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Natural and converted ecosystems in Simlipal Biosphere Reserve: Linkages and impact

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ABSTRACT

The present study was conducted in the Simlipal Biosphere Reserve, Odisha, India, which presents a typical example of Mahanadian bio-geographic zone. Four villages from core zone, three villages in buffer zone and five villages in peripheral zone of the biosphere reserve were selected for the agro-ecosystem linkage study. These tribal villages are located at different altitudes where agriculture is the main source of livelihood. The extent of agriculture area and forests across these villages vary significantly; both total land area and uncultivable land being highest in peripheral villages. The core villages are highly dependent on agriculture and carrying capacity at present seems to be enough to support existing human population. All the tribes in core villages practice sustainable methods of collection of forest produce as per their need. The Non-Timber Forest Products (NTFP), food, firewood and medicinal plants are the main requirements of the forest dependent tribal communities of Simlipal. Data on NTFP, food, fuel wood, kerosene consumption and medicinal plants were collected to understand their significance to the livelihood of tribal communities. The Santal, Kolha and Munda tribals are the resident of core zone villages and are primarily agrarian tribes whose dependency on NTFP is high compared to other tribes. The main wild leafy vegetables are Mata saga (*Antidesma acidum*), Gadri saga (*Alternanthera sessilis*), Pita or Khatta Saga (*Mallotus nudiflorus*), Koilari saga (*Bahunia purpurea*), Saijana saga (*Moringa oleifera*), Mati or Sankha saga (*Rungia pectinata*) etc. The NTFP collected by tribals in core zone are more compared to buffer and peripheral villages and these are used mainly for household consumption besides small scale sale in local markets. However, the peripheral villages are mainly market driven societies as they have easy access to market places. Agriculture in core village is much more energy efficient than buffer and peripheral zones. Because of higher human and livestock population and availability of marketing channel, the total annual consumption of NTFPs was much higher in comparison to peripheral and buffer areas. We collected first-hand field data on medicinal plants collected from the forest by all village communities as per requirement to cure various ailments. We recommend strengthening the protection mechanism in forest blocks surrounding the buffer and peripheral villages and also implementation of policies on eco-development and modern agriculture to reduce dependency on forests.

1. Introduction

In developing countries like India, the rural sector with a high population density and a high level of poverty exerts tremendous pressure on natural resources for food, fodder and fuel. A large number of Asian farmers pursue crop cultivation to get the basic food, cloth, education and health care (IFAD, 2004; UNESCAP, 2002; IFPRI, 2002). The major components of village ecosystem such as land, human being and livestock are always in a delicate balance with energy mediating their inter-relationship (Nisanka and Mishra, 1990a; Reddy, 1981; Revelle,1976; Chandola, 1976). An ecosystem operates under a set of definite rules and regulations and any diversion from that set of rules makes the ecosystem converted or modified. Human activity such as hunting and gathering, agriculture, building of cultural landscapes etc. makes an ecosystem converted. Since the

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agro-ecosystems are modified by human beings to produce foods, fibers and other agro products, these are also termed as converted ecosystems (Conway, 1987). Agro-ecosystem is a producer-consumer system in which human being attempts to direct the flow of energy and materials through crop, cattle and humans. Therefore, information about the agricultural crops and animals is essential for agro ecosystem analysis. In agro ecosystems, a large number of operations are performed and controlled by man and these require a lot of energy inputs (Buringh,1985). The fuel-based agriculture is not sustainable in long run (Rappaport, 1967,1971). An agricultural system will be considered to be sustainable if its productivity is maintained in long run, natural resources driving the agricultural production processes are conserved and profitability of production and financial income of the farmers are guaranteed (Neher, 1992; Kessler, 1994). The agriculture ecosystems are the most disturbed systems because of typical intense management and disturbance of specific farming practices (Cox and Atkins, 1979). Mitchell (1979) highlighted the basic structure and other functional attributes of labour-intensive Indian agriculture and also developed methodologies to analyse the Indian agro ecosystem. Low productivity of livestock deprives the villagers from livestock-based income this is due to scarce vegetation base and fodder availability (Semwal *et al.*, 2004). Many of the native indigenous crops have become extinct in the areas where Tribals prefer high yielding variety and adopting fuel energy based agricultural practices (Maikhuri *et al*, 2001). Several studies have been done on agro-ecosystems in India (Toky and Ramakrishnan, 1981, 1982; Mishra and Ramakrishnan, 1981, 1982; Singh and Singh, 1984; Singh 1989; Sharma, 1989) and these are useful in increasing our understanding on agro-ecosystems. In Odisha, works of Patel (1985); Fernandes *et al.* (1988); Nisanka and Mishra (1990, a, b); Nayak and Mishra (1991); Sahoo and Mishra (1992); Nayak *et al.* (1993); Patro and Panda (1994); Mishra and Dash (2000); and Dash and Mishra (2001) provide a deep understanding of various factors and their effects on functioning of agro-ecosystems.

Attempts to generate data on structure and functioning of ecosystems of Simlipal Biosphere Reserve are confined to survey, identification and taxonomy (Patnaik and Jena, 1995; Kanungo, 1985; Panigrahi, 1985 and Saxena and Brahmam,1988, 1989), medicinal plants (Panigrahi, 1985; Tribedi *et al.*, 1982), fauna and wildlife (Anonymous, 1990; Prusty and Singh, 1996); Climate (Das, 1989; Sahu, 1985), tribal people and biodiversity (Mahajan, 1983; Rao *et al* (1984) and rare, endangered and endemic flora and fauna (Nayar and Sastry, 1987). Senapati and Sahu (1969) and Das and Das (1997) provided historical information on Similipal. The phytosociological analysis of Simlipal forests has been studied by Mishra (2002), Mishra *et al* (2004), Das (2004, a, b), Das and Das (1968) and Dash (1998) but little attention has been given for systematic research on existence and functioning of converted ecosystems within Simlipal. This study is a systemic analysis of converted ecosystem and changes brought about by human beings covering agriculture, animal husbandry, collection of fuelwood and minor forest products, conversion of forest land for other purposes, tourism etc. An attempt has been made to find out the linkages between tribal and natural resources and to study the practices of settled agriculture cultivation by the tribals in detail with reference to landform, climate and geographical phenomena.

2. Study area and methodology

The Simlipal Biosphere Reserve is located in Mayurbhanj district of Odisha, India. Important townships around Simlipal Biosphere Reserve are Baripada and Udala on Eastern side and Jasipur, Karanjia and Thakurmunda on the Western side. The bordering districts of Mayurbhanj are Balasore and Keonjhar at the east and southwest directions respectively and Midnapore (Westbengal) and Singhbhoom (Jharkhand) at the northeast and northwest directions respectively (Das, 1998). The Mayurbhanj is the largest district of Odisha with an area of about 10,418 sq. km. of which 42.16% (4392.12 sq. km.) is forest area. It is predominantly a tribal district having 56.6% of its population as schedule tribe. A total of 131 castes and tribes were reported in Mayurbhanj District Gazetteers (Senapati and Sahu, 1969). The major tribes residing in Simlipal Biosphere Reserve are Ho or Kol, Kharia, Mankiria or Birbor, Munda, Santals Bhathudi, Bhumij and Manali. The schedule caste and other backward caste of Simlipal Biosphere Reserve include Ghasi, Gaud or Mahakud, Mahato, Teli, Bindhani and Ganda. The Simlipal Biosphere reserve is located between 21^0 30' to 22^0 08' North latitudes and 86^0 05' to 86^0 37' East longitude. The area of Simlipal Biosphere reserve is 4374 sq. Km. of which 845 sq. Km. is core zone of Simlipal tiger reserve (STR), 2129 sq. km. of buffer zone (1905 sq. km. of STR buffer + 77 sq. Km. of Nato reserve forest and 147 sq. km. of Satkosia reserve forest) and 1400 sq. km. of transitional zone or peripheral zone (Srivastava and Singh, 1998).

2.1. The sample villages

There are several villages located inside the Simlipal Biosphere reserve. Most of the villages are established in the valley areas. There are four revenue villages in core zone, 61 revenue villages in buffer zone and about 1200 villages in peripheral zone. A through survey of Simlipal Biosphere Reserve was done to get the representative village

and a total of twelve sample villages were selected for the study. All the four villages from core zone (Jenabil, Jamunagarh, Kabatghai and Bakua. are in Gudgudia Grampanchayat of Jasipur block), three villages out of total 61 villages in buffer zone (Gudgudia village in Gudgudia Grampanchayat, Nawana in Astakumar Grampanchayat and Barehipani in Barehipani Grampanchayat of Jasipur block) and five villages out of 1200 villages in peripheral zone (Jamuani in Podagarh Grampanchayat of Jasipur block, Lulung in Kochilaghati Grampanchayat of Samakhunta block, Kadamsul in Kolialum Grampanchayat of Kaptipada block, Ghodabindha in Hathigora Grampanchayat of Thakurmunda block and village Patiapada in Champajhar Grampanchayat of Thakurmunda block) were selected.

2.2. Geographical settings of sample villages

The village Jenabil is located on the southeastern part of Simlipal plateau and is about 65 Km from the block head quarter Jasipur. The village is called Jenabil, after the name of its first settler Jena Kol. The altitude of Jenabil is 870 m mean sea level(msl) and is surrounded by thick forests. The second village Jamunagarh is at an elevation of about 800 meters msl. Jamunagarh is a valley area surrounded by mixed Sal forest having shunted tree growth. The village Kabatghai is located nearer to the boundary of the core with elevation of Kabatghai is 671 meters from msl and is surrounded by dense woodland forests consisting of mainly Sal. The village Bakua has highest elevation of 918 meters and is also surrounded by Sal dominated dense forests. The buffer zone village Gudgudia, Nawana and Barehipani are located at about 600, 740 and 710 meters from msl, respectively and is surrounded by dense woodland. Barehipani has the famous Barehipani water fall. All these three buffer study villages are important meeting centers of the buffer zone villages.

The peripheral villages represent a mixture of culturally advanced as well as of backward communities. The first Ghodabindha is located at an elevation of 360 meters msl on a slopping ground between two seasonal streams namely Seem Nadi and Chirupad Nadi, surrounded by a degraded but pure stand of Sal. The village Jamuani is located at the northwestern foothill of Simlipal, 0.5 Km from buffer zone boundary. One river flows through the village fulfilling the water needs of villagers during summer. The topography of Jamuani village is a rolling plain land having an elevation of about 460 meters msl, The forest surrounding the village is degraded. The village Kadamsul is located at 70 meters msl on the southeastern foothill of Simlipal, in Kolialum grampanchayat of Kaptipada block. The village is well connected with Khunta by means of a fair weather road. The vegetation is more or less degraded scrubland because of prevailing dry weather conditions, coarse textured soil and rugged terrain. The fourth village Lulung is situated at the northeastern foothill of Simlipal surrounded by hills on three sides and can be approached through Rangamatia and Balideha dam. The Palpala River, which passes through Lulung, is the sole source of fishing, bathing and washing of clothes. The adjoining hills are rich in limestone. The last village Patiapada is located in the foothill zone of southwestern flank of Simlipal, Salandi River flows touching the village boundary and the village is surrounded by dense woodland. The terrain of village is undulating plain land having some waterfalls in the forest near the village.

