas aeruginosa*. Fig. 5 (D-F): Antibacterial activity by methanolic extract of *O. debilis* against (D) *Bacillus subtilis* (E) *Streptomyces epidermis* and (F) *Pseudomonas aeruginosa*

could be considered as an alternative to vegetable in case of emergency or food scarcity. The leaves contain sodium (1.12%), calcium (2.5%) and nitrogen (3.5%) as reported by Ibrahim (2012). Moisture content of the powdered leaf of *O. debilis* has been found to be ~11.09% (Junejo *et al.*, 2016). The carbohydrate content was higher in *O. debilis* (6.40 ìg/mg) than that of *O. corniculata* (3.81 µg/mg). The protein content of *O. corniculata* and *O. debilis* was quite comparable in both the species and was found to be 0.29 ìg/mg and 0.31 ìg/mg respectively. The crude fibre content was calculated as 65.5% and 62.00% in *O. corniculata* and *O. debilis* respectively. Traditionally, *O. debilis* is used for treatment of various ailments such as diarrhea (Sarma *et al.*, 2015) but the medicinal use of *O. triangularis* is not known.

Table 1

Phtochemical constituents of different species of *Oxalis*

Chemical structure of reported phytoconstituents	References
Oxalis corniculata	
Flinolenic acid, stearic acid, phenolic acids like p- hydroxybenzoic, vanillic and syringic acid, flavonoids like isoorientin, isovitexin and swertisin, flavons like acacetin and 7,4' – diOMe orientin, flavonols (3', 4'- di OMe quercertin), Corniculatin A.  Iso-orientin  Isovitexin  Swertisin  Corniculatin A	Sarma and Kumari (2014)
Tannins, saponins, terpenoids, glycosides, phenolic compounds, ferritin in integumentary cells of ovule, immature plastids, oxalic acid, ?- sitosterol, betulin, 4-hydroxybenzoic acid, ethyl gallate, 7,5'-dimethoxy- 3, 5, 2'-trihydroxflavone, apigenin, glucoptranoside, Flavanoids, tannins, phytosterols, phenol, glycoseides, fatty acids, galacto-glycerolipid and volatile oil, Phenoic compounds, gallic acid, flavonols and flavonoids, C-glycosylfavones , harmine (7- methoxy-1- methyl-beta-carboline) and harmaline (3, 4dihydroharmine),heptadecyl-5-methoxy-phenol, Alkaloids, carbohydrates and glycosides, phytosterols, phenolic compounds, aminoacids, flavonoids, volatile oil, proteins.  β-sitosterol  Betulin	Raghavendra *et al.,* (2006), Qureshi *et al.,* (2009), Manna *et al.,* (2010), Kumar *et al.,* (2012), Mohan *et al.,* (2015), Panda *et al.,* (2016)

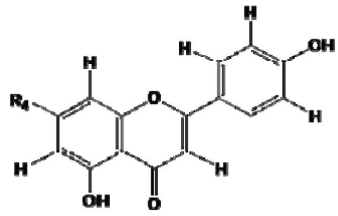
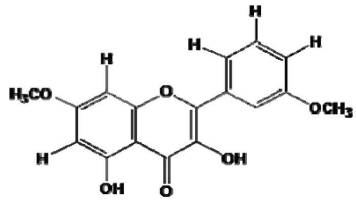
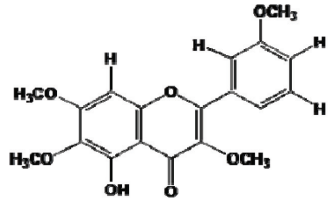
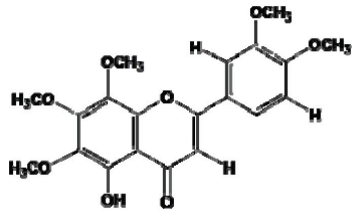
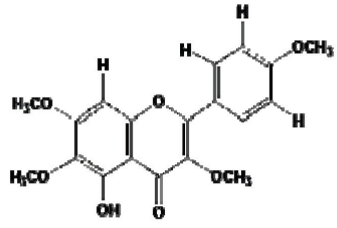
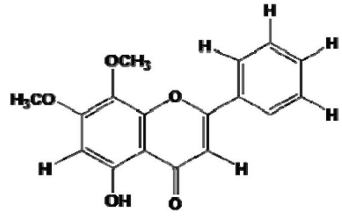
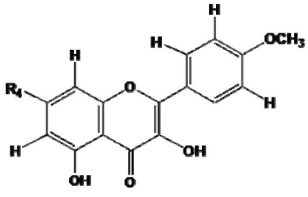
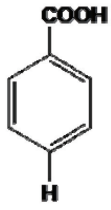
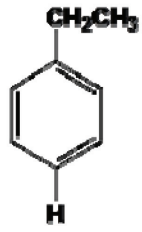
Chemical structure of reported phytoconstituents	References
 Apigenin  7,5'-dimethoxy-3,5,2'-trihydroxyflavone  4', 5-hydroxy-3,6,7-trimethoxy lavone  5-hydroxy-3',4',6,7,8-pentamethoxyflavone  5-hydroxy-3,6,7,4'-tetra-methoxy lavone  5-hyrdoxy-7,8-dimethoxy lavone  3,3',5,7-trihydroxy-4'-methoxy lavone  4-hydroxybenzoic acid  ethyl gallate	
Oxalis debilis	
Ascorbic acid, crude fibre, phenolic compounds. glycosides, carbohydrate, tannins, terpenoids, alkaloids and saponin in aquous extract	Sarma *et al.,* (2015), Panda *et al.,* (2016), Junejo *et al.,* (2016)
Oxalis triangularis	
Ten fatty acid alkyl esters, methyl/ethyl linoleate and linolenate	Uh *et al.,*(2010)

Table 2
Effect of crude extract of *O. corniculata* and *O. debilis* on different microorganisms

Solvent systems used	Pathogen used for antimicrobial test	Antimicrobial effect	References
Oxalis corniculata			
Methanol and ethanol extracts showed significant activity	*Xanthomonas* and fourteen human pathogenic bacteria.	Positive effect	Raghavendra *et al.,*(2006)
Aqueous extract	*S. aureus* and *E. coli*	Positive effect	Handali *et al.,* (2011)
Methanolic extract	*Bacillus subtilis, Streptococcus epidermis, Pseudomonas aeruginosa*	Most effective against *Bacillus subtilis* and *Streptococcus epidermis* as compare to *Pseudomonas aeruginosa*	Panda *et al.,* (2016)
Activity of aqueous ethanol and ethyl ether	*Staphylococcus faecalis, Escherichia coli, P. vesicularis, Aeromonas hydrophilia, Stphylococcus cohni, Serratia ficaria. S. typhi*	Effective against all tested organism	Mohan *et al.,* (2015)
Oxalis debilis			
Methanol extract	*Bacillus subtilis* and *Pseudomonas aeruginosa*	Highly effective against Bacillus subtilis and Pseudomonas aeruginosa	Panda *et al.,* 2016

4. Antibacterial activity

The antimicrobial activities of crude extracts of *Oxalis* species, as reported earlier, have been presented in Table 2. Methanolic extract of *O. debilis* was found to be highly effective against *Bacillus subtilis* and *Pseudomonas aeruginosa* (Panda *et al.*, 2016). There are not much work of antibacterial activity of *O. debilis.* And *O. triangularis* except our work described above (Table 2, Fig. 5).

5. Pharmacological activities

The methanolic, petroleum ether and ethyl acetate extract of *O. corniculata* leads to death of *Eisenia foetida* worm and paralysis as reported by Dighe (2014). It is reported by Chitwood (2002) and Leando (2004) that ethanolic extract of *O. corniculata* has nematoxic activity against phytoparasitic nematodes. Similarly, in case of *O. debilis,* single dose administration of hydro-alcoholic extract up to the level of 5000 mg/kg body weight has showed no toxic symptoms or mortality over any animals up to 14 days of the experimental observations. With the treatment of high dose of hydro-alcoholic extract of *O. debilis,* there is no reduction in body weight of the test animals during the period of experiment, which needs further investigation to identify the responsible component of the plant extract in enhancement of weight (Junejo *et al.*, 2016).

6. Anti-oxidant activity

The most common antioxidants present in herbs and fruits are vitamins C and E, carotenoids, flavonoids and thiol compounds (-SH) etc. Natural antioxidants supplementation through a balanced diet containing adequate herbs could be much more effective than the individual antioxidant uptake such as vitamin C or vitamin E. Methanolic extract of whole plant of *O. corniculata* has been reported to be having significant antioxidant DPPH and nitric oxide radical scavenging activity. The extract has

inhibitory effect against lipid peroxidation. The presence of high phenol content, flavonoids and flavonols has been reported, which is largely responsible for the plant's anti-oxidant activity. Extract also showed *in-vitro* anti inflammatory activity by inhibiting the heat induced albumin denaturation and Red Blood Cells (RBC) membrane stabilization with the IC50 values of 288.04 and 467.14 ìg/ml respectively (Sakat *et al.*, 2010). Similarly, *O. debilis* also possesses anti-oxidant activity. The total phenol content of *O. debilis* was found to be ~1.6 fold higher as compared to *O. corniculata* (Sarma *et al.*, 2015). The ascorbic acid content also reported higher in *O. debilis* (110.75 mg/100 gm) in comparison to *O. corniculata* (92.20 mg/100 gm). *O. debilis* had higher antioxidant activity (IC50=25.82 ìg/ml) than *O. corniculata* (IC50=73.67ìg/ml) (Sarma *et al.*, 2015). However, there is no published report yet on anti-oxidant properties of *O. trianguaris*.

7. Cytogenetics and phylogeny of *Oxalis* species

A number of publications are available on the floral morphology, pollen viability and DNA ploidy level of *Oxalis* species (Castro *et al.*, 2007; Luo *et al.*, 2006; Tsai *et al.,* 2010). *Oxalis pes-caprae* is a widespread invasive weed, which is reported as heterostylous with trimorphic flowers and a self- and morph-incompatible reproductive system in its native habitat (southern Africa) but in most of the areas invaded, only a pentaploid short-styled morphotype that reproduces mainly asexually by bulbils is reported. The low or null sexual reproduction success of this species in the area of invasion studied seems related with the high frequency of monomorphic populations, the unequal proportion of floral morphs in dimorphic populations and the presence of different ploidy levels between short-styles and long-styled morphs (Castro *et al.*, 2007). As generally perceived, the successful establishment of exotic species through vegetative propagation has been largely correlated with their invasion success (Godfrey *et al*., 2004; Lloret *et al*., 2005). When a single floral morphs is introduced in new area, the sexual attributes to the fitness of the newly established plant or population is low or null as reported in *Oxalis pes-caprae* (Castro *et al.*, 2007), *O. debilis* (Luo *et al.*, 2006) and *O. corymbosa* (Tsai *et al.,* 2010).

Cytogenetic studies revealed a great deal of variability in the basic number of chromosomes (from x = 5 to x = 12), as well as variation in the degree of ploidy level, ranging from 2x to 8x (Marks, 1956; Cronquist, 1981; de Azkue and Martínez, 1983, 1988 & 1990). Phylogenetic analysis of ncpGS sequences give a comparison with those of the internal transcribed spacer of nuclear ribosomal DNA (ITS) from different species of the genus *Oxalis* (Mshwiller *et al.*, 1999). *O. corniculata* is octoploids with $2n = 8x = 48$. Pollen germination, pollen tube development, fruit and seed production, seed germination and offspring ploidy levels have been analysed after controlled hand-pollinations to assess self- and morph-incompatibility and production of viable gametes by the 5x S-morph (Costa *et al.*, 2013). According to Tosto and Hopp (2008), the RFLP characterization of nuclear r-DNA units of *O. tuberosa* and four selected diploid species through EcoRI, BamHI, EcoRI and BamHI (double digestion) and EcoRV has produced restriction map for EcoRI and BamHI. Phylogenetic results suggest that the American bulb-bearing *Oxalis* originated in southern South America, dispersed repeatedly to North America, and had multiple transitions from tristyly to distyly (Gardner *et al.*, 2012). Amplifications of the ITS/5.8S region from *Oxalis* species normally resulted in a single product of 700 bp, typical in size for angiosperms (Baldwin *et al.*, 1995). Meiotic studies recorded normal bivalents in *O. bupleurifolia* (n=5), *O. dentata* (n=7), *O. namaquana* (n=14) and *O. purpurea* (n=20+2 univalents). Though cytological studies and determination of ploidy levels in *O. cuneata* (2n=12), *O. cathera* (2n=14), *O. massoniana* (2n=14), *O. hirta* (2n=30), *O. imbricata* (2n=40) and *O. purpurea* (2n=42) have been reported by Rutland (1941), there is no report on cytology and ploidy study of *O. debilis* and *O. triangularis*.