2.3. Methodology and data collection

The primary data was collected through household survey, group discussions, and field check. The sources of secondary data were Census of Odisha, Forest Department, Directorate of Economics and Statistics, Odisha Tourism Department and Revenue Department. The maps and toposheets were also collected from Survey of India and National Atlas Thematic Mapping Organization (NATMO). The household survey is widely used tool for collecting primary data. A household survey was undertaken between October, 2003 to October, 2004. The villages were surveyed in different months of the year on a well-structured questionnaire. The questionnaire was prepared after a thorough review of earlier works of Singh (1989), Sharma (1989), Nisanka and Mishra (1990 a, b), Nayak and Mishra (2002), Mishra and Dash (2000), Sahoo and Mishra (1992), Dash and Mishra (2001) and Nayak *et al.* (1993). Bhasin and Bhasin (1997) provided some basic ideas to design the questionnaire. The survey was intended to find out household structure and family size (male, female and children), living conditions of household's viz. types of houses, number of doors, windows and rooms in each house, major drinking water sources etc., livestock reared by household such as cattle (cow, goat, buffalo, ox, sheep), poultry birds (hen, duck, peacock, dove) and pets (cat, dog) and size of landholdings, major and minor crops grown there. The study generated data on quantity of firewood and kerosene used for cooking and lighting purpose, household consumption of rice, oil and salt etc., major and minor forest produce collected by household and money earned, information on Tribal culture and tradition and basic amenities such as education, food, clothings and health care and their availability to them

3. Results and discussion

The Non-Timber Forest products (NTFP), food, fuel (fuel wood and kerosene) and medicinal plants are the main requirements of the forest dependent tribal communities of

Simlipal. A list of plants with local name, scientific name and common use has been given in Table 1. A zone wise analysis of NTFP, food, fuel wood, kerosene consumption and medicinal plant's use has been done to have information about their importance for the livelihood of Tribal communities (Table 2-5). The Santal, Kolha and Munda tribals are the resident of core zone villages. They are primarily agrarian tribes whose dependency on NTFP is low compared to Kharia Tribes. The NTFP collected by tribals in core zone are mainly used for household consumption and a very little is sold in the market. The main wild leafy vegetables (saga) are Mata saga (*Antidesma acidum*), Gadri saga (*Alternanthera sessilis*), Pita or Khatta Saga (*Trewia nudiflora*), Koilari saga (*Bahunia purpurea*), Saijana saga (*Muringa pubicens*), Mati or Sanka saga (*Pachyrrhizus erosus*) etc. Some of other saga, whose botanical name could not be found, but collected and used by the Tribals in the core zone of Simlipal are Binda saga, Kitagini saga, Kankari saga, Muri Saga, Serali saga, Lendung saga and Leper saga. The range of wild leafy vegetables collection was 0.08 to 1.5 kg/day/household in core zone. The average and total wild leafy vegetables collection was 0.79 kg/day/household and 68 kg/day in core zone (Table 2) and these leafy vegetables are collected for household consumption only. The main wild seeds collected in core zone of Simlipal are Rimiri (*Protium serratum*), Siali (*Bahuinia vahlii*), Kusum (*Schleichera oleosa*) and Jamun (*Syzygium cumini*). The range of wild seeds collection in core zone was between 0.17 to 11.67 kg/day/household. The average and total wild seeds collection was 1.17 kg/day/household and 100.38 kg/day in core zone (Table 2). Among wild seeds collected in core zone Kusum seed are sold in market and rests are used for domestic consumption. The wild fruits collected in core zone of Simlipal are Anola (*Phyllanthus emblica*), Harida (*Terminalia chebula*), Bahada (*Terminalia bellirica*), Aamba (*Mangifera indica*), Jackfruit (*Artocarpus heterophyllus*) and Raiphal (*Dillenia pentagyna*). The range of wild fruits collection in core zone was 0.17 to 18.61 kg/day/household. The average and total wild fruits collections were 2.60 kg/day/household and 224 kg/day in core zone (Table 2). Among wild tubers and roots only Jungle alu or Mati alu and Boitadu (*Dioscorea* species and *Curcuma* species) are the main collection in core zone. The collection of wild tubers and roots in core zone ranged between 1.00 to 3.33 Kg/day/household. The average and total wild tubers and roots collection are 0.54 kg/day/household and 47 kg/day in core zone (Table 2). Champa (*Magnolia champaca*) is the main wild flower collected in core zone. Honey is collected in two seasons. A number of mushrooms such as Bada chhatu, Rutuka, Handiphuta, Gobara, Tundi, Bhurkunda kana, Dha kana, Kendu kana, Jamu kana, Panasa kana, Achundi kana, Mayurachualia, Angarpoda, Palua, Siali kana, Jadi kana, Champa kana, and Aamba kana are collected. Honey and mushrooms are collected for both domestic as well as for sale in haat. The range of grasses, flowers, honey and mushrooms collection in core zone was 0.08 to 1.50 kg/day/household. The average and total grasses, flowers, honey and mushrooms collected in core zone was 0.59 kg/day/household and 51 kg/day. Collection of Chana grass (*Imperata cylindrica*) was surprisingly avoided by core zone villagers (Table 2). Rice and edible oil are two staple foods of core zone villagers. The annual rice consumption by households per hectare of cropland owned was 7.51, 8.07, 37.37, and 45.29 quintals in Jenabil, Bakua, Jamunagarh and Kabatghai villages respectively (Table 3). However, the annual edible oil consumption by core zone villagers was 6, 16, 26 and 42 kg/hectare in Bakua, Jenabil, Jamunagarh and Kabatghai villages respectively (Table 3). The fuel wood consumptions by household per ha of crop land owned in core zone villages were 25,807; 39,805; 73,768 and 3, 93,607 kg/ha/year in Bakua, Jenabil, Jamunagarh and Kabatghai respectively. The range of fuel wood consumption was 25,807 to 3,93,607 kg/ ha/ year (Table 3). The average fuel wood consumption in core zone was 1,33,247 kg/ ha/year. The high fuel wood consumption in Kabatghai may be due to inhabitant of Santal tribe who are relatively more advanced and their paddy processing method requires heavy fuel wood. Kerosene is the only source of lighting in all the core zone villages of Simlipal. The kerosene consumptions (liter/hectare of crop land/ year) by core zone villagers were 31, 36, 122, and 195 in Bakua, Jenabil, Jamunagarh and Kabatghai villages respectively (Table3).

The Kharia and Mankiria tribals are the main hunters and gatherers of Simlipal. Most of the Kharias resides in Gudgudia village of buffer zone and some villages in peripheral zone. Mankiria live a life of Transhuman while Kharias are settled. Gudgudia villagers are provided with Indira Awas by Kharia and Mankiria Development Authority (KMDA). Kharias take agriculture as their subsidiary occupation as they have no technical knowhow of agricultural practices. The life of these primitive hunters and gatherers is comparatively harder than the people dependent on agriculture. They also preprocess the Non timber forest products NTFPs to fetch higher price in the market. The NTFP collected by Kharia are mainly wild vegetables, wild seeds, wild sap and gums, wild fruits, wild tubers and roots, wild grasses. Medicinal plants and fishes also substantiate the income of these Tribal people. A number of wild leafy vegetables collected from the forest are mainly used for domestic consumption. These are Sanka saga (*Rungia pectinata*), Gadri saga (*Alternanthera sessilis*), Pita saga or Panigramari (*Mallotus nudiflorus*), Koilari saga (*Bauhinia purpurea*), Saijana saga (*Moringa oleifera*), Mati or Mata

saga (*Antidesma acidum*) and Zinka saga etc. The range of wild leafy vegetables collection in buffer zone varied from 0.1 to 0.6 kg/day/household. The average and total wild leafy vegetables collections were 0.13 kg/day/household and 332 kg/day in the buffer zone (Table 2). The principal wild seeds collected by Kharias of Gudgudia village in buffer zone are Rimiri (*Protium serratum*), Siali (*Bauhinia vahlii*), Ghurdu (*Gardenia gummifera*), Kusum (*Schleichera oleosa*), Sal (*Shorea robusta*), Kendu (*Diospyros melanoxylon*), Jamun (*Syzygium cumini*), Bela (*Aegle marmelos*), Jambira (*Citrus medica*) and Kandhia etc. The average daily collection of wild seeds ranged from 0.67 to 0.23 kg/day/household, except Sal (5 kg/day/household) that have some market value. The total wild seeds collected in the buffer zone were 1481 Kg/day (Table2). Panaria gum (*Elaeocarpus stipularis*) and Siali gum (*Bauhinia vahlii*) are the main wild saps and gum collected by the Tribals of buffer zone. The daily collection of saps and gums was about 1 kg or liter/day/ household. The total wild sap and gum collected in buffer zone was around 2554 kg/day (Table 2). A number of wild fruits collected from the forests by Kharias include Chara Koli (*Buchanania cochinchinensis*), Chunkari (*Ziziphus rugosa*), Ghuira (*Vachellia leucophloea*), Mahua (*Madhuca longifolia* var. *latifolia*), Anola (*Phyllanthus emblica*) and Rajara. The average daily collection of wild fruits ranged from 0.2 to 1 kg/day/household and total wild fruit collected in the buffer zone was 639 kg/ day (Table 2). Mundai alu, Pinkara, Rasa, Laddu, Pittadu and Karu alu are main wild tubers collected by Kharias from the forests of Simlipal. Most of the wild tubers belong to *Dioscorea* genus and the main species collected are *Dioscorea glabra*, *D. pubera*, *D. bulbifera* and *D. belophylla* etc. The average daily collection of these wild tubers and roots ranged from 0.2 to 0.5 kg/day/household, except jungle alu (*Curcuma* species) 7 kg/day/household. Pala or arrowroot prepared from these wild tubers and roots are used for domestic consumption as well as for sale. The total wild tubers and roots collection in buffer zone was 2809 kg/ day (Table 2). Chana grass (*Imperata cylindrica*) is the main wild grass collected by Kharias, used to lay off roofs of huts. The average daily collection of Chana grass was 10 kg/day/household. The total collection of Chana grass in buffer zone was about 6385 kg/day (Table 2). A number of Mushrooms as Bada chhatu, Rutuka, Handiphuta, Gobara, Tundi, Bhurkunda kana, Dha kana, Kendu kana, Jamu kana, Panasa kana, Achundi kana, Mayurachualia, Angarpoda, Palua, Siali kana, Jadi kana, Champa kana, and Aamba kana are collected by the villagers of buffer zone. The flowers honey and mushrooms collection was also done only for domestic consumptions. The staple food of buffer zone villages are rice and edible oil. The annual rice consumption by households per hectare of cropland owned were 4.28, 10.39 and 13.48 quintals for Barehipani, Nawana and Gudgudia villages respectively (Table 4). However, the annual edible oil consumption by buffer zone villages was 3.99, 5.99 and 91.00 kg/hectare in Barehipani, Gudgudia and Nawana villages respectively (Table4). Rice is a major crop in Odisha and it commands over 78% of total cropped area in Odisha. The average rain fed rice yield in hilly Tribal villages of Odisha is less than Meghalaya (Toky and Ramakrishnan, 1981) and coastal villages of Odisha (Nisanka and Mishra, 1990b). However, all these Indian villages are better than most western based villages (Spedding, 1975; Pimental and Pimental, 1979; Pimental *et al.*, 1973) from an energetic point of view. The fuel wood consumptions by households per ha of cropland owned in buffer zone villages were 15,612; 84,686 and 1,20,433 kg/ha/year in Barehipani, Nawana and Gudgudia respectively (Table4). The range of fuel wood consumption was 15,612 to 1,20,433 kg/ ha/ year. The average fuel wood consumption in buffer zone was 73,577 kg/ ha/year. Very high fuel wood consumption in Gudgudia village was because of high requirements to fulfill the needs of ever-growing population and fuel wood intensive paddy processing methods. The kerosene consumption in buffer zone villages were 26, 42, and 47 (liter/ hectare of cropland owned/year) in Barehipani, Nawana and Gudgudia villages respectively (Table 4). In village ecosystem, fuel energy consumption is derived directly or indirectly from biomass (Nisanka and Mishra, 1990, b). The fuel wood collection requires a good deal of human energy, because of depleting forest resources, which is used for cooking and boiling to feed human as well as cattle.

In peripheral zone, there is no definite pattern with respect to time or space for collection of NTFP (Table 2), so data on NTFP from families are not available. The distance and market demand are the main deciding factors for NTFP collections. Few villagers collect Sal leaves, Mahua Flowers (*Madhuca longifolia* var. *latifolia*), Anola (*Phyllanthus emblica*), Kusum (*Schleichera oleosa*) and Karanj seeds for sale in the market. Wild leafy vegetables like Sajana saga, Khatta saga, Koilari saga etc are collected for domestic consumption. Very rarely they collect wild fruits like Mango, Jackfruit, or wild flowers like Champa (*Magnolia champaca*). Some of the peripheral villagers are constrained to go up to 30 Km deep in the forest for NTFP collections. Sal leaves are stitched in to leaf cups and plates there by providing job for about six to eight months to rural Tribal women in the peripheral zone. The traders usually visit the peripheral areas to procure the processed NTFP like Sal seeds, Karanj seeds, Mahua, etc. Rice constitutes the staple food of peripheral zone villagers. The annual rice consumption by households per hectare of cropland owned were 5.74, 8.13,

10.72, 12.89, and 72.41 quintals in Patiapada, Ghodabindha, Kadamsul, Jamunai and Lulung villages respectively (Table 5). However, the annual edible oil consumptions by peripheral zone villages were 1.99, 3.00, 5.00, 5.99 and 19.99 kg/hectare/ year in Kadamsul, Patiapada, Lulung, Ghodabindha and Jamuani villages respectively (Table5). The fuel wood consumptions by household per hectare of cropland owned in peripheral zone villages were 21,453; 30,374; 51,912; 58,093 and 59,388 kg/ha/year in Patiapada, Jamuani, Ghodabindha, Kadamsul and Lulung respectively (Table 5). The range of fuel wood consumption was 21,453 to 59,388 kg/ ha/ year. The average fuel wood consumption in peripheral zone was 44,244 kg/ ha/year (Table 5). The high fuel wood consumption in Lulung may be due to intensive paddy processing and illegal selling and/or transport of fuel wood to neighboring townships. The Kerosene consumption in peripheral zone villages were 31, 45, 51, 65 and 68 (liters/ha of cropland owned/ year) in Patiapada, Jamuani, Kadamsul, Ghodabindha and Lulung villages respectively (Table 5). The fuel consumption is a complex phenomenon affected by agricultural practices as well as by various cultural, social and economic factors (Fernandes *et al*, 1988). Most of households in villages of Odisha use dung cakes due to its easy availability (Nisanka and Mishra, 1990, b; Krishna, 1984). The Tribal villages in India derive most of total village energy from the forest (Nayak *et al*, 1993; Gangwar and Ramakrishnan, 1989; Rabindranath *et al.*, 1981). Therefore, Indian village ecosystem mainly depends on dung cakes, agricultural residue and plant litter on one hand and animal energy for agriculture on other. In India the 80 million animals provide two third of power requirement of villages (Shiva, 1991). The difference in the use of biomass energy could be attributed to difference in per capita income, socio-economic conditions, availability of resources and technical knowhow (Rao *et al*, 2005). The main cropping practices being used in the Tribal village of India are valley cultivation, shifting cultivation (Guda Bari) and home garden cultivation. Minor millets, red gram and niger are the crops of shifting cultivation (Guda Bari), vegetables and species are the main crops of bari or the field near the house while paddy dominates in valley agriculture. Human and drought power are major energy input in valley agriculture while human labour is the only source of energy in shifting cultivation. The bullocks are the dominant draughts power in rural India accounting for 83% of animal work (Revelle, 1976). In hilly Tribal villages, almost all agricultural yields are consumed within village and remaining need is fulfilled by the forest (Dash and Mishra, 2001).

A number of medicinal plants are collected from the forest by all village communities as per requirement to cure various ailments. Some of the plants having important medicinal constituents are given in Table 1. These medicinal plants are used as Ayurvedic drugs in a variety of ailments. The local Vaidyas prepares the medicine for a number of health related problems starting from simple headache, or stomach ache to snake bite. The local Tribal people prefer the Vaidyas for their day to day health related problems. In case of severity the patients are sent to hospitals located at the town in periphery. Medicinal and aromatic plants, which the locals extracted for their own uses have good market. But the people in villages do not market these products in general mainly due to restrictions and also lack of support. Similar situation exists in central Himalayan villages and several researchers have recommended for Supporting people to cultivate these plants through eco-development activities and providing assured market opportunities that will help in reducing extractions from wild and ensure economic opportunities with the access to market for this produce (Maikhuri *et al*. 2001a & 2003a).

Fishes are relished by all communities of Simlipal but quantitative data on fish collection and consumption was not available with communities. The principal fishes captured in the rivers/ponds of Simlipal are Seula (*Channa marulius*), Magura (*Clarias batrachus*), Kau or Kari (*Anabas testudineus*), Korandi (*Puntius species*), Kantia (*Mystus seenghala*), Gadisa (*Channa panctatus*), Tudi or Turi (*Mostocembelus armatus*), Balia (*Wallago attu*), Bakura (*Catla catla*), Rohi *(Labeo rohita*), Singi (Heteropheurtes fossils) and Chenga or Chengo (Chhana gachua) etc. All of these fresh water fishes are captured mainly for household consumption. The Tribals also lack the technical knowhow to preserve fishes for future consumption. The only restriction for fishing is that the fishes should not be captured during their breading period.

From the above data of three village ecosystems, it is evident that Forests are a vital source of energy to village ecosystems which play a crucial role in making them sustainable. Tribal are always presented as the encroachers and destroyers of natural resources. But that is not true in case of Simlipal Biosphere reserve. In fact, they are the managers of forests who are managing it since ages for their livelihood. Now a days the primary hunters and gatherers of Simlipal Biosphere reserve are facing stiff competition from other tribes. On agricultural front they are the learners. The major dependency of village ecosystem on forest is in the form of fuel wood. In central Himalayan region also the Fuel and fodder are the major dependencies of people on the forests (Rao *et al*., 2005). Fence wood and non-timber forest resources (NTFP) plays a crucial role in making the village ecosystem sustainable by providing employment to

the unskilled labour force, alternative source of food, medicine and other household requirement. In Simlipal Biosphere reserve the Tribals and forest are intimately connected and the Tribal traditions and customs are very tightly interwoven with nature and Tribal people always pay due regard and respect to the natural laws or rules of the nature. A number of their deities are from natural assets such as Mountains, Trees, Sun, Earth, Rivers etc. They always live in fear of the wrath of deities in the event of break of laws knowingly or unknowingly. There are several rituals, traditions and customs (beliefs) which prohibits the Tribal people to over exploit the forest. The kols or Ho's that form the majority in core, buffer and peripheral zone villages of Simlipal Biosphere reserve, had a large number rituals / traditions for resource conservation and their proper utilization. The Kharia and Mankiria are the main hunters and gathers of Simlipal Biosphere reserve while all the other tribes including kols are agrarian communities. Some of the rituals / traditions governing the natural resources, conservation and proper utilization are noteworthy. No forest produce can be collected without observing Kabadi or Jungle Puja by Kharias and Maghuani Puja by other tribes. In other wards nobody dares to enter into the forest after Makara festival till the completion of Maghuani Puja. During pregnancy, both wife and husband refrain from entering into the forest until the pollution is over. In case of death of a Tribal, all the kins and family members are tabooed to enter into the forest till the period as mandated comes to an end. All the Kharias practice judicious methods to collect forest produce such as they don't dig out all the underground roots and tubers, instead they left some part of it for regeneration, leafy vegetables and medicinal produce are collected as per the need. The bee hives situated on tall trees on cliffs cannot be exploited without prior propitiation of hill sprits. Places of origin of hill streams are considered a sacred place and no forest produce extraction activities are allowed and there. Milking of cow is considered as a sin among kols. Making of nuisance in the forest and wearing red colour cloth is prohibited as it will attract wild animals as elephant, tigers etc. In most of the tribes the non-vegetarian diet is prohibited in the days of pollution.

Among NTFPs, the number of wild leafy vegetables wild fruit and mushrooms collected in core zone was higher than buffers and periphery. Relocation of Kharias in the buffers zone had increased the human pressure on NTFPs and other forest resources as they are facing stiff competition from fellow Kols and other tribes. The average daily collection of wild leafy vegetables and wild seeds and fruits per households was high but the total collection in the villages of buffer zone was higher than villages of core zone. The wild saps and gums are not collected in core zone. The collection of wild tubers and roots, wild grasses, flowers, honey and mushrooms was also high in buffer zone which is due to Kharias Tribes and a large population of other tribes. In peripheral zone, the market exposure of the tribes transformed their life style to some extent. The peripheral villagers have to covers large distance to collect NTFPs. The fuel wood consumption was highest in core zone followed by buffer zone and peripheral zone. In rural areas of India about 180 million tons of biomass fuel is used for cooking through insufficient and smoky cook stoves and cooking and lighting energy constitute 75 % of total energy used in India (Rajvanshi, 2003). The average fence wood consumption was highest in buffer zone followed by peripheral zone and core zone. Technically unskilled Kharias and Kolhas make a high fence wood energy input in buffer zone. In buffer zone, the villagers have high dependency on forest as they lack other sources of livelihood, besides having comparatively skilled labour force as far as primary operations are concerned. Though the market provides very less energy to the village ecosystem but that is very crucial as it meets demand for food, edible oils, kerosene, salt, fertilizer, pesticides, seeds of hybrid varieties (Sarakari Dhan) etc. The villagers of Simlipal Biosphere reserve purchase these items from the respective block head quarter and local weekly markets/haat. The high dependency on market in peripheral zone is due to good roads, and comparatively better economic conditions of Tribals. There are linkages between rural peripheral villages and local market town and a good connectivity by means of roads convey a set of benefits like lager market for agricultural and non-agricultural products, improved access to agricultural inputs, improved access to amenities as health, education, safe drinking water, modern building material for houses, additional source of livelihood such as remittances and markets for farm labour and better access to goods for consumption, new opportunities to sell goods and services.

In Simlipal Biosphere Reserve, the villagers sell their agricultural and non-agricultural (mostly forest products) products in local markets. A large part of these marketing follow the barter system as it improves their cultural ties also. The most sold products are edible oils, honey, ropes, mats, forest leaves, leaf cup plates, Mahua flowers, Sal, Kusum, Karanj seeds, Sal and Siali gum etc. The capacity of forest seems to have reached its optimum or may have crossed its limit in buffer zone to support huge population. The dependency on forest components is highest in buffer zone followed by peripheral zone and core zone of Simlipal Biosphere reserve. The Tribal tradition plays a key role in structure and functioning of agro ecosystem in Tribal villages. Women perform the major manual works including all domestic activities (Patel, 1985). The native people are

in an utter state of helplessness and non-responsive towards the resource management (Joshi *et al.,* 1997). A policy intervention to provide food grains to meet the requirements of these villages better eco-development services to discourage collection of wild germplasm and take up alternate livelihood works, will reduce pressures in SBR. The tradition of growing the paddy crop only is a serious obstacle, which needs to be discouraged in order to infuse some ecologically sustainable and economically viable forms of agriculture. The practice of straying cattle into the fields and forests restricts the regeneration of growing of forest crops. The misconception behind not milking of cows in Kol tribes is that they consider it as sin and hence are deprived of livestock based income and energy. The wide spread use of intoxicating drinks are the main evils behind the civil crimes in Tribal pockets of Odisha. It also weakens the economic conditions of the households (Nayak, 2003). The ever-increasing human pressure on the forest in buffer and periphery requires more attention in terms of making innovative policies and programmes to benefit these communities.