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Macrolichen diversity of Mahendragiri hills of Odisha State, India

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ABSTRACT

The present paper enumerates 16 species of foliose lichens belonging to seven genera and five families from Mahendragiri hills of Odisha state. Of these, six species namely, *Bulbothrix setschwanensis*, *Leptogium coralloideum*, *L. cyanescens*, *L. saturninum, Parmotrema crinitoides* and *Pyxine sorediata* are reported here as new distributional records for the state of Odisha.

1. Introduction

Mahendragiri hills are located in Paralakhemundi subdivision of Gajapati district of Odisha in the Eastern Ghats region. These hill ranges lie between 84°102 E - 84°302 E longitude and 18°502 N - 19°052 N latitude (Krishnamurthy *et al*., 2014) and the highest peak has an elevation of 1501 meter. Mahendragiri is recognized as a biodiversity hot spot due to the presence of a large number of medicinal plants, endangered taxa and phytogeographically interesting plant and animal species. The flowering plant and fern diversity of the region is quite rich and has been studied by several workers in the past (Gamble, 1892; Gamble & Fischer, 1915-1935; Mukherjee, 1935; Kapoor, 1964 and Saxena & Brahmam, 1978). However, in spite of the rich lichen flora of Mahendragiri, the region has been poorly explored and only five lichen species namely, *Bacidia* sp., *Coccocarpia palmicola*, *Coccocarpia pellita*, *Heterodermia* sp. and *Letrouitia* sp. have been reported from this hill range (Nayak *et al.,* 2016). Of these, *Cococarpia palmicola, Cococarpia pellita* and *Heterodermia* sp. are known to be macrolichens. As a part of the ongoing research project entitled "Inventory of Macrolichen diversity of Odisha State" implemented by the Botanical Survey of India, the author conducted two field tours in Parlakhemundi Forest Division in 2016, collected macrolichens and identified the specimens. The voucher specimens collected from Mahendragiri hills have been deposited in the Herbarium of Botanical Survey of India, Deccan Regional Centre, Hyderabad (BSID). A total of 16 macrolichen species belonging to seven genera and five families have been identified and preserved. Of these, six species viz. *Bulbothrix setschwanensis*, *Leptogium coralloideum*, *L. cyanescens*, *L. saturninum, Parmotrema crinitoides* and *Pyxine sorediata* turned out to be new distribution records for the state of Odisha. All the identified taxa are enumerated here with citation of specimens examined. The species reported as new to the State of Odisha are marked by an asterisk (*) in the enumeration.

2. Materials and methods

The morphological features were studied under stereo-zoom microscopes (Olympus SZ61 and Nikon SMZ1500) and anatomical characters were examined with compound microscope (Magnüs MLX-Tr). Colour spot tests were performed by using K, C and PD reagents. The lichen substances were identified by Thin Layer Chromatography

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(TLC) using solvent system A, following White & James (1985). All specimens were examined under UV light (365 nm).

3. Enumeration of taxa

Bulbothrix isidiza (Nyl.) Hale, Phytologia 28: 480. 1974. (PARMELIACEAE)

Specimens examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, N 18°56.7582, E 084°20.5282, alt. *c*. 566 m, 08 July 2016, *G. Swarnalatha* 261 (BSID); Ramgiri Range, N 18°582 29.83, E 084°222 13.33, alt. *c*. 1379 m, 12 Sep. 2016, *G. Swarnalatha* 571 (BSID).

****Bulbothrix setschwanensis*** (Zahlbr.) Hale, Phytologia 28: 480. 1974 (PARMELIACEAE)

Specimen examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, N 18°56.7582, E 084°20.5282, alt. *c*. 566 m, 08 July 2016, *G. Swarnalatha* 260 (BSID).

Coccocarpia palmicola (Spreng.) Arv. & D.J. Galloway, Bot. Not.. 132: 242. 1979. (COCCOCARPACEAE)

Specimens examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, N 18°58.3522 , E 084°22.2042, alt. *c*. 1369 m, 09 July 2016, *G. Swarnalatha* 278 (BSID); Ramgiri Range, near Burkotha on the way to Mahendragiri, 12 July 2016, *G. Swarnalatha* 393 (BSID); Ramgiri Range, N 18°582 21.43, E 084°212 35.73, alt. *c*. 1086 m, 11 Sep. 2016, *G. Swarnalatha* 522 (BSID); Ramgiri Range, N 18°582 20.83, E 084°212 32.83, alt. *c*. 1087 m, 11 Sep. 2016, *G. Swarnalatha* 554 & 555 (BSID).

Dirinaria applanata (Fée) D. D. Awasthi in D. D. Awasthi & M. R. Agarwal, J. Indian Bot. Soc. 49: 135. 1970. (CALICIACEAE)

Specimens examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, N 18°56.4922, E 084°20.1502, alt. *c*. 603 m, 10 July 2016, *G. Swarnalatha* 301 (BSID); Ramgiri Range, N 18°56.5102, E 084°19.6112, alt. *c*. 569 m, 12 July 2016, *G. Swarnalatha* 345 & 346 (BSID).

Dirinaria consimilis (Stirt.) D. D. Awasthi in D. D. Awasthi & M. R. Agarwal, J. Indian Bot. Soc. 49: 135. 1970. (CALICIACEAE)

Specimen examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, on fallen twigs, N 18°582 20.93, E 084°212 34.73, 12 Sep. 2016, *G. Swarnalatha* 581 (BSID).

Heterodermia diademata (Taylor) D. D. Awasthi, Geophytology 3: 113. 1973. (PHYSCIACEAE)

Specimen examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, N 18°582 22.23, E 084°212 50.83, alt. *c*. 1195 m, 12 Sep. 2016, *G. Swarnalatha* 570A (BSID).

Heterodermia speciosa (Wulf.) Trevis., Atti della Società Italiana di Scienze Naturali 11: 614. 1868. (PHYSCIACEAE)

Specimen examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, N 18°58.3522, E 084°22.2042, alt. *c*. 1369 m, 09 July 2016, *G. Swarnalatha* 279 (BSID).

Leptogium austroamericanum (Malme) C.W. Dodge, Ann. Missouri Bot. Gard. 20: 419. 1933. (COLLEMATACEAE)

Specimen examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, N 18°58.3472, E 084°21.4092, alt. *c*. 1026 m, 11 July 2016, *G. Swarnalatha* 333 (BSID).

****Leptogium coralloideum*** (Meyen & Flot.) Vain., Suomal. Tiedeakat. Toim., ser. A, 6(7): 110. 1915 (COLLEMATACEAE)

Specimens examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, N 18°582 21.43, E 084°212 35.73, alt. *c*. 1086 m, 11 Sep. 2016, *G. Swarnalatha* 523 (BSID).

****Leptogium cyanescens*** (Rabenh.) Körb., Syst. Lich. Germ.: 420. 1855. (COLLEMATACEAE)

Specimen examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, N 18°582 22.43, E 084°212 45.23, alt. *c*. 1155 m, 11 Sep. 2016, *G. Swarnalatha* 514A (BSID).

****Leptogium saturninum*** (Dicks.) Nyl., Acta Soc. Linn. Bordeaux 21: 272. 1856. (COLLEMATACEAE)

Specimen examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, N 18°582 22.43, E 084°212 45.23, alt. *c*. 1155 m, 2016, *G. Swarnalatha* 514B (BSID).

****Parmotrema crinitoides*** J. C. Wei, Enum. Lich. China : 177. 1991. (PARMELIACEAE)

Specimen examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, N 18°56.7582, E 084°20.5282, alt. *c*. 566 m, 08 July 2016, *G. Swarnalatha* 258 (BSID).

Parmotrema praesorediosum (Nyl.) Hale, Phytologia 28: 338. 1974. (PARMELIACEAE)

Specimen examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, N 18°56.7582, E 084°20.5282, alt. *c*. 566 m, 08 July 2016, *G. Swarnalatha* 257 (BSID).

Parmotrema ravum (Krog & Swinscow) Sérus. in Vìzda, Lich. Sel Exs.: 3 1983. (PARMELIACEAE)

Specimen examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, N 18°562 21.03, E 084°192 38.63, alt. *c*. 605 m, 2016, *G. Swarnalatha* 485 (BSID).

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Specimens examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, near Laxmipur, N 18°562 25.43, E 084°172 26.43, alt. *c*. 499 m, 08 Sep. 2016, *G. Swarnalatha* 441 & 442 (BSID).

****Pyxine sorediata*** (Ach.) Mont. in Sagra, Hist. Cuba Bot. 9: 188. 1824. (CALICIACEAE)

Specimen examined: Odisha: Gajapati District, Parlakhemundi Forest Division, Ramgiri Range, N 18°56.4922, E 084°20.1502, alt. *c*. 603 m, 10 July 2016, *G. Swarnalatha* 298 (BSID).

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Cytotoxic potential of bark extract of *Hymenodictyon orixense* (Roxb.) Mabb. - a medicinally important tree, on root meristematic tissues of *Allium cepa* L.

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ABSTRACT

There exist a large number of bioactive compounds in plants, out of which a few have been examined and these continue to be important sources of cytotoxic agents. Now-a-days, worldwide effort has been made to find out new cytotoxic compounds from plants. The present study deals with cytotoxic effect of anthroquinone isolated from *Hymenodictyon orixense* bark extract on *Allium cepa* root meristems. Two concentrations (20µg and 50 µg) of bark crude extract of *H. orixense* were studied under 6 h and 24 h of treatment on root meristematic tissues. The direct effect of cytotoxic chemicals of bark methanolic extract revealed significant reduction of mitotic index. The mitotic index reduced significantly (~1.7 to ~2.5 fold) in 24 h of treatment at 20 ìg/ml and 50 ìg/ml concentration respectively as compared to control. Different cytological abnormalities like clumping of chromosomes, DNA fragmentation, spindle arrest, diplochromatin, chromosome erosion and chromosome break were observed. The preliminary investigation showed that this plant-derived anthraquinone can destroy the cells in micromolar concentration and hence may be a potential anticancer drug.

1. Introduction

The use of medicinal plants by human is very ancient and WHO estimated that about 80% of the world population needs herbal medicines to cure diseases. *Hymenodictyon orixense* (Roxb.) Mabb. [Syn: *H. excelsum* (Roxb.) Wall.], belonging to the family Rubiaceae, is a deciduous tree up to 20 m tall having dark grey bark, simple and opposite leaves, terminal racemose inflorescence. It is found mostly in semi-evergreen forests throughout the Western Ghats up to 500 meters, but also occur in moist and dry deciduous forest patches of Mayurbhanj, Kalahandi, Keonjhar, Nayagarh, Phulbani, Sonepur, Gajapati, Sambalpur, Balangir and Koraput districts of Odisha.

The stem bark contains tannin, toxic alkaloids, hymenodictine, aesculin, an apioglucoside of scopoletin and hymexelsin (Rao *et al*., 1988). Anthraquinones, rubiadin and its methyl ether, lucidin, 2-benzylxanthopurpurine, anthragallol, soranjidiol and morindone have also been known from the roots of this plant (Rastogi & Mehrotra, 1996). Leaves contain acetylene fatty acids, triglycerides and triterpenes (Anonymous, 1948). Accumulation of scopoletin has been reported from this plant in addition to other related coumarins and coumarin glycosides such as scopolin, esculetin and esculin (Swe, 2008). A variety of biological activities such as anti-inflammatory, anti-allergic and anti-angiogenesis have been reported from this medicinal plant.

There are only a few reports on the cytotoxic properties of different extracts of *Hymenodictyon orixense* (Khairunnisa and Karthik, 2014). Molecular docking studies revealed that anthraquinones and scopoletin obtained from the plant have anticancer property mainly for prostate cancer, which needs to be validated (Rahman, 2015). The bark of *H. excelsum* is used as an astringent and febrifuge and for treatment of fever and tumors, while the leaves are used to treat ulcers, sialitis, sore throat, tonsillitis and inflammatory conditions in traditional medicine system (Nareeboon *et al*., 2009).

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Although chromosome number of *Hymenodictyon orixense* has been determined as n=33 indicating hexaploidy (Bedi, 1991), no detailed study on genetic variability in terms of ploidy level and phytochemical constituents has been done till date. Keeping in view that very scanty reports on cytotoxic and anti-inflammatory properties of the coumarin derivatives are available in different parts, we report here the cytotoxic activity of bark extract of *Hymenodictyon orixense* on *Allium cepa* root meristems.

2. Materials and methods

2.1 Plant material

The plant material consists of dried powdered bark of *Hymenodictyon orixense,* collected from Hatiasila village of Nuagaon block, Nayagarh District, Odisha State and identified with the help of Flora of Orissa (Saxena & Brahmam, 1995). The herbaria specimens and dried bark samples were deposited in the Herbarium of the Department of Botany, Utkal University, Bhubaneswar, Odisha.