Table 1.

List of plants of Simlipal Biosphere Reserve with local name, scientific name and common use.

Serial No	Local Name	Botanical name	Uses
1	Achchu	*Morinda coreia*	Medicinal plant
2	Agni Jahar	*Clausena excavata*	Medicinal plant
3	Amba	*Mangifera indica*	Multipurpose plant
4	Ambada	*Spondias mangifera*	Edible sour fruits
5	Amla	*Phyllanthus emblica*	Multipurpose plant
6	Aprajeta	*Clitoria ternatea*	Medicinal plant
7	Arjuna	*Terminalia arjuna*	Medicinal plant
8	Asan	*Terminalia alata*	Timber/fuel plant
9	Ashok	*Saraca asoca*	Medicinal plant
10	Ata	*Anaona squamosa*	Fruits are eaten
11	Athandi	*Combretum roxburghii*	Medicinal plant
12	Bagh-lucha	*Martynia annua*	Medicinal plant
13	Bana kapas	*Azanza lampas*	Medicinal plant
14	Bana kapasia	*Kydia calycina*	Produces fibre
15	Bana khira	*Xylia xylocarpa*	A medicinal plant
16	Banabasanga	*Wendlandia tinctoria*	Medicinal plant
17	Banahaldi	*Curcuma* sp.	Rhizome is eaten
18	Banamalli	*Barleria strigosa*	Medicinal plant
19	Baragad	*Ficus benghalensis*	Multipurpose plant
20	Barbhanga	*Cissus quadrangularis*	Medicinal plant
21	Barhakani	*Pterospermum acerifolium*	Medicinal plant
22	Beguni	*Vitex negundo*	Fuel plant
23	Bela	*Aegle marmelos*	Fruits are eaten
24	Bhada	*Terminalia bellirica*	Medicinal plant
25	Bhalia	*Semecarpus anacardium*	Therapeutic *Uses*
26	Bhuin champa	*Ochna obtusata*	Medicinal plant
27	Bhuin kadam	*Sphaeranthus indicus*	Medicinal plant
28	Bhuin neeba/ Kalmegh	*Andragraphis paniculata*	Medicinal plant
29	Bhurkunda	*Hymenodictyon orixense*	Medicinal plant
30	Bichhuati	*Mucuna pruriens*	Herbal drug
31	Borokoli	*Ziziphus jujuba*	Seeds are eaten

32	Brahami	*Bacopa monneiri*	Medicinal plant
33	Chai	*Piper retrofractum*	Medicinal plant
34	Champa	*Magnolia champaca*	Flowers are used
35	Champati	*Poyalthia cerasoides*	Pharmacological value
36	Chana grass	*Imperata cylindrica*	Used for thatching
37	Chara koli	*Buchanania cochinchinensis*	Seeds are eaten
38	Chattu	Macrofungi	Bulbs are eaten
39	Chhachina	*Alstonia scholaris*	Medicinal plant
40	Chimura, Anantamul	*Hemdesmus indicus*	Medicinal plant
41	Chirata	*Swertia chirata*	Medicinal plant
42	Chiuri	*Garcinia xanthochymus*	Ayurvedic medicine
43	Chunkuli	*Ziziphus rugosa*	Fruits are eaten
44	Dambaru	*Gardenia latifolia*	Medicinal plant
45	Deku sindur	*Buttneria herbacea*	Medicinal plant
46	Dhaw	*Anogeissus latifolia*	Ayurvedic medicine
47	Diba guda	*Elaeagnus latifolia*	Medicinal plant
48	Dimuri	*Ficus racemosa*	Fruits are eaten
49	Dom sal	*Miliusa velutina*	Ayurvedic medicine
50	Eksira	*Schrebera swietenioides*	Ayurvedic medicine
51	Gadri saga	*Alternanthera sessilis*	Leaves are eaten
52	Gaisira	*Asparagus racemosus*	Ayurvedic medicine
53	Gambhar	*Gmelina arborea*	Medicinal plant
54	Ganga siuli	*Nyctanthes arbortrists*	Medicinal plant
55	Ghurudu	*Gardenia gummifera*	Fruits are eaten
56	Gillri Phula	*Indigofera cassioides*	Flowers are used
57	Gilo	*Entada phaseoloides*	Ayurvedic medicine
58	Girdhini	*Sterculia urens*	produces fibre
59	Goosebery	*Phyllanthus emblica*	Medicinal plant
60	Guadhania	*Millettia extensa*	Folklore Medicine
61	Gudi Koim	*Mitragyna parvifolia*	Bark And Roots
62	Guhira	*Vachellia leucophloea*	Fruits are eaten
63	Haldu	*Haldina cordifolia*	Medicinal plant
64	Harada, kasaphal	*Terminalia chebula*	Medicinal plant
65	Hijala, Jinjala	*Barringtonia acutangula*	Medicinal plant
66	Hopo	*Cochlospermum religiosum*	Flosses used as kapok
67	Jada bindhi	*Ricinius communis*	Seeds provide oil
68	Jamurol	*Syzygium jambos*	Ayurvedic medicine
69	Jambira	*Citrus medica*	Fruits are eaten
70	Jamun	*Syzygium cumini*	Fruits are eaten
71	Jangli angura	*Cissus vitiginea*	Medicinal plant
72	Jarhi	*Ficus amplissima*	Medicinal plant
73	Jattiko	*Woodfordia fruticosa*	Ayurvedic medicine
74	Jautha	*Artocarpus lacucha*	Medicinal plant
75	Jhili Phula	*Shuteria involucrata*	Flowers are used

76	Jia	*Cipadessa baccifera*	Medicinal plant
77	Jia	*Lannea coromandelica*	Ayurvedic medicine
78	Kadam	*Neolamarckia cadamba*	Ayurvedic medicine
79	Kadiputtu saga	*Murraya koenigii*	Leaves are eaten
80	Kalchia	*Glochidion zeylanicum*	Medicinal plant
81	Kamalagundi	*Mallotus philippinensis*	Yields oil
82	Kanta alu	*Dioscorea glabra*	Rhizome is eaten
83	Kanta mali	*Ventilago denticulata*	Medicinal plant
84	Kanta parasi	*Embelia tsjeriam-cottam*	Medicinal plant
85	Karanj	*Pongamia pinnata*	Multipurpose plant
86	Karati	*Melastoma malabathricum*	Medicinal plant
87	Kari Durkuri	*Erycibe paniculata*	Medicinal plant
88	Karuna	*Psydrax dicoccos*	Medicinal plant
89	Kasa alu	*Dioscorea pubera*	Rhizome is eaten
90	Kasi	*Bridelia retusa*	Medicinal plant
91	Kath kusum	*Garuga pinnata*	Medicinal plant
92	Kedar Jhawar	*Cleome monophylla*	Medicinal plant
93	Kedar Patta	*Skimmia laureola*	Medicinal plant
94	Kendu	*Diospyros melanoxylon*	Multipurpose plant
95	Khejur	*Phoenix sylvestris*	Economic use
96	Koilari saga	*Bauhinia purpurea*	Leaves are eaten
97	Koorsana	*Celastrus paniculata*	Medicinal plant
98	Kuchila	*Strychnos nux-vomica*	Medicinal plant
99	Kuduchi	*Holarrhena pubescens*	Medicinal plant
100	Kukur gadia	*Dioscorea puber*	Rhizome is eaten
101	Kultha	*Grewia tilliaefolia*	Produces fiber
102	Kulthi	*Macrotyloma uniflorum*	leguminous plant
103	Kumbi	*Careya arborea*	Produces fiber
104	Kusum	*Schleichera oleosa*	Multipurpose plant
105	Lodha	*Symplocos racemosa*	Multipurpose plant
106	Macha ranka	*Pavetta indica*	Medicinal plant
107	Madan mast	*Hybanthus enneaspermus*	Medicinal plant
108	Magaki	*Ailanthus excelsa*	Multipurpose plant
109	Mahua	*Maduca longifolia* var. *latifolia*	Multipurpose plant
110	Makar kendu	*Diospyros malabarica*	Multipurpose plant
111	Mamuri saga	*Flacourita ramontchi*	Leaves are eaten
112	Mata saga	*Antidesma acidum*	Leaves are eaten
113	Moi	*Lannea coromandelica*	Antimicrobial
114	Munda noi	*Argyreia nervosa*	Medicinal plant
115	Mutri lata	*Smilax zeylanica*	Medicinal plant
116	Muturi	*Flemingia* sp.	Ayurvedic medicine
117	Nageshwar Phula	*Mesua ferrea*	Fruits are eaten
118	Neem	*Azadiracta indica*	Medicinal plant
119	Noi palasa	*Butea superba*	Ayurvedic medicine

120	Padashi	*Cleistanthus collinus*	Ayurvedic medicine
121	Pai jam	*Syzygium nervosum*	Fruits are eaten
122	Palasa	*Butea monosperma*	Medicinal plant
123	Palka Juti	*Rhinacanthus nasutus*	Medicinal plant
124	Palo	*Curcuma aromatica*	Fruits are eaten
125	Panaria gum	*Elaeocarpus stipularis*	Gum is eaten
126	Panasa	*Artocarpus heterophyllus*	Fruits are eaten
127	Pani alu	*Dioscorea oppositifolia*	Medicinal plant
128	Panjan	*Ougeinia oojeinensis*	Ayurveda herb
129	Paruli	*Stereospermum chelonoides*	Great medicinal value
130	Phir-phira	*Ichnocarpus frutescens*	Medicinal plant
131	Phutguri	*Streblus taxoides*	Medicinal plant
132	Piasal	*Pterocarpus marsupium*	Timber/fuel plant
133	Pipal	*Ficus religiosa*	Multipurpose plant
134	Pita alu	*Dioscorea bulbifera*	Rhizome is eaten
135	Pita saga, Panigamari	*Mallotus nudiflorus*	Leaves are eaten
136	Rai Phula	*Dillenia pentagyna*	Flowers are used
137	Raj jehula	*Elaeodendron glaucum*	Medicinal plant
138	Ram Dantani	*Smilax lanceifolia*	Medicinal plant
139	Rimili	*Protium serratum*	Medicinal plant
140	Rimiri	*Garuga pinnata*	Seeds are eaten
141	Rohini	*Soymida febrifuga*	Medicinal plant
142	Saijana saga	*Moringa oleifera*	Leaves are eaten
143	Sal	*Shorea robusta*	Multipurpose plant
144	Salai	*Boswellia serrata*	Provides gum
145	Sanchi kurchi	*Wrightia tomentosa*	Traditional medicine use
146	Sanka saga	*Rungia pectinata*	Leaves are eaten
147	Sarpagandha/Patalgaruda	*Rouvolfia serpentina*	Medicinal plant
148	Satawari	*Asparagus racemosus*	Medicinal plant
149	Setakata arak	*Cleome gynandra*	Medicinal plant
150	Siali	*Bauhinia vahlii*	Multipurpose plant
151	Sidha	*Legerstromia parviflora*	Multipurpose plant
152	Simili	*Bombax ceiba*	Multipurpose plant
153	Sinkari/pichrangi/mura	*Helicteres isora*	Medicinal plant
154	Sirish	*Albizia odoratissima*	Multipurpose plant
155	Sisoo	*Dalbergia latifolia*	Multipurpose plant
156	Sujuni	*Dalbergia paniculata*	Multipurpose plant
157	Sunari	*Cassia fistula*	Medicinal plant
158	Sunsuni saga	*Marsilea quadrifolia*	Leaves are eaten
159	Suturi	*Vigna radiata*	Leguminous plant
160	Tal	*Borassus flabellifer*	An Ayurvedic Herb
161	Tentuli	*Tamarindus indica*	Fruits are eaten
162	Toon	*Toona ciliata*	Multipurpose plant
163	Tunga alu	*Dioscorea belophylla*	Rhizome is eaten

Table 2.

Average daily collection (Kg/day) of Non Timber forests Products by villagers in Simlipal Biosphere Reserve

		PERIPHERAL ZONE	BUFFER ZONE		CORE ZONE	
S/n	Items		Period of collection	Av. daily collection	Period of collection	Av. daily collection
1	**Wild leafy vegetables**					
a	Sanka saga	NA	Jan.- March	0.60	Jan.-March	1.25
b	Gadri saga	NA	Jan.- March	0.10	Jan.- March	0.67
c	Pita/ Khatta saga	NA	Jan.- June	0.27	Jan.- June	1.00
d	Zinka saga	NA	Jan.-June	0.27	NA	
e	Koilari saga	NA	April- May	0.33	Oct.- Nov.	1.00
f	Saijana saga	NA	year Round	0.10	year Round	1.50
g	Mati/ Mata saga	NA	Jan.- June	0.17	January- June	1.00
h	Binda saga	NA	NA	0.00		1.50
i	Kitagini saga	NA	NA	0.00	Dec.- Feb.	1.00
j	Kankiri saga	NA	NA	0.00	April to May	1.33
k	Muri Saga	NA	NA	0.00	NA	0.50
l	Serali Saga	NA	NA	0.00	NA	0.17
m	Lendung Saga	NA	NA	0.00	NA	0.08
n	Leper Saga	NA	NA	0.00	NA	0.10
	Av. collection	NA	NA	0.13	NA	0.79
	Total collection	NA	NA	332	NA	68.00
2	**Wild seeds**					
a	Rimiri	NA	June	0.07	June	0.17
b	Siali	NA	February	0.23	February	0.33
c	Khandia	NA	December	0.07	NA	0.00
d	Jambiro	NA	December	0.07	NA	0.00
e	Zelleri	NA	year round	0.07	NA	0.00
f	Ghurdu	NA	February	0.17	NA	0.00
g	Kusum	NA	August	0.17	August	11.67
h	Kendu	NA	April	0.17	NA	0.00
i	Sal	NA	June	5.00	NA	0.00
j	Jamun	NA	August	0.17	August	0.67
k	Bela	NA	August	0.17	NA	0.00
	Av. collection	NA		0.58		1.17
	Total collection			1481.00		100.38
3	**Wild sap and Gums**					
a	Panaria	NA	March-April	1.00	NA	0.00
b	Simili gum	NA	March-April	1.00	NA	0.00
	Av. collection	NA		1.00		0.00
	Total collection	NA		2554.00		0.00

4	**Wild fruits**					
a	Chara	NA	June	0.20	NA	0.00
b	Chunkari	NA	June	0.20	NA	0.00
c	Ghuera	NA	June	0.20	NA	0.00
d	Rajara	NA	November	0.20	NA	0.00
e	Mahua	NA	March	1.00	NA	0.00
f	Anola	NA	February	1.00	February	0.33
g	Harada	NA		0.00		0.17
h	Bahada	NA		0.00		0.17
i	Mango	NA		0.00		8.33
j	Jackfruit	NA		0.00		18.61
k	Raiphal	NA		0.00		1.00
	Av.collection	NA		0.25		2.60
	Total collection			639.00		224.00
5	**Wild Tubers and Roots**					
a	Mundai alu	NA	April	0.50	NA	0.00
b	Pinkara	NA	December	0.20	NA	0.00
c	Rasa	NA	December	0.20	NA	0.00
d	Ladu	NA	December	0.20	NA	0.00
e	Pitaddu	NA	December	0.20	NA	0.00
f	Jungle/Mati alu	NA	Feb.- March	7.00	Feb.- March	3.33
g	Karu alu	NA	July	0.50	NA	0.00
h	Baitadu	NA		0.00		1.00
	Av. collection			1.10		0.54
	Total collection			2809.00		47.00
6	**Wild grasses, Flowers, Honey and Mushrooms**					
a	Channa ghasa		January	10.00	NA	0.00
b	Champa			0.00		0.08
c	Honey			0.00		0.77
d	Chattu			0.00		1.50
	Av.collection			2.50		0.59
	Total collection			6385.00		51.00

NA=Not available

Table- 3.

Food, fuel and kerosene consumption in core zone of Simlipal Biosphere Reserve

S/n	Parameters	Jenabil	Jamunagarh	Kabatghai	Bakua
1	Fuel wood (Quintals/ha/year)	398.05	737.68	3936.07	258.07
2	Rice (kg/ha/year)	7.51	37.37	45.29	8.07
3	Edible oil (kg/ha/year)	15.99	26.00	42.02	5.99
4	Kerosene (liters/ha/year)	36.00	122.00	195.00	31.00

Table- 4.

Food, fuel and kerosene consumption in buffer zone of Simlipal Biosphere Reserve

S/n	Parameters	Gudgudia	Barehipani	Nawana
1	Fuel wood (Quintals/ha/year)	1204.33	156.12	846.86
2	Rice (kg/ha/year)	13.48	4.28	10.39
3	Edible oil (kg/ha/year)	5.99	3.99	91.00
4	Kerosene (liters/ha/year)	47.00	26.00	42.00

Table 5.

Food, fuel and kerosene consumption in peripheral zone of Simlipal Biosphere Reserve

S/n	Parameters	Jamuani	Ghodabindha	Kadamsul	Lulung	Patiapada
1	Fuel wood (Quint./ha/yr)	303.74	519.12	580.93	593.88	214.53
2	Rice (kg/ha/year)	12.81	8.13	10.72	72.14	5.74
3	Edible oil (kg/ha/year)	19.99	5.99	1.99	5.00	3.00
4	Kerosene (liters/ha/year)	45.00	65.00	51.00	68.00	31.00

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Bioprospecting of fungal endophytes from plants of the family Zingiberaceae and their pharmaceutical applications

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ABSTRACT

Endophytic fungi are thought of as the hidden members of the microbial world that are widely recognised as a rich resource of potential chemical compounds with biological activities.The primary role of the bioactive substances produced by endophytes is to aid the host plants in withstanding abiotic and biotic stresses, which is ultimately beneficial to the survival of the host. Most of these endophytic fungi are from the Ascomycota, Oomycota, Zygomycota, and Basidiomycota groups.Research into endophytes has revealed that they have the potential to yield useful pharmaceuticals. Endophytic fungi are regarded as an under-utilized sourcethat hasn't been used enough to find new drugs and compounds. Endophytic fungi exhibit antibacterial, anticancer, immunomodulatory, antiviral, anti-diabetic, antioxidant, anti-inflammatory and other biological activities, which are being exploited for pharmaceutical application using biotechnological tools. The present review focuses on the biopharmaceutical potential of endophytic fungi from an important group of medicinalplant belonging to the family Zingiberaceace.

1. Introduction

Endophytes are described as polyphyletic groups of microorganisms which may be actinomycetes, bacteria or fungi (Kusari *et al*., 2012). Endophytic fungi (EF) are a group of fascinating host-associated fungal communities that thrive asymptomatically the internal tissues of healthy plants without causing any diseases to the host (Amirita *et al*., 2012). They colonise the host either as obligate associates or as facultative associates with lower and higher plants (Deshmukh *et al*., 2006). Endophytes communicate information to higher plants and have evolved biochemical pathways that produce novel bioactive substances. Therefore, they offer chances for discovering products and processes with medical and biotechnological applications (Bielecka *et al*., 2022). In recent years, accumulated research on endophytic fungi has revealed their wide-ranging ecological distribution, biodiversity and multidimensional interactions with host plants and other microbiomes in the symbiotic continuum (Alam *et al*., 2021).

It is believed that there are about one million different species of endophytic fungi that can be found in nature (Faeth and Fagan, 2002). According to Schultz, the endophytic fungi are mainly classified into three main ecological groups: (a) pasture or balansicaeousendophytic fungi; and (b) non pasture endophytic fungi; (c) mycorrhizal endophytic fungi (Faeth and Fagan, 2002).

Their affiliation can be compulsory or discretionary and does not, thus, cause direct damage to its host. Endophytes have dynamic relationship with its host plants, which include mutual relationship and antagonistic relationship**.** Plants primarily restrict endophyte growth and the endophytes undergo various mechanisms to steadily adapt into the living environment (Dudeja *et al*., 2012). In order to maintain a sustainable symbiotic association, a range of compounds are produced by the endophytes such as gibberellins, indoleacetic acid, alkaloids, isocoumarin derivatives, quinones, chlorinated metabolites, flavonoids, terpenoids, phenol and phenolic acids which facilitates the

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growth of the host plant and help them better survive in their environment. The presence of endophytes encourages plant nutrient absorption and thus contribute towards host plant's enhanced growth. Endophytes are considered as plant growth promoters by producing various broad range of phytohormones like auxins, gibberellins, IAA, cytokines etc. Besides plant growth, endophytes are known to induce growth in terms of increase in plant height, shoot diameter and shoot weight (Yates *et al*., 1997). Apart from plant growth factors, endophytes generate pharmaceutical agents which have biotechnological potential, like anti-tumor drugs (taxol), anti-fungal drugs (quercine), and various industrially important enzymes (Ting *et al*., 2008; Cao *et al*., 2004). Different enzymes are produced by endophytes include protease, amylase, pectinase, L-asparginase, chitinase, laccase, etc. Endophytes living inside medicinal or crop plants have confirmed the presence of most enzymes (Ayob *et al*., 2016).

During the past two decades, the endophytes are being explored for novel bioactive compounds. Various compounds generated via biosynthetic pathways belong to broad classes of compounds like phenols, tetralones, enniatins, steroids, isocoumarins. Endophytic organisms are actually a reservoir for the discovery of new compounds for development in medicinal and agricultural industries by producing various antibiotics, antioxidants, anti-parasitic and anti-cancer drugs. At present, main focus of exploring endophytes are the potential of these microorganisms in developing secondary metabolites. Since endophytic microbes may synthesis or even mimic the same substance produced by their host plants, bioprospecting of these microorganisms opens up avenues of discovering novel molecules (Venieraki *et al*., 2017).

Notable species like ginger and turmeric belonging to family Zingiberaceae has long been recognized as a potential cure for a variety of conditions, including cramps, digestive issues, arthritis, fever, cough, and cold (Ma, 2012). Zingiberaceae plants have been extensively researched in the scientific literature because of their antimicrobial, anti-apoptosis, anti-tumor, and anti-inflammatory and other biological activities, which can be attributed to the presence of several phyto-compounds in them (Karuppiah, 2012). Anti-microbial resistance (AMR) is a global health challenge on medicinal view point and there is a need for discovery of new antimicrobial compounds from natural sources. In this context, exploitation of fungal endophytes to synthesize novel antibiotics, especially the modified or somewhat similar with Zingiberaceae compounds has great prospect in medical application. The initial step for study about the biopotential of endophytic fungi is the isolation of some antagonistic fungal strains from non-symptomaticand healthy plant parts. Several researchers have successfully isolated and reported many fungal endophytes and evaluated their antibacterial potential. Antagonistic endophytic *Penicillium* sp. has been isolated from *Curcuma longa*, which exhibits inhibitory activity against human pathogenic bacteria such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Rathod, 2013). The results of antagonistic activity of fungal strains towards examined pathogens were unique, demonstrating that fungal strains have varying capabilities in generating antimicrobial chemicals. Chemical compounds made by growing *Pestalotiopsis vaccinii* were thought to be new natural products made only by the strain itself (Wang, 2014; Wang, 2017). Research on application of fungal endophytes from the Zingiberaceae family is still in its infancy. However, earlier studies have revealed endophytic fungi (EFs) as new sources of bioactive chemicals with anticarcinogenic substances (Uzma *et al*., 2018). Therefore, the study of distribution, diversity, and biochemical properties of endophytes is a very important, which will help us to understand and improve plant fitness (Yuan *et al*., 2019). The present review provides a thorough explanation of the biological features of EFs from plants belonging to Zingiberaceae family, including their diversity, distribution, function along with pharmaceutical applications. Besides, the review also highlights the promising therapeutic uses and exhaustive pharmacological information of the species of Zingiberaceae.