The test plant was onion or *Allium cepa* (Liliaceae), which has 16 long chromosomes. This species is an excellent plant material and a useful biomarker for environmental monitoring with many advantages, such as large number of roots from a bulb, low costing plant material, short duration to conduct a test, easy storage and handling, large cells with easily visible long chromosome and ease of observing abnormal phenomena of chromosome during mitosis (Banerjee and Giri 2014).

2.2 Extraction of crude bark extract

The fresh bark of *H. orixense* was collected and dried under room temperature and powered mechanically. The powered bark sample was kept in air tight container until the time of use. The powered bark (50 gm) was exhaustively extracted with 99.8% methanol (200 ml) using Soxhlet apparatus at 40^{o}C. The methanolic extract was filtered and the filtrate condensed under reduced pressure and was concentrated to dryness under controlled temperature (40^{o}C) with the help of IKA RV10 Rotary Evaporator (Germany) fitted with IKA HB10 digital temperature controller, vacuum pump and water chillier (Cole-Parmer).

2.3. Cytotoxic study

Allium cepa var. Deshi was grown in sand in the net-house of the Department of Botany, Utkal University, Vani Vihar, Bhubaneswar and was used as experimental material for cytotoxicity test. After 4-5 days, bulbs with 3- 4 cm long roots were washed in running tap water and subjected to treatment of 0µg/ ml (control), 20µg/ ml and 50 µg/ ml concentration of bark extract dissolved in dimethyl sulfoxide (DMSO) followed by double distilled water and kept for 6 h and 24 h at room temperature. A control experiment was conducted without any bark extract.

2.4. Mitotic index and chromosome study

Root tips from each treatment were collected and fixed in 1:3 acetic acid: ethanol overnight at room temperature. Fixed root tips were treated with 45% acetic acid for 15 min and were stained in 2% aceto-orcin: 1N HCl (9:1) for 4-5 h. Stained root tips were squashed in 45% acetic acid on a clean glass slide. For each treatment ~100 cells from root tips were scored at random from each slide and the data were pooled for each treatment. The mean data were taken from each treatment and each experiment was replicated thrice. Cells from each root tip were scored at different stages of chromosome under Olympus BX56 microscope (Japan) attached with a digital camera. All the observations were recorded for abnormalities during the cell and chromosome division under the provided stress conditions.

2.4 Cell death measurement

The cytotoxicity levels were measured for both treated and control roots by staining them in 0.25% Evan's Blue (w/v) for 30 min (Baker and Mock, 1994). Stained root tips were transferred to 1 ml of N, N-dimethylformamide for 1 h at 37^{o}C. The absorbance of the dissolved Evan's Blue solution was measured at 600 nm in a UV-visible spectrophotometer and plotted in a graph and calculated statistically.

3. Results and discussion

3.1 Mitotic index and chromosomal anomalies

The treated roots became brown in colour and growth was restricted in 24 h of direct treatment in 50 µg ml^{-1} as compared to control or 6 h of treatment. The concentration of the bark extract and the time of exposure played an important role in reduction of mitotic index in a dose-dependent manner. Mitotic index (MI) decreased progressively with increase in concentration as well as the duration of the treatment (Table 1). The MI was 40.21 and 28.49 respectively in 6 h and 24 h treatment in 20 ìg/ml, which was slightly higher that 50 ìM ìg/ml concentration. The mitotic index dropped significantly by about ~1.7 to ~2.5 fold in 24 h of treatment at 20 ìg/ml and 50 ìg/ml concentration respectively when compared to that of control. Control root tip cells showed normal mitosis. However, C-mitoses, chromosome bridges, chromosome fragments, chromosomal clumping, chromosome stickiness, chromosome break and chromosome erosions were recorded (Fig. 1 a-h). Spindle fiber abnormality (SFA) included C-

mitosis, chromosome stickiness, chromosomal clumping; and chromosomal abnormality (CA) included chromosomal breaks and chromosomal bridges (Fig. 1). Laggard chromosomes and distorted chromatin (Fig. 1e) were observed in direct treatment. The frequency of chromosome break and chromosome erosions increased significantly with increasing concentration of bark extract and prolongation of treatment time. Very condensed chromosomes with mitotic effects in the cell as well as chromosome fragments were noticed in 50 ìg/ml (Figs. 1 b & c). Sticky chromosome bridges were found in low doses *i.e.* 20 ìg/ml of extract. Chromosomal stickiness as well as break and erosion are usually irreversible (Fig. 1 g & h) and could be due to the toxic effects of plant extracts leading to cell death. Like mitotic index, more metaphase percentage was noticed at lower concentration compared to other concentrations. Likewise, the percentage of anaphase also varied in different concentrations. The oxidative damage by bark extract might have induced various chromosomal abnormalities, which are dose-dependent (Table 1). The highest percentage of abnormalities was noted with 50 *ì*g/ml treatment of extract as compared to treatment with 20 *ì*g/ml.

A comparative analysis of all abnormalities percentage of different hours of treatment showed that a comparatively high percentage of metaphase cells were damaged as compared to anaphase. Metaphase and anaphase displayed various types of chromosome abnormalities like spindle fiber anomalies leading to pretreatment effect, chromosome break, chromosome break with lagging chromosomes, chromosome erosion, chromosome clumping, sticky chromosomal bridge formation and C-mitosis formation. The maximum number of chromosome breaks and erosions was found in the 50 *ì*g/ml treatment for 24 h. At 20 *ì*g/ml, a number of abnormalities were also found with significant percentages. The chromosomal abnormality percentage showed dose- and time-dependent increase. The treated root tips showed abnormalities of spindle formation in low dose that leads to metaphase arrest and separation due to direct bark extract treatment. Chromosomal break was observed in 50 *ì*g/ml treatment, while 20 *ì*g/ml treatment produced early separation of chromosomes in anaphase. Chromosomal damages were prominent resulting in chromosomal erosion and intense break of chromosome at 50 *ì*g/ml treatment. That clearly indicated that methanolic extracts have potential carcinogenic

Table 1

Effect of crude bark extract of *Hymenodictyon orixense* on mitotic index of *Allium cepa* root apical meristems

Treatment	20 µg/ml		50 µg/ml	
	Mitotic index Mean (±S.D.)	% Cell aberrations Mean (±S.D.)	Mitotic index Mean (±S.D.)	% cell aberrations Mean (±S.D.)
Control	48.17±1.23	1.21±0.56	46.25±0.98	1.02±0.67
6 h	40.21±2.14	58.27±2.13	35.49±2.56	64.70±1.29
24 h	28.49±1.56	72.21±1.59	22.31±2.09	83.14±1.78

chemicals, which can kill the cells. Hence, isolation and extraction of individual chemicals, separation and testing of their efficacy at cellular level are very important considerations for discovery of cytotoxic molecules. Since this extract has serious impact on cell cycle, the extract in crude form may have potential anticancer activity.

Root is the most sensitive and accessible part of *Allium cepa*. The inhibition of root growth upon exposure to bark extract clearly showed cytotoxic effect of plant extract and consequent chromosomal aberrations similar to heavy metal stress (Zhang *et al*., 2009). Chromosome stickiness is a lethal type of aberration besides chromosome fragments and bridges, which was also observed in the present study. Increased frequency of chromosome bridges and presence of more chromosome fragments in the cells might be due to chromosome replication and enhanced protein synthesis in roots induced under plant extract stress. The active molecules of bark extract might be interfering with calmodulin, a calcium modulating protein, located in the mitotic spindle by influencing the uptake of Ca^{++} and causing abnormal processes of chromosome movements leading to mitotic abnormalities (Liu *et al*., 1995).

There are not many reports on biotoxicity in plants causing chromosomal abnormalities such as chromosome break, lagging, erosion and effect on cell division. Various types of abnormalities were noticed by treating the *Allium cepa* cells with methanolic bark extract of *Hymenodictyon orixense* at the cell and tissue levels, affecting the elongation

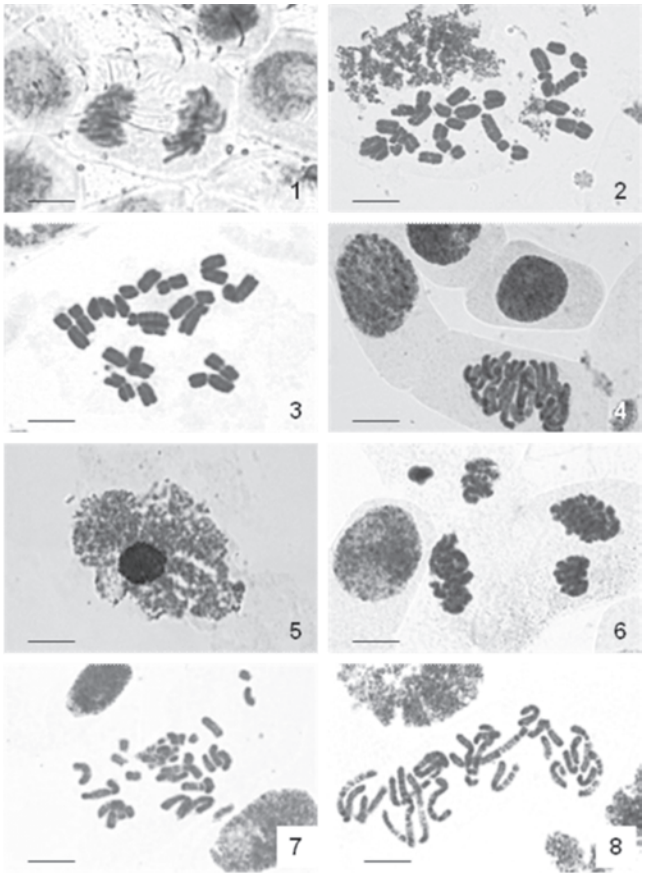

Fig. 1: Cytotoxic effects of crude extract of *Hymenodictyon orixense* on *Allium cepa* root tip cells- (a): Sticky chromosome bridge at 20 Bar = 20µg/ml concentration at 6 h direct treatment. (b-c): Chromosome condensation with spindle arrest with metaphase effect at 20 µg/ml concentration at 24 h and chromosome break at 50µg/ml concentration at 24 h treatment. (d-e): Nuclear clumping and nuclear chromatin decondensation and fragment at 50µg/ml concentration at 24 h treatment. (f-h): Chromosome break and erosion at 50µg/ml concentration at 24 h treatment.

zones of root apex. The effect of low doses of methanolic bark extract was found to be significant on oxidative damage of chromosome structure. Chromosomal damage includes gross structural changes that are initiated by chromosome breaks and erosion.

3.2 Cell death and cytotoxicity

Increased Evans Blue uptake of 10.25 % was found with treatment of 20 µg/ml and 18.65 % in 50 µg/ml at 24 h treatment as compared to control. However, in 6 h treatment with both the concentrations, no significant cell death was observed. Pronounced cytotoxic effect of bark extract on roots of *A. cepa* in both the concentrations was found to vary with duration of treatment. The uptake of Evans Blue stain by the samples with longer period of exposure was significantly more at higher concentrations in comparison to those exposed to low concentrations for short duration of treatment. The increase in Evan's Blue uptake in the roots of *A. cepa* at different concentrations of bark extract indicates its cytotoxicity effect even at micromolar concentrations, which may be due to mitotic arrest leading to cell death (Arya and Mokherjee, 2014). Our observation on DNA and chromosomal damage in *A. cepa* caused by the crude bark extract of *H. orixense* in the present investigation is comparable with the findings of Figueiró *et al.* (2016) using *Glandularia selloi* leaf extract. Root extracts of *Coccinia grandis* also showed cytotoxic and pesticidal effect (Hasan and Sikdar, 2016). *Rhaphidophora korthalsii*- a root-climber plant used in Chinese traditional medicine for cancer and skin disease, is also reported to have cytotoxic effect on NK cell against the NK sensitive target K562 cell line (Yeap *et al.* 2013). The higher percentage of cell death might be due to higher lipid peroxidation activity that might be leading to membrane instability.

4. Conclusion

The findings of our study establish the cytotoxic and genotoxic effects of crude bark extract of *Hymenodictyon orixense* at very low dose on root tip cells of onion (*Allium cepa*). It can be concluded that onion is sensitive to plant alkaloids similar to animal cells at very low concentrations and therefore, can be used as an indicator for cytotoxic study. The active principles of the methanol fraction of bark extract have high cytotoxic effect, which necessitates detailed study to elucidate the molecular mechanisms of cell death.

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Diversity of wild edible food plants and their contribution to livelihood of tribal people in Nabarangpur district, Odisha

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ABSTRACT

Quite a good number of wild plants are used as food by tribals and other local people living in and around the forests in several parts of India. The diversity of wild species not only offers varieties in family food diet and contributes to household food security but also help in generation of income by selling the excessive food plants in the local markets. The present papers deals with 80 species of wild edible food plants belonging to 56 genera and 41families used by tribal communities of Nabarangpur district of Odisha. This includes 71 species of flowering plants, 7 wild edible mushroom species and 2 species of ferns. Botanical name, local name, habit, parts used and first-hand information on use value of each species has been provided. The aspects of marketing, value addition and conservation of potential wild edible plants of Nabarangpur district have been emphasized.