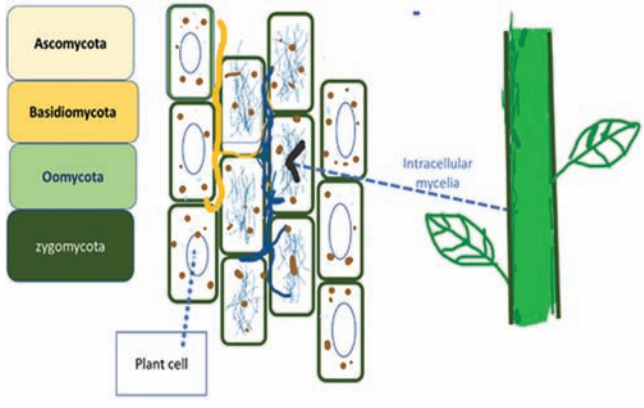

Fig. 1: Classification of endophytic fungi and their occurrence with in plant cell

2. Species and phytochemical diversity of family Zingiberaceae

The monocotyledonous family Zingiberaceae are comprised of a large number of medicinal, aromatic and ornamental plants, which are widely distributed throughout

the tropical and sub-tropical regions of the world (Ghosh *et al*., 2013) with greater species concentration in Southeast Asia (Jantan *et al*., 2003). The family is comprised of over 1200 species under 53 genera (Kress *et al*., 2002). As many as 178 species under 22 genera are reported from North Eastern and peninsular region of India (Jain and Prakash, 1995).The members of Zingiberaceae are both perennial or annual rhizomatous herbs. These aromatic ethnomedicinally important plants are characterised by the presence of volatile oils and oleoresins. The important members under the family Zingiberaceae having medicinaluses belongs to the genus *Amomum, Curcuma, Alpinia Elettaria, Hedychium, Kaempferia* and *Zingiber* (Prabhu *et al.*, 2010). In general, the rhizomes and fruits belong to this group of plant are aromatic and are used as tonic and stimulant. These multipurpose plant family are the rich sources of natural products and chemicals thatare already being utilized as spices, medicines, dyes, perfume and food.

India, traditionally known as 'the spice bowl of the world', is the largest producer, consumer and exporter of ginger. Ginger has rich cultivar and genetic diversity in India, and the cultivars are generally named after localities of occurrence or cultivation. More than 50 cultivars possessing varying yield and quality parameters are grown in India. Kerala holds maximum diversity of ginger followed by Himachal Pradesh and Assam (Ravindran *et al*., 2005). Disease-resistant and high-yielding ginger hybrids are the results of crop improvement programmes and Athira, Rajatha, Mahima, Suprabha, Suruchi, Suravi, Himagiri, Karthika, Varada are some popular varieties developed in India.

Endophytic fungi live in rhizomes of plants of ginger family and the metabolites that these fungi make may have an effect on the bioactivity of ginger extracts and the quality of the spice. These metabolites are not removed by washing, so they may also have an effect on human health. These metabolites are produced against host-specific phytopathogens and may demonstrate antibacterial, antifungal, cytotoxic, anticancer, antioxidant, and anti-inflammatory activities.

Though fungi are capable of colonising intercellular or intracellular regions of host plant, they are more likely to do so at the plant's roots than at its leaves or stems. Apoplastic fluid from the host is the primary nutrition supply for colonisation in aerial organs, allowing for healthy reproduction of EFs (Schulz *et al*., 2002). During an infection, fungi form three distinct types of interactions with their hosts: communalistic (non-beneficial/virulent endophytes), mutualistic (beneficial endophytes) and pathogenic (virulent pathogens). The nature of these interactions differs depending on the physiological status of the host plants or the specific conditions that they are exposed to. *Alpinia, Amomum, Curcuma, Elettaria, Hedychium, Kaempferia* and *Zingiber* are the interesting genera of Zingiberaceae which contain aromatic spice plants with very high medicinal values (Joy *et al*., 1998). Among them, few species are cultivated but majority of the species occur in the wild. Each of the above-mentioned genera are briefly discussed below.

2.1. Genus Alpinia

Alpinia Roxb. is the most widespread and largest genus belonging to subfamily Alpinioideae under the Zingiberaceae with about 230 species. Members of this genus are mainly distributed in India, Indonesia, Arabic gulf areas, Egypt, Malaysia, Japan, southeast Asia, the Pacific, Samoa, the Caroline Islands, Australia and northern New South Wales, China and Sri Lanka (Larsen *et al*., 1998; Smith, 1990). Most of the species grow in open and sunny places, low- to mid-elevation forests and brushwood. In India, the species of these genus are found in the Himalaya and Southern region of Western Ghats (Khare *et al*., 2007).These are perennial herbs with oblong-lanceolate leaves, tuberous root, greenish white flowers. (Gupta *et al*., 2010). While the rhizomes are source of essential oils (Jirawan *et al*., 2005), the young shoots and flowers are used as a spice (Arambewela *et al*., 2006) and condiments. In Asian countries, *Alpinia* species are used in cooking (*A. galanga*) and medicinal purposes (e.g., *A. officinarum* Hance). The plant is mostly used in the traditional system of medicine viz. Ayurveda, Unani, Chinese and Thai folk medicines (Yang *et al*., 1999). The active phytochemical constituents of these plants support medicinal and pharmacological properties.

2.2. Genus Curcuma

Curcuma is a genus with some 120 species occurring from Indochina, Malaysia, India, northern Australia and Thailand (Leong, 2007; Larsen, 1996; Maknoi, 2006). It is a genus of perennialherbs whose rhizomes are fleshy, aromatic and usually varies from light brown to different shades of yellow (Maknoi, 2007). India is the top producer of *Curcuma* rhizomes followed by Southeast Asia, Thailand, Central and Latin America, and Taiwan.

Many of its members have medicinal uses such as treating digestive disorders including dyspepsia and colic due to its anti-inflammatory, anti-viral, anti-bacterial, anti-fungal, and anti-oxidant properties. The anti-cancerous activity from extract of *Curcuma longa* is under trials. *Curcuma* is of great economic importance, with *Curcuma longa* as the top notched in the genus along with *C. amada*, *C. angustifoli*a and *C. zedoria, C. caesia* are common species.

2.3. Genus Zingiber

The genus *Zingiber* is comprised of about 85 species of herbs mostly grown in Asia, Africa, central andsouth America (Sabulal *et al*., 2006). These plantae prefer moist, tropical conditions (Jayashree *et al*., 2015). Phytochemical investigation of the rhizomes of several *Zingiber* species has revealed that they possess various pharmacological and physiological effects due to the presence of bioactive compounds such as gingerols, shogaols, diarylheptanoids, phenylbutenoids, flavanoids, diterpenoids and sesquiterpenoids (Sivasothy *et al*., 2011). In addition, shogaols, dehydrated gingerol derivatives, are the predominant pungent constituents in dried ginger (Jiang *et al*., 2006).These plants are used as common ingredients in traditional medicines. The rhizomes have been used successfully to treat a variety of medical conditions, including gastrointestinal distress, motion sickness, vomiting, epilepsy, bronchitis, common cold, bruises, wounds, liver complaints, rheumatism, muscular pains, atherosclerosis, migraine headaches, high cholesterol, ulcers, and stomach discomfort (Shukla *et al*., 2007). Phenolic compounds in ginger root, especially gingerols, have been shown to have chemopreventive effects linked to their antioxidant and anti-inflammatory properties (Shukla *et al*., 2007).

2.4. Genus Hedychium

The genus *Hedychium* has 87 species, which have worldwide distribution (Plant list, 2010). *Hedychium spicatum*, found from Himachal Pradesh to Arunachal Pradesh, is useful in the treatment of liver complaints, fevers, vomiting, diarrhoea, inflammation, pains and snake bite.The rhizomes of *H. spicatum* are reported to harbour twenty-eight fungal strains and about 84% of endophytic fungi isolated belonged to the phylum Ascomycota. Endophytic fungal genera such as *Fusarium* and *Penicillium* were found to be common to all the plant parts. *Hansfordia biophila* is an endophytic fungus isolated from *Hedychium acuminatum* that produces tannin (Hastuti *et al*., 2018).

2.5. Genus Elettaria

The genus *Elettaria* is represented by few species of perennial herbs with rhizomes.They grow in India, Sri Lanka, Malaysia, and Indonesia (Wills, 1967). In India, the species are indigenous to the most southern tropical evergreen forests of the Western Ghats of India (Ravindran, 2002). Among them, Cardamom (*Elettaria cardamomum*), also known as Indian cardamom or small cardamom, has been used worldwide for culinary purposes and the older forms of medicine. Essential oils and other high-value antioxidant and gastroprotective bioactive metabolites from its fruits are primarily responsible for its unique aroma and function as a functional food as well as a nutraceutical and medicine (Hamzaa and Osman, 2012).

2.6. Genus Amomum

Amomum, another genus of Zingiberaceae, is represented by over 150 species, mostly in Asia and Oceania (Cai *et al*., 2021). They are perennial herbs with distichous leaves and elongating pseudo-stems (Syazana, 2018). Volatile oils, as one of the essential components of the genus *Amomum*, have been extensively studied. Till date, more than 160 non-volatile compounds have been isolated from this genus, including flavonoids, terpenoids, diarylheptanoids, *etc*. In China, India, Thailand and Nepal, 16 species of the genus *Amomum* are utilised in traditional medicine. Recent exploratory research on the pharmacological qualities of the *Amomum* species revealed their efficacy as anti-inflammatory agents (Jin *et al*., 2016), cure for stomach diseases (Kumar *et al*., 2014), cancer (Zhang *et al*., 2015), hepatopathy diseases (Kim *et al*., 2015) and malaria (Heilmann *et al*., 2001).

3. How endophytic fungi colonise within host plants

It is well established that endophytic fungus colonisation is not a random event and happens because of chemotaxis, or specialised molecules produced by the host plant. At the same time, different types of secondary metabolites like saponin and essential oils from medicinal plants, are made as a way to fight off pathogens which acted as barriers against the colonisation of endophytic fungi. To circumvent this, the endophytic fungi must secrete the corresponding detoxifying enzymes such as cellulases, lactases, xylanases and proteases for the degradation of these secondary compounds before they reach the defence systems of the host-plants. Once within the tissues of a host-plant, endophytic fungi assume a quiescent (latent) state, either for the entire lifetime of the host plant (neutralism) or for an extended period of time (mutualism or antagonism), until environmental conditions are favourable for endophytic fungi or the ontogenetic state of the host changes to the fungi's advantage (Sieber, 2007).

Different interactions have been developed between endophytic fungi and their host plants through a distinct fungus-host interaction during the lengthy period of co-existence and evolutionary processes: (i) a continuum of mutualism, (ii) antagonism, and (iii) neutralism. The genetic background, nutrient level, and ecological habitats of medicinal host plants are thought to be pressure-choice factors on the population structure of endophytic fungi, which in turn confer some kinds of benefits, such as induced growth, increased resistance to disease, and/or herbivores,

as well as accumulated bioactive components (Firakova *et al*., 2007). For this reason, the mutual relationship between endophytic fungi and their host plants might impose specific effects on the formation of particular types of bioactive substances which have been explored for human benefits. To the best of our knowledge, there has been very little research on the commercialization of fungal endophytes isolated from Zingiberaceous plants.

4. Diversity of endophytes associated with the plants of the family Zingiberaceae

Endophytic fungi associated with different species of Zingiberaceae have been isolated from meristems, leaves, roots, stems, rhizomes by different workers. Generally, they are isolated by surface sterilization followed by culturing them from either crushed tissue extract or culturing through plant tissues on suitable media (Hata *et al*., 2008). Members of family Zingiberaceae have been explored for a diverse group of endophytic fungi. Eleven fungal strains have isolated from *Curcuma longa* plant by Septiana (2017). About 33 fungal isolates have been isolated from six species of Zingiberaceae viz. *Zingiber officinale, Curcuma domestica, Kaempferia rotunda, Curcuma xanthorrhiza, Curcuma mangga* and *Curcuma zedoaria* (Praptiwi *et al*., 2016).

Members of 10 fungal genera have been reported from *Alpinia officinarum* by Shubin *et al*. (2014). Endophytic fungi such as *Trichoderma* sp., *Mycelia sterilia, Penicillium* sp., *Alternaria* sp., *Penicillium* sp., *Fusarium* sp., *Aspergillus* sp., *Bipolaris* sp. and *Nigrospora* sp. have been isolated from *Hedychium coronarium* by Uzma *et al*. (2016). Similarly, species of *Colletotrichum, Fusarium, Bipolaris, Pithomyces, Mucor, Alternaria, Mycelia, Rhizopus, Cladosporium* are reported from *Hedychium flavescens* (Uzma *et al*., 2016). Bussaban *et al*. (2001) reported occurrence of *Eupenicillium crustaceum, Fusarium* spp., *Glomerella* spp., *Phomopsis* spp., *Phyllosticta* spp., *Pyricularia* spp., *Talaromyces flavus*, taxa of Xylariaceae, *Mycelia sterilia* from *Amomum siamense.*

5. Isolation of endophytic fungi from species of Zingiberaceae

Surface sterilization is the most needed technique which is used for the isolation of endophytic fungi from ginger family. Surface sterilized by immersing in 70% ethanol for 1 min. Then rinse the samples three times with sterile distilled water and soak them again in hypochlorite solution (NaOCl) 0.5% for 5 min and again rinse them in sterile water for six times. Allow the samples to become surface-dry by putting them on sterilized filter paper for 12 hours and it should be carried out in biosafety cabinet (Hallmann *et al*., 2006). Place all the sterilized segments of each plant part on PDA containing chloramphenicol antibiotic (0.5 g/l) or streptomycin to inhibit the bacterial growth. Seal the Petri dishes with parafilm to prevent desiccation of the medium and incubate them in dark at 28 °C for 7-21 days. Continuously monitor the fungal growth. When the fungi grow out from the plant organ, then cut the hyphal tip arising from the fungal colony and transfer them to fresh PDA to enhance the normal sporulation for better identification. Every precaution should be practiced while performing the endophytic fungal isolation, as there are many chances of existence of epiphytic fungi during isolation.

Fungal identification was accomplished by observing their morphological characteristics. Besides, PCR and DNA sequencing are novel techniques employed to identify endophytic fungus. Identification of fungal endophytes can best be accomplished by amplification of Internal Transcribed Spacer (ITS) regions, which are repeating units of DNA encoding ribosomal RNA.

6. Biopharmaceutical potential of endophytic fungi from Zingiberaceae

Endophytic fungi are regarded as rich sources of numerous bioactive secondary metabolites and phytohormones that promote plant growth and enable plants to withstand biotic or abiotic stresses (Tan and Zou, 2001; Chadha *et al*., 2014). Apart from the benefits to the host plant, the endophytes have also proved as an outstanding source of SMs and bioactive antimicrobial products**.** Endophytic fungi can biosynthesize some of the most important commercially exploited SMs, including antibiotics, anticarcinogenic, cytotoxins, insecticides, and allelopathic compounds (Schneider *et al*., 2008).They have a lot of commercial potential in the pharmaceutical, medical, agricultural, nutraceutical, cosmetic, flavour, and fragrance industries. This makes EFs an interesting topic for endophytism research (Hyde and Soytong, 2008).The potential of endophytic microorganisms as untapped resources for innovative drugs has aroused a great deal of attention (Strobel, 2002). The ginger species are well-studied for their biological properties such as anti-microbial, anti-oxidant, anti-tumor, antihyperglycemic, anti-hyperuricemic, anti-inflammatory, anti-larvae, anti-aging and functional properties as a topical medicine attributed to presence of alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins, and terpenoids (Juwita *et al*., 2018).

Hypomontagnella monticulosa (Zg 15SU) isolated from *Zingiber griffithii* showed potent cytotoxic activity (Lutfia *et al*., 2021). Bioactive compounds such as a-cyclocitral, cembrene A, laurenan-2-one, sclareol, 2Z, 6E farnesol, cembrene, isocomene and α-curcumene obtained

from endophytic fungi *Arthrinium* sp. (MFLUCC16-1053) account for their antimicrobial andantioxidant activities, which were isolated from *Zingiber cassumunar* (Pansanit *et al*., 2018). *Zingiber zerumbet* rhizomes harbour *Fusarium solani* and *F. oxysporum* which has antipythium activity (Keerthi *et al*., 2016). Similarly, the phenolic compounds produced by the fungal endophyte *Bipolaris specifera, Alternaria tenuissima, Aspergillus terreus, Nectria haematococca* and *Fusarium chlamydosporum* from *Zingiber nimmonii* have potential antioxidant properties (Das *et al*., 2017). *Eurotium* sp. isolated from *Curcuma longa* produces asparginase that has anticancer as well as antimicrobial properties (Jalgaonwala *et al*., 2010; Jalgaonwala *et al*., 2014). The antimicrobial and oxidant activity of 33 fungal isolates obtained from *Zingiber officinale, Curcuma domestica, Kaempferia rotunda, Curcuma xanthorrhiza, Curcuma mangga* and *Curcuma zedoaria* have been reported (Praptiwi *et al*., 2016). MM2 compound isolated from endophytic fungi *Aspergillus terreus* (RTN3) from young stems of *Alpinia chinesis* has shown potent anticancer activity and inhibitory effects on *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (Vo *et al*., 2020). The ability of endophytic fungus *Trichoderma afroharzianum* isolated from *Hedychium coronarium* in producing chitinase has been reported (Munir *et al*., 2019). The major bioactive compounds synthesized from endophytic fungi and their bioactivities are summarized below in Table 1.

Although the pharmacological activities of Zingiberaceae species have been extensively described, not much is known about bioprospecting of their endophytic microorganisms. Endophytic mycoflora are regarded as an exceptional pharmaceutical bioresource as they produce a wide range of substances known as secondary metabolites (Strobel, 2003). As bioactive compounds produced by endophytic community are believed to be regulated by the positive effect of plants, these plants with medicinal characteristics are regarded as a significant resource for studying fungal endophytes (Venkateswarulu *et al*., 2018). Endophytes have been found to be good sources of biologically active products that are interesting for certain health care uses (Strobel *et al*., 2001; Wiyakrutta *et al*., 2004). Apart from the benefits to the host plant, the endophytes have also proved as an outstanding source of secondary metabolites and bioactive antimicrobial products. Although there are many different endophytic fungi of documented pharmaceutical benefits isolated from family Zingiberaceae, we have complied a list of some of the most important ones in Table 1.

Table 1.

List of endophytic fungi isolated from plants of family Zingiberaceae showing their pharmaceutical and industrial activities.

Sl. No.	Host plant	Endophytic fungi	Chemical compound / Enzyme	Pharmaceutical activities / Industrial application	References
1	*Zingiber griffithii*	*Hypomontagnella monticulosa* Zg15SU	Sesterpenoid	Anticancer	Lutfia *et al*., 2021
2	*Zingiber nimmonii*	*Bipolarisspecifera, Alternaria tenuissima, Aspergillus terreus, Nectria haematococca* and *Fusarium chlamydosporum*	Phenolic compounds	Antioxidative	Das *et al*., 2017
3	*Zingiber cassumunar*	*Arthrinium* sp. MFLUCC16-1053	Bcyclocitral, 3Ecembrene A, laurenan-2- one, sclareol, 2Z, 6Efarnesol, cembrene, βisocomene and γcurcumene	Antibacterial and antioxidant activities	Pansanit *et al*., 2018

Sl. No.	Host plant	Endophytic fungi	Chemical compound / Enzyme	Pharmaceutical activities / Industrial application	References
4	*Zingiber zerumbet*	*Fusarium solani* and *F. oxysporum*		Antipythium activity	Keerthi *et al*., 2016
5	*Curcuma longa*	*Eurotium* sp.	Asparginase	Anticancer	Jalgaonwala *et al*., 2014
6	*Curcuma longa*	Fungal isolates	-	Antibaterial and antifungal	Jalgaonwala *et al*., 2010
7	*Alpinia calcarata*	*Cylindrocephalum* sp.	Amylase	-	Sunitha *et al*., 2012
8	*Elettaria* sp.	*T. harzianum* and *T. atroviride*	Antifungal enzyme	Antifungal	Munir *et al.*, 2019
9	*Alpinia chinesis*	*Aspergillus terreus*	MM1	Anticancer	Vo *et al*., 2020
10	*Alpinia chinesis*	*Aspergillus terreus*	MM1	Antimicrobial against *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA)	Vo *et al*., 2020
11	*Elettaria* sp.	*Trichoderma harzianum*	-	Antagonistic activity	Munir *et al*., 2019
12	*Elettaria* sp.	*Trichoderma atroviride*	IAA production	-	Munir *et al*., 2021
13	*Hedychium coronarium*	*Trichoderma afroharzianum*	Chitinase	-	Munir *et al*., 2019
14	*Hedychium coronarium*	*Trichoderma afroharzianum*	-	Fusarium oxysporum	Munir *et al*., 2019
15	*Curcuma longa*	*Arthrobotrys foliicola, Cochliobolus kusano, Daldinia eschscholz, Fusarium oxysporum, Fusarium proliferatum, Fusarium solani, Fusarium verticillioides, Phanerochaetechrys osporium, Phaeosphaeria ammo philae*		Inhibit growth of thehistamineproducing bacteria	Septiana *et al*., 2017
16	*Zingiber officinale*	*Curvularia affinis*	-	Antimicrobial against *Fusarium oxysporum*	Ginting *et al*., 2013
17	*Zingiber officinale, Curcuma domestica, Kaempferia rotunda, Curcuma xanthorrhiza, Curcuma mangga* and *Curcuma zedoaria*	33fungal isolates	-	Anti-oxidant and antimicrobial	Praptiwi *et al*., 2016
18	*Hedychium coronarium*	Endophytic fungal strain	*Staphylococcus aureus*	Antimicrobial	Lutfia *et al*., 2018

7. Conclusion and future prospective

The current study shows that endophytic fungal metabolites are important targets for finding and making new drugs. Currently, the quest for novel biologically active metabolites from plant endophytes has broadened in light of the changing perspectives on medicinal substances and the rising demand for non-hazardous medications. We are losing the battle against therapeutic antimicrobial drugs due to development of resistance over the period of time. Endophytes, on the other hand, are a viable alternative since they contain an abundance of unique bioactive chemicals with virtually limitless potential biological activities. Numerous studies have reported novel, beneficial bioactive compounds exhibiting other biological properties, such as anti-diabetic, anti-inflammatory, antiprotozoal, anti-tuberculosis, insecticidal, immune-modulatory, antiviral, anticancer activities, anthelmintic, etc., that were successfully isolated from endophytic fungi. Since the turn of the century, endophytic fungi have been the subject of intense study in the pharmaceutical industry due to their abundance and pervasive distribution. Although bioactive chemicals and the usage of certain medicinal plants in traditional medicine have been extensively explored, little is known about the bioactivities of its associated endophytic fungus. Despite this, limited research has been conducted on the valuable bioactive compounds from endophytic fungi. Thus, the screening of endophytic mycoflora for the possible SMs synthesis can help in the establishment of their pharmaceutical functions for sustainable human health and effective against antibiotic resistance.