1. Introduction

The majority of the tribal communities of India live close to or within forests and depend on wild products and biomass for food and energy needs (Mohapatra and Sahoo, 2010; Bahuguna, 2000; Mahapatra and Mitchell, 1997). Such communities have distinct socio-cultural traditions and food habits. Wild edible plants provide staple food for indigenous people, serve as complementary food for other local inhabitants and offer an alternative source of cash income through sale in local market places (Shrestha & Dhillon, 2006). These are important nutrient and vitamin supplements for indigenous people and contribute to reduce the vulnerability of local communities to food insecurity by providing a buffer in times of food shortage (Misra *et al*., 2008). Historically, tribal and rural people identified and collected plants for food and medicine from forests and developed a range of processing methods in accordance with their needs. With modernization and settled agriculture, this knowledge is becoming lost, a trend that may lead to decreased diversity of indigenous diets and poorer nutrition (Dwebe and Mearns, 2011). Site specific studies have recorded consumption of wild edibles by tribals and the rural poor in a few locations in India (Mahapatra & Panda, 2012; Sundriyal *et al*., 2004; Misra *et al*., 2008), but generally, information on edible indigenous plants is scattered in botanical monographs, informal notes and tribal oral traditions. The useful properties of non domesticated crops known in local communities requires proper study and documentation in order to validate, quantify and spread this useful knowledge (Edison *et al*., 2006). In the present study, information on the use values of whole plants, leaves, flowers, fruits, tubers, seeds of plants used by Gond, Kandha, Paraja and other tribes of Papadahandi, Umerkot, Dabugaon and Jharigaon blocks of Nabarangapur district has been provided.

2. Methodology

2.1. Study area

The ethnobotanical study of wild edible food plants were conducted in the Nabarangpur district, lying on the

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western part of the Odisha state of India. The district is surrounded on the north and west by Bastar and Raipur districts of Chhattisgarh state, and on the east and south by the Kalahandi and Koraput districts of Odisha. The district is more or less an elevated plateau of Eastern Ghats with valleys and peaks. It lies between 19^{0}10'42" and 20^{0}6'12 N" latitudes and between 81^{0}51'30"and 82^{0}52'36" E longitudes (Fig. 1). The maximum temperature of the district is 40^{0}C during the month of May, while the lowest temperature falls down to 9^{0}C during January. The average annual rainfall is 1423 mm. In the present piece of work, special emphasis was given to collect first-hand information on wild edible food plants used by Gond, Paraja, Kandha and Bhottonda tribes of Papadahandi, Umerkot, Dabugaon, Jharigaon revenue blocks of the district.

2.2. Data collection

An extensive field survey was conducted in four tribal dominated revenue blocks namely, Papadahandi, Umerkot, Dabugaon, Jharigaon of Nabrangapur district of Odisha during July 2012 – October, 2013 covering various forest types, forest-fringe villages, adjoining farmlands and hamlets. The aim of the fieldwork was to study the diversity of wild food plants of the region and to collect field level first-hand information from the beneficiaries on their utilization, processing, value addition and marketing through local channels. Primary data were collected through interviews of key informants and random households and through distribution of questionnaires. Local forest officials and key informants helped in collection of plant samples to

Fig.1: Location map of Nabarangpur district of Odisha

authenticate the identity of the plants and relate the same to vernacular/ local names.

2.3. Collection of voucher specimens and identification

The plant specimens (whole plants and plant parts) were collected from the field with the help of tribal people and were preserved as voucher specimens. The herbarium specimens were dried and preserved using the techniques described in Jain and Rao (1967) and Bridson & Forman (2013). The plant specimens were identified in consultation with the Botany of Bihar and Orissa (Haines, 1921-1925), The Flora of Orissa (Saxena and Brahamam, 1994-1996) and matching with authentic herbarium specimens in different Indian Herbaria. The herbarium specimens have been deposited in the Herbarium of Regional Plant Resource Centre (RPRC), Bhubaneswar.

3. Results and discussion

A thorough exploration of the forests, forest-fringe villages and homesteads in four revenue blocks namely, Papadahandi, Umerkot, Dabugaon, Jharigaon of Nabrangapur district of Odisha revealed that a total 80 wild plant species are consumed as food items by local tribals (Table 1). These 80 species belong to 56 genera under 42 families and includes 71 species of flowering plants (51 dicots and 20 monocots), 7 wild edible mushroom species and 2 species of ferns. In terms of habit, trees are represented by 32 species, shrubs by 8 species, climbers by 14 species and herbs by 19 species. Besides, 7 wild edible mushrooms are used by tribal people of the area. The family Dioscoreaceae was the most dominant family with 10 species of wild edible plants followed by Moraceae with 5 tree species.

Table 1

Diversity and traditional uses of wild edible plants by the tribals of Nabarangpur District, Odisha

Sl.No.	Botanical name	Odia Name	Family	Part (s) used	Habit	Use values and mode of consumption
1	*Abelmoschus moschatus* Medic.	Banabhendi, Khatabhendi	Malvaceae	Fruits, Leaves, Flowers	S	During scarcity of food, flowers are fried with other leafy vegetables and eaten. The fruits are sour in taste and chutney or fried vegetables are prepared out of it. Leaves are also consumed after boiling and frying.
2	*Alternanthera sessilis* (L.) R. Br. ex DC.	Madaranga	Amaranthaceae	Leaves	H	Young shoots and tender leaves are collected, cooked with spices and other leafy vegetables and eaten.
3	*Amaranthus spinosus* L.	Kanta leutia	Amaranthaceae	Leaves	H	Young shoots and tender leaves are cooked and consumed as leafy vegetables.
4	*Amorphophallus paeoniifolius* (Dennst.) Nicolson	Olua	Araceae	Tuber, Stem	H	The tubers are boiled and fried with oil for consumption. During boiling, tamarind is added to reduce irritation of mouth. The stems are also fried with oil and added to make curry either alone or with fish and prawn.
5	*Annona reticulata* L.	Ramphala	Annonaceae	Fruits	T	The white or cream-colored, granular, sweet, aromatic pulp is edible.
6	*Annona squamosa* L.	Sitaphala	Annonaceae	Fruits	T	The ripe fruits are of sweet taste and pleasant aroma; directly taken as food.
7	*Artocarpus heterophyllus* Lam.	Panasa	Moraceae	Fruits, Seeds	T	The sweet, aromatic fruiting perianths are eaten. The unripe fruits are used as vegetables or for making pickles. After drying, the seeds are eaten after roasting or boiling, also taken as a vegetable.
8	*Artocarpus lacucha* Roxb. ex. Buch.-Ham.	Jeuta	Moraceae	Fruits, Seeds	T	The sweet sour pulp is eaten raw when ripe..

Sl.No.	Botanical name	Odia Name	Family	Part (s) used	Habit	Use values and mode of consumption
9	*Averrhoa carambola* L.	Karmanga	Geraniaceae	Fruits	S	The ripe sweet and sour fruits are eaten fresh and used for preparation of pickles and chutneys
10	*Azadirachta indica* A. Juss.	Nima	Meliaceae	Flowers, Leaves	T	The tender leaves and flower buds are fried and eaten, sometimes with other vegetables.
11	*Bambusa bambos* (L.) Voss	Kantabaunsa	Poaceae	Young Shoots	H	The tender sprouting rhizomes are chopped, boiled, fried with oil and curry is made out of it or after adding other vegetables or fishes with it. Often, the chopped stems are sun-dried and stored for use at the time of food scarcity
12	*Bauhinia purpurea* L.	Barada saga, Koliari saga	Caesalpiniaceae	Leaves	S	The tender new leaves are collected, fried or boiled with other vegetables and consumed as a leafy vegetable.
13	*Bauhinia vahlii* Wight & Arn.	Siali, Sialipatra	Caesalpiniaceae	Seeds	C	The seeds are roasted and eaten.
14	*Buchanania lanzan* Spreng.	Chara	Anacardiaceae	Fruits	T	The ripe fruits, which are tasty and sweet, are eaten. The seed kernels are also eaten.
15	*Careyota urens* L.	Salapa	Arecaceae	Stem, Juices	T	The sap extracted from the inflorescence before sun rise is consumed as a health drink and an alcoholic beverages is made using the sap by fermentation.
16	*Cassia tora* L.	Sanachakunda	Caesalpiniaceae	Leaves	H	Tender leaves are fried or boiled along with other vegetables and eaten.
17	*Celastrus paniculata* Willd.	Pengu	Celastraceae	Leaves	C	Young leaves are consumed as leafy vegetables.
18	*Celosia aregentea* L.	Nahanga	Amaranthaceae	Leaves	H	The tender leaves are cooked with other leafy vegetables and eaten.

Sl.No.	Botanical name	Odia Name	Family	Part (s) used	Habit	Use values and mode of consumption
19	*Chenopodium album* L.	Bathua	Chenopodiaceae	Leaves	H	The leaves are used as vegetables.
20	*Colocasia esculenta* (L.) Schott.	Saru	Aracea	Leaves, Tubers	H	The tubers are roasted or boiled in water and eaten. The petioles and leaves are also used as vegetable as such or after sun-drying.
21	*Commelina benghalensis* L.	Kansiri saga	Commelinaceae	Leaves	H	Young shoots and tender leaves are consumed after frying or boiling with/without other leafy vegetables.
22	*Commelina kurzii* C. B. Clarke	Kansiri	Commelinaceae	Leaves	H	Young shoots are eaten as vegetables.
23	*Cordia dichotoma* G. Forst.	Guala koli	Ehertiaceae	Fruits	S	The sweet and mucilaginous ripe fruits are edible. The sticky jellies like substances from the fruits have several medicinal uses.
24	*Costus speciosus* (Koenig) Sm.	Keu, Gaigobara	Zingiberaceae	Rhizomes	H	Boiled rhizomes are consumed during food shortage. The rhizomes are sliced, cooked with other vegetables and eaten.
25	*Dendrocalamus strictus* (Roxb.) Nees	Baunsa karadi	Poaceae	Young Shoots	H	The young shoots are chopped, boiled or fried with oil to make a type of curry with other vegetables. The chopped stems are sun-dried and stored for use at the time of scarcity of food.
26	*Dillenia aurea* Sm.	Rai, Karmata	Dilleniaceae	Fruits	T	The ripe fruits are consumed as such. The fruits are also used to make chutney/pickles.
27	*Dillenia pentagyna* Roxb.	Rai	Dilleniaceae	Fruits	T	The fruits are sour in taste and used to prepare chutneys or curries. Ripe fruits are edible.
28	*Dioscorea alata* L.	Khamba alu	Dioscoreaceae	Tubers	C	The tuber is boiled and curry is prepared with it with other vegetables.

Sl.No.	Botanical name	Odia Name	Family	Part (s) used	Habit	Use values and mode of consumption
29	*Dioscorea belophylla* Voigt ex Haines	Bhatkanda	Dioscoreaceae	Tubers	C	The tubers are boiled and consumed as vegetables; also as a food item during the period of food shortage.
30	*Dioscorea bulbifera* L.	Pita alu	Dioscoreaceae	Tubers	C	The tubers are sliced and washed repeatedly by keeping overnight in running water. Next morning, the slices are boiled/ fried and eaten. This is also a distress food in lean period of the year.
31	*Dioscorea glabra* Roxb.	Kanta alu, Pindalu	Dioscoreaceae	Tubers	C	The tubers are collected, washed and cooked as vegetables.
32	*Dioscorea hispida* Dennst.	Banya alu	Dioscoreaceae	Tubers	C	The tubers are sliced, kept in running water overnight and then cooked as an ingredient of the curry. The tuber is also preserved after drying to be consumed as a famine period.
33	*Dioscorea oppositifolia* L.	Pani alu, Pitalakanda	Dioscoreaceae	Tubers	C	The tuber is cooked with other vegetables for preparation of curry. This tuber is also consumed during the period of food shortage.
34	*Dioscorea pentaphylla* L.	Masia kanda, Karaba	Dioscoreaceae	Tubers	C	Washed and sliced tubers are cooked as vegetable.
35	*Dioscorea pubera* Blume	Kasa kanda	Dioscoreaceae	Tubers	C	The tubers are used as vegetables and cooked using oil and spices. Also preserved in dried form for future use during critical periods.
36	*Dioscorea tomentosa* Koenig ex Spreng.	Taraga	Dioscoreaceae	Tubers	C	Tubers are boiled and washed repeatedly and then cooked as vegetable.
37	*Dioscorea wallichii* Hook.f.	Pita alu	Dioscoreaceae	Tubers	C	The tubers are sliced, boiled and cooked for preparation of curry.
38	*Diospyros melanoxylon* Roxb.	Kendu	Ebenacaeae	Fruits	T	Ripe fruits taste sweet and are edible.

Sl.No.	Botanical name	Odia Name	Family	Part (s) used	Habit	Use values and mode of consumption
39	*Diplazium esculentum* (Retz.) Sw.	Lengudi saga	Athyriaceae	Leaves	H	The leaves are collected during rains and eaten as a leafy vegetable; sold in local tribal markets.
40	*Enydra fluctuans* Lour.	Hidimicha	Asteraceae	Leaves	H	The leaves and tender parts are fried or cooked and consumed as leafy vegetable. Sometimes the leaves are used in preparation of "Pakoda".
41	*Ficus hispida* L.f.	Dimiri	Moraceae	Fruits	T	Immature fruits are used as vegetables for making curries, while ripe fruits are consumes raw.
42	*Ficus racemosa* L.	Dumar, Dumri	Moraceae	Fruits	T	The ripe fruits are eaten after removal of maggots and insects.
43	*Ficus semicordata* Buch-Ham.ex Sm.	Podha Koli, Bhuin dimiri	Moraceae	Fruits	T	Immature fruits are used as vegetables.
44	*Gardenia gummifera* L. F	Ghurudu, Bhurudu koli	Rubiaceae	Fruits	T	The fleshy pulp of the fruit is eaten.
45	*Indigofera cassioides* Rottl. ex DC.	Girili, Giridi phul	Fabaceae	Flowers	S	The flowers are cooked as a vegetable, either alone or mixed with fish and dry fish. The dry flowers are sometimes preserved to be used at the time of need.
46	*Ipomoea aquatica* Forssk.	Kalama saga	Convolvulaceae	Leaves	H	The tender leaves are collected from wetlands, rice fields, cooked and consumed. Sometimes other leafy vegetables are also added to it.
47	*Lentinus fusipes* Cooke & Massee	Baunsa chattu	Polyporaceae	Mushroom	MUSH	The freshly collected mushrooms are used as vegetable for making tasty curries or consumed after frying or roasting in fire. Also these mushrooms are sun-dried and preserved for future use.
48	*Leucas aspera* (Willd.) Link.	Gayasha saga	Lamiaceae	Leaves	H	Fresh and tender leaves and shoots are eaten as leafy vegetable.

Sl.No.	Botanical name	Odia Name	Family	Part (s) used	Habit	Use values and mode of consumption
49	*Leucas cephalotes* (Roth) Spreng.	Gayasha saga	Lamiaceae	Leaves	H	Leaves and young shoots are cooked and eaten.
50	*Madhuca indica* Gmel.	Mahula	Sapotaceae	Fruits, Flowers	T	The fruits are eaten raw or cooked. The dry flowers are boiled in water with salt and eaten; they are also fermented using traditional methods to make country liquor called "Mahuli". The dry flowers are powdered and fried with linseed seed and eaten directly.
51	*Mangifera indica* L.	Amba	Anacardiaceae	Fruits	T	The fruits are used to make pickles, chutneys and a powder named "Amchur". The ripe fruits are used for preparation of jam, jelly, marmalades, squashes and drinks. The seed kernels are cleaned, washed, powdered and used by tribals as food item during food scarcity period.
52	*Manihot esculenta* Crantz	Kathalu, Simuli kanda	Euphorbiaceae	Roots	T	The tuberous roots are eaten raw or cooked as vegetables to make curry.
53	*Marsilea minuta* L.	Sunsunia saga	Marsileaceae	Leaves	H	The tender leaves are collected and cooked as leafy vegetable.
54	*Moringa oleifera* Lam.	Sajna saga, Munga	Moringaceae	Leaves	S	The leaves are commonly used as a vegetable. The fruits are the popular vegetables among the tribals and flowers are also edible, when cooked.
55	*Olax psittacorum* (Lam.) Vahl	Bhadabhadalia	Oleaceae	Leaves	C	The leaves are fried and consumed as vegetable.
56	*Paderia foetida* L.	Pasaruni, Gandhuli	Rubiaceae	Leaves	C	The young leaves are used for preparation of curry along with patato or brinjal. This is considered to be useful for stomach problems.
57	*Phoenix acaulis* Buch.-Ham. ex Roxb.	Bhuin khajuri	Arecaceae	Fruits	T	The fruits with scanty and sweet pulp are consumed as such. The central pith of the stems are rich source of starch and are consumed raw due to sweet taste.

Sl.No.	Botanical name	Odia Name	Family	Part (s) used	Habit	Use values and mode of consumption
58	*Phoenix sylvestris* (L.) Roxb.	Khajuri	Arecaceae	Fruits	T	The ripe sweet fruits are eaten. The sugary sap of the plant is used as a country liquor after fermentation.
59	*Phyllanthus acidus* (L.) Skeels	Narakoli	Euphorbiaceae	Fruits	T	Fruits are sour but eaten raw; sometimes pickles are also prepared from the fruits.
60	*Phyllanthus embilica* L.	Aonla	Euphorbiaceae	Fruits	T	The fruits are acidic and rich source of vitamin-C. The fruits are used by tribals to prepare pickles and the dry fruits are used as medicine.
61	*Pithecellobium dulce* (Roxb.) Benth.	Akasakaian, Sima Kaian, Bilati Kaian	Mimosaceae	Fruits	T	The sweet arils of ripe fruits are edible. The pulp is also used in fish curry to give a sour taste. Rarely, chutney is also made from the fruit.
62	*Protium serratum* (Wall. ex Colebr.) Engl.	Rimili, Sarupatri mai, Limbur	Burseraceae	Fruits	T	The fruits are acidic in nature and sour in taste. Ripe fruits are eaten raw and chutney is prepared out of it. The fruits are sold in local market places.
63	*Schleichera oleosa* (Lour.) Oken	Kusuma	Sapindaceae	Fruits	T	The ripe fruit pulp is edible and has acidic taste. Seeds are good source of cooking oil.
64	*Semecarpus anacardium* L. f.	Bhalia	Anacardiaceae	Fruits, seeds	T	The fleshy orange coloured peduncles are eaten when ripe. The seed kernels are eaten after roasting, which taste like almond.
65	*Sesbania sesaban* (L.) Merr.	Jayanti	Fabaceae	Leaves , Flowers	S	Leaves are fried with other leafy vegetables and eaten.
66	*Shorea robusta* Gaertn. f.	Sal, Sargi, Salua	Dipterocarpaceae	Seeds	T	The seeds are roasted and eaten at the time of food scarcity.
67	*Spondias pinnata* (L. f.) Kurz	Ambada	Anacardiaceae	Fruits	T	Unripe fruits are used as vegetables, while the ripe fruits are eaten raw and also used to make chutneys and pickles.
68	*Syzygium cumini* (L.) Skeels	Jamu	Myrtaceae	Fruits	T	The ripe fleshy fruits are commonly eaten. The seed power is used against diabetes.

Sl.No.	Botanical name	Odia Name	Family	Part (s) used	Habit	Use values and mode of consumption
69	*Tamarindus indica* L.	Kainan, Tentuli	Caesalpiniaceae	Fruits	T	The ripe and unripe fruits are sour and are used for preparing chutneys, pickles, curries and several other preparations. The seed powder is also eaten at the time of need.
70	*Tamilnadia uliginosa* (Retz.) Tirv.& Sastre	Tolaka, Pendra	Rubiaceae	Fruits	T	The ripe fruits are eaten; also as a vegetable.
71	*Termitomyces eurrhizus* (Berk.) Heim.	Nada chhatu	Lyophyllaceae	Mushroom	MUS	Freshly collected mushrooms are fried or boiled to make curry with spices and other vegetables.
72	*Termitomyces heimii* Natarajan	Sravana chhatu	Lyophyllaceae	Mushroom	MUS	These are used for preparation of curries with other vegetables. Also sold in local markets during rainy season.
73	*Termitomyces medius* R. Heim. & Grasse	Bali chattu	Lyophyllaceae	Mushroom	MUS	It is fried and consumed; also used to make curry with other vegetables.
74	*Termitomyces microcarpus* (Berk.& Broome) Heim	Hunka chattu	Lyophyllaceae	Mushroom	MUS	After collection, the mushrooms are cleaned properly, fried or boiled and eaten
75	*Trapa natans* L.	Trapa natans	Onagraceae	Fruits	H	The white starchy portion of the fruits are sweet in taste and mostly eaten raw.
76	*Tuber rufum* Pico	Rutka chattu	Tuberaceae	Mushroom	MUS	Curries are made after peeling off outer cover; sold in local markets @ Rs.200-300/- per kg.
77	*Volvoriella volvaceae* (Bul. ex Fr.) Singer	Kuta chhatu	Pluteaceae	Mushroom	MUS	Used for preparation of curries; sometimes with salt and spices.
78	*Ziziphus rugosa* Lam.	Tinkoli, Chunkoli	Rhamnaceae	Fruits	S	Ripe fruits are sweet and are eaten raw. The salted and dried fruits are made to pickles.
79	*Ziziphus jujuba* Mill.	Barakoli	Rhamnaceae	Fruits	T	Fruits are consumed raw, cooked or as pickles.
80	*Ziziphus oenoplia* (L.) Mill.	Kanteikoli, Burukoli	Rhamnaceae	Fruits	S	The ripe fruits are sweet and sour in taste and consumed raw, mainly by children.

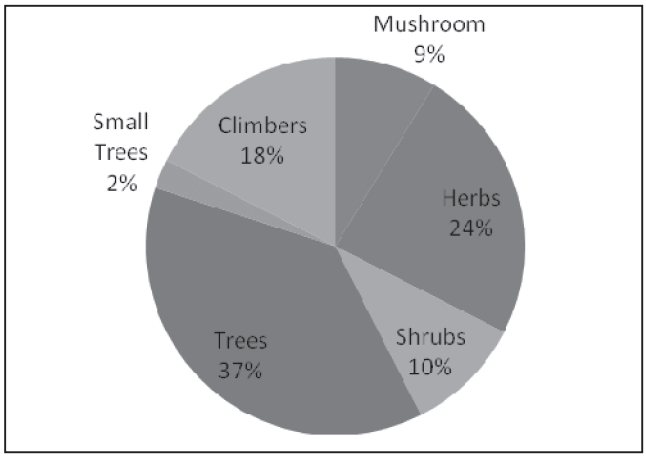

Fig. 2: Habit wise distribution of wild edible food plants studied in Nabarangpur district.

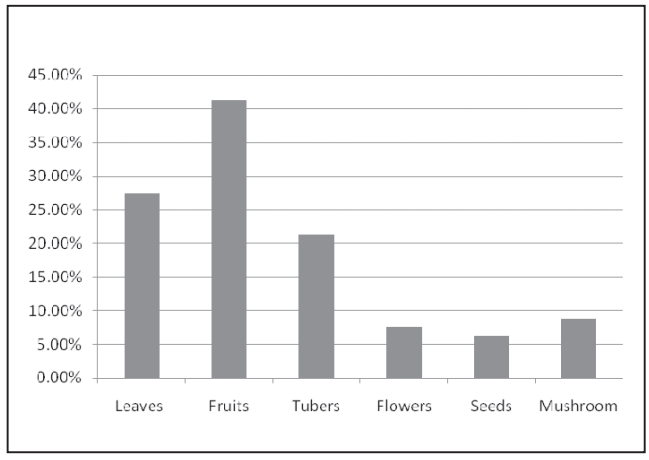

Fig. 3: Category wise use of wild edible food plants of Nabarangpur district, Odisha

Caesalpiniaceae and Anacardiaceae had 4 species each. Of the 80 plant species now listed, 33 species (41.25%) yield edible fruits, 20 species are used as leafy vegetables and 17 species have edible tubers/ rhizomes/ roots. Besides, flowers of 6 species and seeds of 5 species are consumed as edible plant parts (Fig. 3). Species such as *Abelmoschus crinitus, Amorphophallus paeoniifolius*, *Artocarpus heterophylla, Moringa oleifera, Mangifera indica* etc. have multipurpose uses. A number of leafy vegetables like *Alternanthera sesilis*, *Celosia argentea*, *Commelina* spp., *Leucas* spp. grow as weeds in cultivated fields and homesteads and are used as vegetables by all communities.

During the survey, it could be observed that, the tribals of Nabarangapur district prefer some wild edible plants over others because of their multipurpose use and long-term association. Some of them are *Tamarindus indica, Mangifera indica, Amorphophallus paeoniifolius, Artocarpus heterophyllus, Phoenix* spp*., Cassia tora* etc. Collection and harvesting of wild edible plants is a seasonal activity and depends on the availability of fruits in the locality and time of ripening of fruits. The tubers of *Dioscorea* spp. are mainly collected after rainy season, while fruits of *Mangifera indica* and *Diospyros melanoxylon* are collected during summer. Most of the leafy vegetables like *Cassia tora*, *Cleome viscosa*, *Celosia argentea, Commelina* spp.etc are found in abundance during rains and consumed in quantity. All kinds of mushrooms are available in the wild during this period and are commonly sold in markets throughout the season. Tribal people of Nabarangapur district have faily good knowledge on preservation and storage of wild edible plants such as mushrooms, mango kernels, seeds of Mahua (*Madhuca indica*), Char (*Buchanania lanzan*), Kusum (*Schleichera oleosa*), fruits of tamarind (*Tamarindus indica*) and few others. Of the products available in local market, tamarind fruits, Kusum seeds, yams of *Dioscorea* spp., flowers of Mahua and Girili phul (*Indigofera cassioides*), leaves of *Bauhinia* spp. and *Enydra fluctuans* need special mention as they contribute to the livelihood and income generation of the local people.

4. Conclusion

The tribals of Nabarangapur district of Odisha possess fairly good knowledge on utilization, harvesting, preservation and marketing of food plant available in the forests close to them. Some of these edible plants have great economic value and are highly linked with socio-economic development of tribal communities of the state. Though most of them depend on forest products for their livelihood, the traditional knowledge on wild food plants is declining day by day due to over-exploitation of forests, non-availability of resources close to their villages/ hamlets and availability of cultivated fruits and vegetables in local markets. The present study emphasized the need for the protection and conservation of these wild edible plant resources for the benefit of human kind through bioprospecting, quality improvement, domestication, value addition and development of market links.

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Short communication

Two new records of Graphidaceae (Lichenized Ascomycota) from India

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ABSTRACT

Two species of lichen *viz. Fissurina comparilis* (Nyl.) Nyl. and *Graphis erythrocardia* Müll. Arg. (Graphidaceae) collected from Eastern Himalaya are reported here as new distributional records for India.

Eastern Himalaya is one of the eleven bio-geographical zones in India and it forms a part of the globally recognised 'Biodiversity Hotspot'. The region lies between 26°272 - 29°302 N latitudes and 87°592 - 97°302 E longitudes and covers an area of 93,988 sq. km. It covers the states of Arunachal Pradesh, Sikkim and Darjeeling district of West Bengal. During the course of the revisionary studies on Indian *Graphidaceae*, several specimens from the Eastern Himalaya have been collected, analysed and identified. Till date, 94 species of Graphidoid *Graphidaceae*, belonging to 16 genera have been documented (Singh and Swarnalatha, 2011a, b; Singh *et al.,* 2011; Swarnalatha, 2016 a, b; Swarnalatha, 2017 a, b). In the present paper two species namely, *Fissurina comparilis* and *Graphis erythrocardia* belonging to the family *Graphidaceae* are reported as new distributional records for India.

Morphological examination of the specimens was carried out under a stereo-zoom microscope (Nikon SMZ 1500), while anatomical characters were examined using a compound microscope (Magnüs MLX - Tr). The chemical composition of lichen was investigated with Thin Layer Chromatography (TLC) in solvent system A, following the method described by White and James (1985). The spot tests were performed with the usual chemical reagents (K, C and P). Further, the lichen specimens were examined under UV light (365 nm). The specimens cited in this paper were studied at the Lichen Laboratory, Botanical Survey of India, Central Regional Centre, Allahabad, during 2011. Identification of the specimens was done following the keys designed by Staiger (2002) and Lücking *et al.* (2009).

The up-to-date nomenclature, morphological description, chemistry, distribution and relevant taxonomic and ecological notes on these two species of lichen of the family Graphidaceae are provided below.

Fissurina comparilis (Nyl.) Nyl., Lich. Nov. Zeland.: 125 (1888). *Graphis comparilis* Nyl., Bull. Soc. Linn. Normandie, ser. 2, 2: 119 (1868). Type: *New Caledonia,* Loyalty, Lifou, *Thiébaut* 1864 (lectotype- H-NYL 7478).

Thallus corticolous, epiphloeodal, continuous to sometimes cracked according to the nature of bark; surface yellowish-green to olive-green, smooth to rough. Thallus in section has prosoplectenchymatous upper cortex and abundant calcium-oxalate crystals below the algal layer. Photobiont *Trentepohlia*.

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Fig. 1: *Fissurina comparilis* (Nyl.) Nyl. Thallus with ascomata. (Scale = 1 mm)

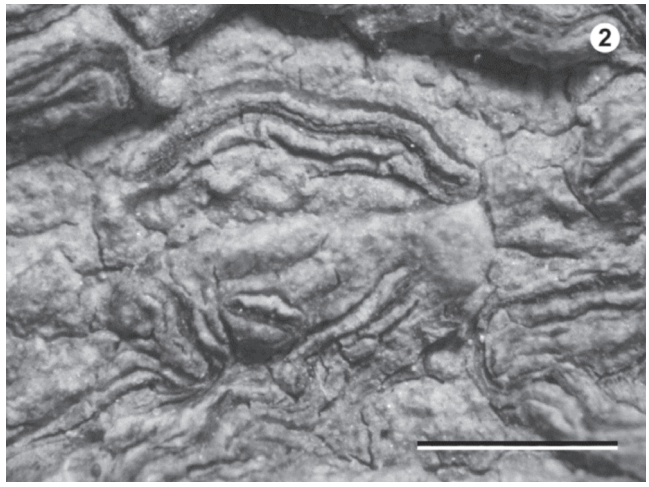

Fig. 2: *Graphis erythrocardia* Müll. Arg. Thallus with ascomata. (Scale = 1 mm)

Ascomata lirelliform, concolorous, numerous, simple to sparsely branched, curved, fissurine to immersed or erumpent, up to 6 mm long, 0.2-0.35 mm broad, acute to acuminate at the ends; disc open, slit like, epruinose; excipulum complete, apically carbonised but laterally and basally uncarbonized and brown; laterally covered by thalline margin; labia convergent, entire; hymenium hyaline, not inspersed, I-; epihymenium indistinct to thin, *c.* 5 ìm thick; subhymenium hyaline; paraphyses simple, *c.* 1.5 ìm thick, tips simple; periphysoides not observed. Asci ellipsoid. Ascospores 8 per ascus, ellipsoid, transversely septate, constantly 4 locular, with thickened septa and lenticular lumina, halonate, 14-20 × 6-9 ìm, hyaline.

Chemistry: Thallus K-, C-, KC-, P-, UV-; no lichen substance detected by TLC.

Remarks: This species is characterised by its yellowish-green to olive-green coloured thallus; concolorous, fissurine to immersed or erumpent, lirelliform ascomata; apically carbonized excipulum; halonate, hyaline, transversely septate, 4 locular ascospores and lacking lichen substances in thallus. *F. comparilis* is somewhat similar to *F. khasiana* in anatomical characters but later species differs from former by having constictic and stictic acids in thallus.

Distribution: Africa, Australia (Queensland), Central America (Costa Rica), Cameroon and New Caledonia; it is reported here from India for the first time.

Note: Earlier this specimen was identified as *Fissurina inquinata* Knight & Mitten by Dubey *et al.* (2007). *F. inquinata* is characterised by 4-5 locular ascospores and presence of stictic acid in thallus, but *F. comparilis* has constantly 4 locular ascospores and lacks lichen substances.

Specimen examined: INDIA: Arunachal Pradesh, West Siang district, Doji-16 km from Along towards Basar, 26 March, 2006, *U. Dubey* 06-006386 (LWG).

Graphis erythrocardia Müll. Arg., Bot. Jb. 20: 280. 1894. Type: Tanzania, *Holst* 3081 (holotype-G). *Graphis vinosa* Müll. Arg., Type. Australia, Queensland, Thursday Island, 1887, *C. Knight* 341 (Lectotype G).

Thallus crustose, corticolous, epiphloeodal, irregular, *c.* 3.8 cm across, prothallus indistinct; surface pale-grey to grey or blackish-grey, wrinkled, uneven, matt, finely cracked. Thallus in section 160-290 ìm thick above the bark, ecorticate, abundant calcium oxalate crystals present in the thallus. Photobiont *Trentepohlia*.

Ascomata lirelliform, black, numerous, crowded, distributed all over the thallus, simple, immersed to often erumpent, straight to curved or flexuous, blunt at the ends, (-0.3) 1-5 mm long, 0.2-0.6 mm broad; disc closed, epruinose; excipulum complete, laterally carbonised, thin, uncarbonised brown at base, slopping lateral thalline margin; labia convergent, entire, covered with ashy-white layer; hymenium hyaline, not inspersed, I-, 70-145 ìm high; epihymenium dark brown, 9-12 ìm thick; subhymenium hyaline, 12-16 ìm thick; paraphyses simple. Asci 8 spored, clavate, 60-80 × 22-29 ìm. Ascospores transversely septate, sub-biseriate to aggregate, distoseptate, (-24) 30-57 × 8-12 ìm, 6-14 locular, hyaline, I+ blue violet.

Chemistry: Thallus K+ red, C–, KC–, P+ yellow, UV; norstictic acid (major) and connorstictic acid (trace) detected by TLC.

Remarks: This species is characterised by immersed to often erumpent, simple lirelliform acsomata; laterally carbonised excipulum; convergent, entire labia; hyaline, transveresly septate, medium sized ascospores; presence of

norstictic acid (major) and connorstictic acid in its thallus. In anatomical characters *G. erythrocardia* resembles *G. longispora,* but later is distinguished by its saxicolous habit, elongated, much branched lirellae, larger ascospores and presence of salazinic acid in additional to norstictic acid.

Distribution: Australia and Tanzania; it is now reported here from India.

Specimens examined: INDIA: Arunachal Pradesh, Lohit, Tezu, Near Lohitpur Assam Rifle camp, alt. *c.* 250 m, 02 Jan. 1984, *K.P. Singh* 4222/A (ASSAM); West Bengal, Darjeeling district, Lava, 10 June 1983, *K.N. Roy Choudhary* 4722 (CAL).

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Short communication

Pityrogramma Link. (Pteridaceae) – A new generic record for Odisha, India

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ABSTRACT

Pityrogramma calomelanos (L.) Link of the fern family Pteridaceae collected from Mahandragiri hills, Gajapati district, Odisha is reported here as new distributional record for the state of Odisha. The genus *Pityrogramma* Link is also reported for the first time from within the geographical boundary of the state.

India has a rich and varied pteridophytic flora due to its unique geographical location, diversified topography, variable climatic conditions and migration of species of different phytogeographical regions. The pteridophytes in India are distributed in different biogeographic regions from plains to mountains, coastal regions, arid regions, riverine ecosystems etc. with major concentration in the Himalayas, Western Ghats, Eastern Ghats and Panchmarhi Biosphere Reserve. The total number of ferns and fern allies occurring wild in India has been variously estimated between 1200 to 1000 species (Dixit, 1984; Chandra, 2000, Chandra *et al*., 2008; Fraser-Jenkins, 2012). According to Fraser-Jenkins (2012) the total number of pteridophyte species present in India is c. 1100 and one-third of them *i. e.* 337 taxa are considered to be threatened or endangered.

The *Pityrogramma* Link is a small genus of 14 species (Tryon, 1962) and 3 species are reported to occur in India (Beddome, 1883; Dixit, 1984). Holttum (1954), while reporting the occurrence of two species of *Pityrogramma* viz. *P. calomelanos* (L.) Link and *P. chrysophylla* (Swartz) Link from Malaya, remarked that *P. calomelanos* is found throughout the tropics and probably, it is dispersed by men. This species has long been under cultivation in the name of 'Silver Fern'. It is reported to be abundant in the plains of Assam and West coast of South India, where the young fronds are used as leafy vegetables (Nayar, 1959). Occurrence of *P. calomelanos* from Garo hills of Meghalaya and Kamrup district of Assam has been recorded by Baishya and Rao (1982) and Handique and Konger (1986) respectively.

During the floristic studies on the Mahandragiri hills, Gajapati district, Odisha, the authors collected some interesting specimens of pteridophytes belonging to the genus *Pityrogramma* Link (Pteridaceae). Through consultation of literature and study of type and other herbarium specimens available at K and NY, we identified it as *Pityrogramma calomelanos* (L.) Link, which has earlier been reported from different parts of India, other than the state of Odisha (Dixit, 1984, Nayar and Geevarghese, 1993; Barbhuiya and Singh, 2014). Therefore, the present occurrence of the species in Odisha is a new generic record of fern for the state. Nomenclature, botanical description, distribution, details of specimens studied and notes on ecology etc. have been provided below along with photographs of plant and plant parts (Fig. 1). The voucher specimen has been deposited in the herbarium of Regional Plant Resource Centre (RPRC), Bhubaneswar, Odisha, India.

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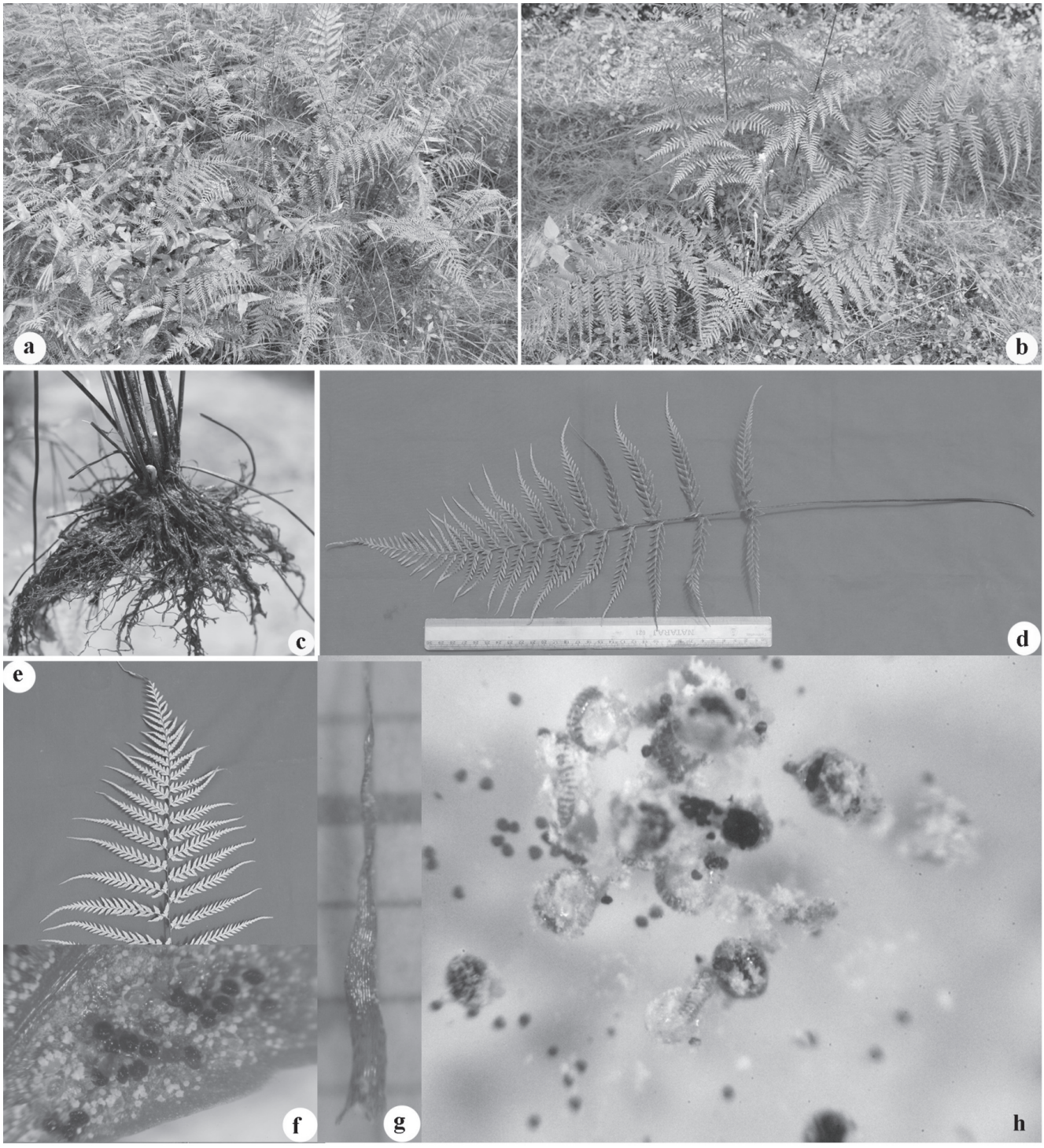

Fig. 1: *Pityrogramma calomelanos*(L.) Link (Pteridaceae): a. Habitat, b. Habit, c. Rhizome, d. Frond, e. Abaxial of pinnae, f. Enlarge of sori, g. Rhizome scale, h. Sporangia with spores.

Pityrogramma calomelanos (L.) Link, Handb. Gewachse 3: 20. 1833; Baishya, & Rao, Ferns & Fern Allies of Meghalaya State, India 146. 1982; Dixit, Census of Ind. Pteridophytes 79. 1984; Handique, & Konger, J. Assam Sci. Soc. 31 (2): 70. 1989; Nayar & Geevarghese, Fern Fl. Malabar 99. 1993; Barbhuiya, & Singh, J. Threat. Taxa 6(9): 6252. 2014; Dudani, *et al.,* Indian Journ. Plant Sciences 3(1): 33. 2014. *Acrostichum calomelanos* L. *Sp. Pl. 2: 1072. 1753. Ceropteris calomelanos* (L.) Link, *Fil. Spec. 141. 1841. Ceropteris serrata* Fée, *Mém. Foug. 8: 81. 1857. Gymnogramma calomelanos* var. *aureoflava* Hook. *Gard. Ferns t. 50. 1862. Pteris calomelanos* (L.) Bedd. Ferns Brit. India t. 22. 1863. *Pellaea calomelanos* (L.) Link, Fil. Sp. 61. 1841; Bedd. Handb. Ferns Brit. India 104. 1883.

Gymnogramma calomelanos var. *denudata* Harr. *J. Linn. Soc. Bot. 16: 37. 1877. Neurogramma calomelanos* (L.) Diels *Nat. Pflanzenfam. 1(4): 264. 1899. Ceratopteris calomelanos* (L.) Underw. *Bull. Torrey Bot. Club: 632. 1929.*

Plants terrestrial; rhizomes erect or ascending, covered by paleae, pale brown, narrowly lanceolate. Frond erect, 60-120 cm long, with the stipe erect, 15-50 cm long, 2-4 mm thick, dark brown, covered by silvery farina when young, covered by a scale, golden brown, 4 mm long, 0.5 mm width, linear, apex tapering. Lamina erect, obovate, 20-60×10-20 cm, covered by dense silvery farina on lower surface; rachis similar to stipe and bearing several loosely placed alternate primary pinnae which are oblong to ovate, 5-15×2-5 cm, facing nearly upwards, and with the secondary rachis adaxially grooved; secondary pinnae pinnatifid and acuminate in the apical region but pinnate below with many subopposite or alternate, sessile ultimate pinnae having the basiscepic base, narrowly decurrent, elongate-ovate to rhomboidal, 10 - 15×3-5 mm, with bluntly acute apex and serrate margin. Sporangia on lower side of pinnae, along the veins; stalk shorter than the capsule and with 32-60 spores, triangular or hemispherical.

Ecology: It grows on humus-rich mountain slopes usually near streams in dense forest at an altitude of about 300m above MSL.

Specimens examined: India, Odisha, Gajapati District, Mahaendragiri hill top, 20-08-2015, *C. Kalidass & P. Murugan,* 9483 (RPRC) N 18Ú58'270'', E 084Ú21'238'' and Alt. ±1002m above MSL.

World distribution: The species is native to America; now widely naturalized in many tropical regions.

Distribution in India: Tamil Nadu, Kerala, Karnataka, Andaman & Nicobar Islands, Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura, Sikkim and Odisha (present report).

Notes: Tryon *et al.* (1975) presented a well-documented chromosome count of n = 116 (x = 29) for *Pityrogramma calomelanos* from Brazil, in disagreement with previous counts for that species.

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Short communication

Andrographis longipedunculata (Sreem.) L. H. Cramer (Acanthaceae)- an addition to the flora of Odisha

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ABSTRACT

Andrographis longipedunculata (Sreem.) L. H. Cramer (Acanthaceae) is reported here as a new distributional record for the state of Odisha, India. A detailed note on the botany, nomenclature, ecology, phenology and distribution of this species is provided in this paper.

The family Acanthaceae (including Avicenniaceae and Mendonciaceae), with about 212 genera and 3175 species, is a large flowering plant family in the world. Most of the species of the family are distributed in the tropical and subtropical regions of the world and only a few in temperate regions (Mabberley, 2008). In India, Acanthaceae is represented by 593 species under 47 genera (Karthikeyan *et al.,* 2009) and 93 species belonging to 29 genera are reported to occur in Odisha (Saxena, & Brahmam, 1995). Recently, while revising the family Acanthaceae of Sri Lanka, Cramer (1996) enumerated 32 species of *Andrographis* Wall. ex Nees, which includes two species earlier described under the genus *Neesiella* Sreem. In India, the genus is represented by 28 taxa with greater concentration of species in Peninsular India (Karthikeyan *et al.,* 2009). Of these, 4 species are reported to occur wild in Odisha (Saxena, & Brahmam, 1995).

During floristic studies in Odisha, we collected some interesting specimens of Acanthaceae family from Khandapada Forest Range of Nayagarh District, Odisha. On critical study of their morphological characters and consultation of relevant literature (Kumari, 1987; Pullaiah, & Moulali, 1997; Karthikeyan *et al.,* 2009; Tiwari, & Ravikumar, 2014), the specimens were identified as *Andrographis longipedunculata* (Sreem.) L. H. Cramer. A thorough scrutiny of literature on flora of Odisha (Haines, 1922; Saxena & Brahmam, 1995) revealed that this species has not yet been reported from within the geographical boundary of Odisha State and thus, turned out to be a new plant record for Odisha. A detailed botanical description along with notes on ecology, phenology, distribution and nomenclature are provided below to facilitate easy identification of the species in the field. The herbarium specimens have been deposited in the Herbarium of the Regional Plant Resource Centre (RPRC), Bhubaneswar.

Andrographis longipedunculata (Sreem.) L. H. Cramer in Kew Bull. 51(3): 555. 1996; Karthikeyan *et al.,* Flowering Plants of India, Dicotyledons 1: 3. 2009; Tiwari, & Ravikumar, in Taprobanica 6(2): 132. 2014; Singh, *et al.,* Endemic Vascular Plants of India 67. 2015. *Neesiella longipedunculata* Sreem. in Phytologia 15(4): 271. 1967. *Indoneesiella longipedunculata* (Sreem.) Sreem. in Phytologia 16(6): 466. 1968; K. M. Matthew, Fl. Tamilnadu Canatic 3: 1182. 1983; Kumari in A.N. Henry *et al.,* Fl.

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Tamil Nadu 2: 150. 1987; Moulai, in T. Pullaiah & D. A. Moulali, Fl. Andhra Pradesh 2: 715. 1997. *Erianthera longipedunculata* (Sreem.) M. R. Almeida, Fl. Maharashtra 4A: 41. 2003. (Fig. 1)

Fig. 1: *Andrographis longipedunculata* (Sreem.) L. H. Cramer (Syn: *Neesiella longipedunculata* Sreem.)

Types: India, Maharashtra, Nagpur, *K. Subramanyam* 4696A (Holotype in CAL) and *K. Subramanyam* 4696B-G (Isotype in MH)

Erect herbs, up to 50 cm high; stem quadrangular or angular, unbranched, wholly hispid. Leaves sessile, simple, opposite, decussate, lanceolate or ovate-lanceolate 4-6 × 1-2 cm long, apex acute to sub-acute, obtuse to sub-cordate at the base, margin entire, sub-coriaceous, sparsely hispid on both sides; lateral nerves 5-7 pairs; petiole absent. Inflorescence axillary, simple raceme, branched; peduncle up to 12 cm long; pedicel erect, sub-sessile or 1 mm long, glandular hairy-hispid. Capsule ovoid-elliptic, less than 1.5 cm, glandular hairy-hispid, apex pointed, with persistent calyx *ca*. 1 cm diam., 5-lobed, each lobe, 9 mm long, linear, glandular-hairy. Seeds 4, non-endospermous, glabrous, reticulately pitted.

Flowering and Fruiting*: November - December*

Ecology: The species was found growing in open scrub forests in association with *Andrographis paniculata* (Burm.f.) Wall. ex Nees, *Tridax procumbens* (L.) L., *Caesalpinia bonduc* (L.) Roxb. *emend*. Dandy *et* Exell., *Spermacoce* spp. and other grasses and sedges.

Distribution: INDIA (Maharashtra, Tamil Nadu, Andhra Pradesh, Karnataka, Gujarat, Rajasthan and Odisha).

Specimens examined: INDIA, Odisha State, Nayagarh District, Khandapada Forest Range, Chadeibasa R.F. foot hills towards temple along roads, N20Ú15.345'; E 085Ú10.689', ±95 MSL, Dt. 23. 11. 2016, *Kalidass & Murugan* 9500 (RPRC).

Notes: Andrographis longipedunculata (Sreem.) L. H. Cramer is closely related to *Andrographis echioides* (L.) Nees, but can be distinguished by its elliptic-oblong leaves and much branched and longer inflorescences than the leaves.

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Short communication

Endemic vascular plants of Odisha: A reappraisal

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ABSTRACT

With an estimated 3,000 wild plant species, Odisha is considered as a biodiversity rich state in India. While more than 100 species are listed as classified as threatened plants, only few are strictly endemic and their distribution restricted to the geographical boundary of Odisha State. Thorough scrutiny of published literature, study of herbarium specimens and based on primary field data collected by the authors, 17 vascular plant taxa belonging to 12 genera under 7 families are found to be endemic to Odisha. This includes 4 neo-endemic and 13 paleo-endemic species of plants. Poaceae and Orchidaceae are the families having maximum number of endemic species, 7 and 4 species respectively. Notes on taxonomy and nomenclature of certain endemic plants of Odisha has been provided along with a complete list of 17 endemic taxa of Odisha with notes on their habit, habitat, distribution and conservation status of each of them.

Endemism in plants is a phenomenon or an ecological state in which species are found to occur in restricted areas, isolated by geographical or temporal barriers. Most of the endemic taxa are seen confined to small areas like islands, peninsulas, mountain ranges, plateaus or in distinct phytogeographical regions. Endemism in plant kingdom has become a subject of fascination for the taxonomists and presence of endemic taxa makes their area of occurrence biologically unique and interesting. Besides, endemic species throw some light on the biogeography, centres of speciation, areas of extinction, vicariance and adaptive evolution of the biological entities (Singh *et al*., 2015). It is generally perceived that physical, climatic and biological factors contribute to endemism. The two sub-categories of endemism such as "paleoendemic" refers to species that were widespread earlier but are now restricted to a smaller area and "neoendemic" refers to species that have recently evolved through divergence and reproductive isolation or through hybridization and polyploidy. Endemic taxa can easily become threatened or extinct, if their habitats get modified rapidly and drastically. Species with narrow distribution range and/or fewer individuals are prone to extinction due to changing climatic conditions and competition by alien species. Endemic species have long been targets for conservation efforts because of imminent danger on their survival. Out of 45,000 plant species reported from India so far, 4381 vascular plants belonging to 1007 genera and 176 families are recorded as strict endemics to the Indian political boundary (Singh *et al*., 2015). Of these, 4303 taxa are angiosperms, 12 species are gymnosperms and 66 are pteridophytes. In the present paper, in addition to field observations of the authors, attempts have been made to examine published literature, herbarium specimens and bring out a list of strict endemic species of the state of Odisha.

Saxena and Brahmam (1994-1996) listed 28 plant taxa as endemic to the state of Odisha. Out of them, *Aglaia haslettiana*, *Mucuna minima*, *Cedrela brevipetiolulata*, *Oldenlandia arenaria, Premna calycina, Premna latifolia* var. *mucronata, Liparis vestita* ssp. *seidenfadenii* and *Gardenia gummifera var. gummiferoides* have been reduced to synonyms of *Aglaia lawii, Mucuna pruriens, Toona sureni* var. *celebica, Oldenlandia stricta, Premna barbata*, *Premna mollissima, Liparis vestita* and *Gardenia gummifera* respectively which are widespread and reported to occur in other Indian states and beyond India (Panda & Das, 1997; Singh *et al*., 2015). Identity of *Tephrosia purpurea* var. *maritima,* earlier reported as an endemic infra-specific taxon, is doubtful as no herbarium specimens are available in any of the herbaria. Some species such as *Habenaria panigrahiana* var. *panigrahiana, Odisha cleistantha, Rhynchosia hainesiana* and *Lasiococca comberi*, previously described as endemic to Odisha, have subsequently been

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reported to occur in other Indian states. *Combretum albidum* G. Don var. *cooperi* (Haines) Saxena & Brahmam was a new combination based on *C. ovalifolium* Roxb. var. *cooperi* Haines. This variety was described by H. H. Haines on the basis of Cooper's collection from Kalahandi, Odisha and the taxon has not been recollected till date. Similarly, *Diospyros ebenum* var. *acuminata* Haines described on the basis of his own collections of H. H. Haines from Champagarh forest, Puri (*Haines* 4094) and Angul (*Haines* 2510) and later collected by Mooney (*Mooney* 2874) before 1950 from Khandapada, Nayagarh need to be re-located and its population assessed.

Oryza jeyporensis Govindsw. & Krishanam., a wild rice described from Jeypore tract, has been reduced to a synonym of widespread *Oryza rufipogon* Griff. Similarly, *Heritiera kanikensis* is an unresolved name and no specimen is available in any herbaria to authenticate its identity. *Zeuxine lindleyana,* earlier considered to be endemic to Odisha, has recently been reported to occur in north India. Similar is the case with two previously described endemic species of *Strobilanthes* of Odisha. The herbarium specimen of *Strobilanthes circarensis* Gamble available at K was collected by J. S. Gamble (21779 Dt. January, 1890) from Peddavalsa, Visakhapatnam district of Andhra Pradesh and therefore, this species can no more be treated as a strict endemic species of Odisha. Later, it is reported from Visakhapatnam hills, Peddavalsa, Endrica and Vantala of Andhra Pradesh. Besides, it is kept as an "unresolved" species in the "The Plant List 2013" (version 1.1.) (http://www.theplantlist.org). Similarly, in case of *Strobilanthes jeyporensis* Bedd., the herbarium specimen available at K (Acc. No. K000882916) was collected by R. H. Beddome from Golkonda hills of Andhra Pradesh and therefore, cannot be considered as endemic to the state of Odisha. This is also an "unresolved" species as per "The Plant List 2013" (version 1.1.) (http://www.theplantlist.org).

While enumerating the endemic orchids of peninsular India, Jalal & Jayanthi (2012) reported the occurrence of 12 endemics from Odisha, of which 4 species are reported as strict endemic species to the state**.** However, two of them namely, *Odisha cleistantha* and *Habenaria panigrahiana* var. *panigrahiana* have been found to occur outside the geographical boundary of the state too. *Liparis espeevijii* S. Misra has been reduced to a synonym of *Liparis vestita* Rchb. f., which is distributed from NE India to Thailand. Similarly, *Cirrhopetalum panigrahianum* (S. Misra) S. Misra is now considered as a synonym of *Bulbophyllum sarcophyllum* (King & Pantl.) J. J. Sm. – an orchid also known to occur in Myanmar and Nepal.

Misra (2012 and 2014) described a two new orchid species *Liparis udaii* Misra and *Zeuxine mooneyi* Misra from Rebana forest of Keonjhar, Odisha, which are also considered neo-endemic taxa. Only few individuals of these two species occur in the type locality and need to be conserved *in situ*.

Cycas circinalis var. *orixensis* Haines, earlier considered as endemic to Odisha has been reduced to a synonym of *Cycas sphaerica* Roxb., which also occurs in East Indian states of Andhra Pradesh and Tamil Nadu. Recently, Singh *et al*. (2015) described a new species *Cycas nayagarhensis* from Nayagarh of Odisha and raised *Cycas circinalis* var. *orixensis* to the rank of a species as *Cycas orixensis* (Haines) Singh & Khuraijam. With this, now there are 2 more additions to endemic cycads of Odisha, of which one is neo-endemic and require further field level inventory.

Chorghe *et al*. (2015 and 2016) described two new grass species namely *Tripogon mahendragiriensis* and *Themeda odishae* from Mahendragiri hills of Gajapati district, Odisha and are, therefore, to be considered as neo-endemics. Besides, they rediscovered five endemic grass species of Odisha such as *Dimeria mooneyi*, *Dimeria orissae, Dimeria mahendragiriensis*, *Themeda saxicola* and *Themida mooneyi*, four of which were not collected after its first collection by H. F. Mooney before 1950.

List of 17 strict endemic species of Odisha along with notes on taxonomy and nomenclature, habit, habitat, distribution and conservation status has been provided in Table 1.

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Table 1

The list of endemic plant species of Odisha with notes on habit, distribution and conservation status

Sl. No.	Species	Family	Habit	Distribution	Conservation status	Remarks
1	*Aspidopterys tomentosa* A. Juss. var. *hutchinsonii* (Haines) R. C. Srivastava	Malpighiaceae	Climber	Collected by H. H. Haines in 1920 from Bhanjam hills, Mayurbhanj.	Not assessed	Type specimen (Haines 4181) is available at K and CAL; not recorded after its first collection by H. H. Haines.
2	*Cycas orixensis* (Haines) Singh & Khuraijam	Cycadeaceae	Tree	From Ganjam district in South to Mayurbhanj in North	Population studies in Odisha are being carried out.	Earlier known as *Cycas circinalis* var. *orixensis* Haines, recently elevated to the rank of species.
3	*Cycas nayagarhensis* Singh, Radha & Khuraijam	Cycadeaceae	Tree	Recently described as a new species from Nayagarh district.	Only 100 individuals are reported in two hills of Nayagarh district	Population inventory need to be made and identity need be confirmed.
4	*Dimeria mahendragiriensis* Ravi, Saxena & Brahmam	Poaceae	Herb	Collected from Mahendragiri hills, Gajapati district; not known to occur in any other locality of Odisha.	Population studies are yet to be conducted.	The species has been recently collected from its type locality by Chorghe *et al*. (2015).
5	*Dimeria mooneyi* Raizada	Poaceae	Herb	Sunabeda plateau	Population studies not done	The type specimen available at K (*Mooney*, 3652) was collected by H. F. Mooney from Sonabera village, Sambalpur district (?) in September-October, 1949. Chorghe *et al*. (2015) collected the species from Sunabera plateau recently.
6	*Dimeria orissae* Bor	Poaceae	Herb	Sunabeda plateau	Population inventory needs to be undertaken.	The type specimen (previously identified as *Dimeria connivens* Hack. by H. F. Mooney) as available at K (*Mooney*, 2758) was collected by H. F. Mooney

Sl. No.	Species	Family	Habit	Distribution	Conservation status	Remarks
						from Pipokhri, Keonjhar district on 1.10.1946. Later, described as a new species by N. L. Bor. Recent collections have been made by Chorghe *et al.* (2015) from Odisha.
7	*Eria meghasaniensis* (S. Misra) S. Misra	Orchidaceae	Epiphytic herb	Only known to occur on mountain peaks of Meghasani and Khairiburu hills of Similipal	Only 200-300 plants are found in natural habitats.	Recent collections have been made from the type locality and attempts are underway to propagate the species.
8	*Eriolaena hookeriana* Wt. & Arn. var. *viridis* Haines	Sterculiaceae	Tree	Karlapat Wildlife Sanctuary	Population studies are yet to be conducted by RPRC	Recent collections have been made from Karlapat.
9	*Flacourtia indica* (Burm. f.) Merr. var. *innocua* (Haines) Saxena & Brahmam	Flacourtiaceae	Shrub	Khandagiri hills, Khurda	Population size and phyto-sociology not known.	Recent collection has been made from Ranpur, Nayagarh District by Odisha Biodiversity Board
10	*Homonoia intermedia* Haines	Euphorbiaceae	Shrub	Collected and described from Mahanadi river bed at Tikarpada by H. H. Haines in 1917; not known to occur anywhere.	The species need to be searched for in its type locality.	Herbarium specimen (Haines, 2505) collected from Tikarpada on 13 March, 1917 and specimen (Haines, 2505a) collected from the same locality in April, 1917 are available at K.
11	*Habenaria panigrahiana* var. *parviloba* S. Misra	Orchidaceae	Herb	Reported from Bhanjanagar, Ganjam by S. Mishra	Size and structure of population not known.	No collection has been made in recent times. But *H. panigrahiana* var. *panigrahiana* has been reported from Odisha, Andhra Pradesh and Tamilnadu
12	*Liparis udaii* S. Misra	Orchidaceae	Herb	Khajuridhi Forest Block in Sundargarh district	Population data not available.	Described as a new species by S. Mishra.

Sl. No.	Species	Family	Habit	Distribution	Conservation status	Remarks
13	*Themeda saxicola* Bor	Poaceae	Herb	Koraput district	Population studies has to be conducted	The type specimen (4241 Dt. 25.10.1950) collected by H. F. Mooney from Laxmipur, Koraput is available at K. Recently the species has been collected from its type locality (Chorghe *et al*., 2015).
14	*Themeda odishae* Chorghe, Prasad, Prasanna & Rao	Poaceae	Herb	Mahendragiri hill, Gajapati	More population level information is required.	Reported as a new species by Chorghe *et al*. (2016) from Mahendragiri hills.
15	*Themeda mooneyi* Bor	Poaceae	Herb	Koraput district	Population status has to be determined.	Type of the species collected by H. F. Mooney (4064 Dt. 10.10.1950) from Pottangi, Koraput and his other collection (4064 Dt. 10.10.1950) from Turia Konda, Deomali, Koraput are available at K. Chorghe *et al*. (2015) collected the species from its type locality after 65 years.
16	*Tripogon mahendragiriensis* Chorghe, Dey, Prasad, Prasanna & Rao	Poaceae	Herb	Mahendragiri hills, Gajapati	Population studies are yet to be conducted.	A recently described species from Mahendragiri (Chorghe *et al*., 2015).
17	*Zeuxine mooneyi* S. Misra	Orchidaceae	Herb	Rebana forest, Keonjhar	Size and structure of population have to be studied.	A neo-endemic species recently described by S Misra (2014).