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Yield variability of Sub1 introgressed late-duration rice varieties of Odisha as evidenced by OJIP transient analysis

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ABSTRACT

In current climate changing environment, selection of suitable rice genotype is very important for sustainable rice productivity. Genetic diversity of seven late-duration rice (*Oryza sativa* L.) varieties (Swarna, Swarna Sub1, TDK, TDK Sub1, Bahadur, Bahadur Sub1 and a check variety Mrunalini) of Odisha were analyzed for yield attributing traits and photosystem II activity related to yield.100-grain weight was significantly high in TDK (2.95 g) with low grain number per panicle and TDK Sub1 (2.89 g) with significantly high fertile grain number. Significantly high harvest Index was recorded in TDK Sub1 with second highest plot yield of 49.39 q ha^{-1} which was 1.26-fold more than check variety Mrunalini. However, the highest yield was obtained in TDK (1.33-fold) followed by Swarna (1.14-fold) as compared to Mrunalini. Sub1 gene introgression yielded better in Bahadur *Sub1* which was not found in Swarna Sub1 and TDK Sub1. A significant positive correlation was noticed between 50% days to flowering *vs.* grain yield per plant (r = 0.96) followed by panicle number (r = 0.94). Plot yield was highly correlated with panicle length, panicle number, fertile grain percentage, flag leaf length, flag leaf area and grain yield per plant. Significant alteration of chlorophyll *a* florescent activity was noticed among the studied genotypes. O-J-I-P analysis revealed variable fluorescence intensity at 'J' and 'I' transient point was significantly higher in TDK and TDK Sub1 as compared to check variety Mrunalini which corroborate with highest yield among the studied varieties.The comparatively low productivity of Swarna and Swarna Sub1 might be due to low fluorescent intensity at 'J' and 'I' transient point as compared to check variety. Total performance index on absorption basis was fund significantly high in Swarna and Swarna Sub1 as compared to TDK and TDK Sub1 with higher yield performance. Cluster analysis confirmed close relation of Bahadur and Bahadur Sub1 as well as Swarna and Swarna Sub1. TDK and TDK Sub1 found distantly related as evident by PCA analysis which confirmed a high amount of genetic alteration due to Sub1 introgression in this variety with high grain number and high plot yield. The identified genetically distinct genotype TDK and TDK Sub1 could be selected for breeding partner with high photosynthetic activity, high grain weight with more grain number per panicle following conventional rice breeding program.

1. Introduction

Rice is used as primary food for most of the Indians besides the Asiatic populations of the world (Nahar *et al*., 2018). Most of the mid-early rice varieties are low yielder and cultivated in rainfed environment having less drought tolerant characters (Panda *et al*., 2022). Yield-limiting stress for rainfed rice ecosystems recorded significant yield loss that produce ~ 42 M ha in Asia (Hu *et al*., 2019). Frequent cyclone and flash flood caused havoc due to the growing global climate change specifically in Odisha (Donde *et al*., 2019; Ansari, 2021). So, the estimation of photosynthetic efficiency of rice in the current climatic condition is very important for more quality rice production and to evolve better breeding strategy under the growing population growth of the country. The response of genotypic efficiency against varied climatic conditions stimuli different physiological processes and mainly photosynthetic efficiency of different genotypes and prompts a number of physiological parameters in plants, that help to cope with

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various environmental conditions (Sahoo *et al*., 2019). A large number of rice landraces compatible with varied agro-ecological conditions have been evolved time to time and a number of submergence introgressed varieties of different duration of maturity of rice have been developed in India which are being used against different climatic conditions as Megha variety (Das, 2018). To check the yield potential of rice varieties of late duration Sub1 introgressed varieties of rice grown in Odisha is very much essential to explore the genetic variability with regard to photosynthetic activity. Several attempts have been made to synthesize heterogeneous populations of rice taking into account different yield attributes to segregate the bulk population of rice (Das, 2018). Thus, a comparative analysis of the efficiency of Photosystem-II has been measured with yield parameters to correlate the genotypic effect on photosynthesis for higher yield and greater physiological activity among the late duration varieties of rice.

Chlorophyll molecules capture light energy during photochemical reactions of photosynthesis and the excess energy is dissipated as heat (Non-Photochemical Quenching) or emitted as chlorophyll *a* fluorescence (Maxwell *et al*., 2000). Increasing photosynthesis leads to a decrease in extra energy dissipation. Thus, the efficiency of the photochemical reactions and the degree of heat dissipation is estimated by measuring the yield of chlorophyll *a* fluorescence which induces OJIP transient (Stirbet and Govindjee, 2011; Tsai *et al*., 2019). OJIP transient analysis in submergence tolerance rice was reported earlier in some varieties of Odisha Panda and Sarkar (2012). The Photosystem II (PSII) activity in form of chlorophyll *a* fluorescence could be used as a screening technique to distinguish high yielding genotypes with distinct useful yield attributing traits for parental selection in rice breeding programme. However, the study on chlorophyll *a* fluorescence in different late-duration of rice of Odisha has not been reported earlier. We report a comparative study of PSII activity of different late-duration rice varieties of Odisha by the measuring chlorophyll *a* fluorescence activity to know its contributiontowards the yield potential of different varieties of rice along with their respective Sub1 introgressed genotypes to develop future parental selection strategies in conventional rice breeding plans.

2. Materials and methods

2.1 Plant materials

Six late-duration varieties (Swarna, Swarna Sub1, TDK, TDK Sub1, Bahadur, Bahadur Sub1) rice varieties along with a check variety (Mrunalini) were tested in field in Kharif season for the year 2020-21 in the experimental firm of Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India. Rice varieties were planted in a randomized block design with a spacing of 15 × 15 cm and 20 cm between plant to plantand in between row respectively. Farm Yard Manure was applied at the rate of 4.0 tones ac^{-1} in the field before 4 to 6 weeks of seed sowing and 10 kg of $ZnSO_4$ ac^{-1} was applied during the last puddling stage. NPK (0.5:0.5:0.5) kg^{-1} 100 sq. m was applied for robust seedling growth and NPK of 100:60:60 was applied during 30 days after transplantation, at active tillering stage, and at panicle initiation stage in the field. Morphological characters of vegetative and reproductive stages of rice plants were collected as per Table 1. Data of all parameters are the mean of two plots (replicas) for all rice varieties.

2.2 Yield attributes

Randomly three plants in each replication were selected for all the morphological characters of each rice variety. Days to 50% flowering and days to maturity were recorded for each variety during one season. The various agronomic characters considered for this study have been presented in Table 1. Detail procedure for measurement of agronomic traits of rice were taken as per our earlier paper (Das *et al*., 2021).

2.3 Chlorophyll a fluorescence analysis

Parameters related to Chlorophyll *a* fluorescence were measured by a Multifunction Plant Efficiency Analyzer (M – PEA) (Hansatech Instruments Ltd, UK) following the method of Strasser *et al*. (2010) standard method. The leaf samples were illuminated with continuous red light after a dark adaptation of 20 min from the middle part of a leaf blade using leaf clips. The Chl *a* fluorescence transient shows the initial (F_0) to the maximal (Fm) fluorescence value, which has been recorded at O-step (20 μs), J-step (2 ms), I-step (30 ms), and P-step (0.5–1 s) (Šimiæ *et al*., 2014). The transients in leaves were induced by red light (peak at 650 nm) of 3,000 ìmol (photon) m^{-2} s^{-1} provided by an array of light-emitting diodes with a light pulse exposure time of 1 sec. Photosynthetic data were collected for 118 points in 1 sec for each leaf sample. The fluorescence signal was recorded with a maximum frequency of 105 points s^{-1} (each 10 μs) within 0 to 0.3 ms, after which the frequency of recording gradually decreased collecting a total of 118 points within 1s. PSII behavior was determined on the basis of parameters recorded and derived from the OJIP transients based on Energy Fluxes in chloroplast membranes. The chlorophyll *a* fluorescence parameters and equations of the OJIP-test were given in Table 1 (Tsimilli-Michael and Strasser, 2008).

2.4 Statistical analysis

All the represented mean data are collected from replicas of three samples. Analysis of variance as well as Duncan's Multiple Range Test were made for all mean morphological and biochemical data (Sokal and Rohlf, 1995). UPGMA (Unweighted Pair Group Method with Arithmetic Mean) is a simple agglomerative (bottom-up) hierarchical clustering method. The similarities of presence or absence of the different agronomic characters were calculated in each variety from the mean values of all the agronomic parameters.

3. Results

3.1 Variation in agronomic characters

The agronomical characters of seven studied rice varieties have been given in Table 1. Days to 50% flowering varied from 98 in Bahadur Sub1 to 111 in Bahadur. The range of mean plant height varied from 86.65 cm in Swarna to 111.5 cm in Bahadur. The panicle length was minimum in 21.42 cm in Mrunalini to 26.29 cm in TDK Sub1 whereas panical number ranged from 8 in Bahadur Sub1 to ~12 in Swarna and Swarna Sub1. The fertile grain number was recorded ~89 per panicle in TDK to ~111 per panicle in Mrunalini. Fertility percentage showed a range from ~62% in Swarna to ~89% in TDK Sub1. 100-grain weight significantly varied among the varieties with a prominent difference between Bahadur and Bahadur Sub1 (Table 1). Plot yield found highest in TDK (52.65 qt ha^{-1}) to lowest in Bahadur (32.58 qt ha^{-1}). The Sub1 introgressed varieties showed lower plot yield as compared to its normal varieties (Table 1).

Table-1

Mean performance of seven late-duration genotypes of rice with respect to yield attributing characters*

Variety	DF	PH	PL	PN	FGN	F%	100-GW	HI	FLL	FLA	GYP	PY
Swarna	100.5b	86.65e	22.83b	12.34a	108.5d	61.94d	1.91a	36.0b	22.08d	18.86d	13.6a	44.77c
Swarna Sub1	103b	86.9e	21.75b	12.34a	109.5d	71.22c	1.91a	40.0a	19.08d	15.22e	16.5a	41.44d
TDK	101b	93.65d	23.5b	7.83b	89.5e	82.68b	2.95a	35.0b	26.17c	24.7c	11.61b	52.65a
TDK Sub1	99.5c	99.6c	26.92a	7.66b	134c	88.82a	2.89a	41.0a	29.83b	29.76b	15.15a	49.39b
Bahadur	111a	111.5a	26.67a	8.5b	169a	82.46b	2.01a	34.0b	24.33c	30.78b	14.21a	32.58f
Bahadur Sub1	98c	108.55a	25.08a	8b	149.5b	81.81b	1.97a	35.0b	27.9b	30.22b	13.88a	36.36e
Mrunalini	108a	105.5b	21.42b	9.5a	111.5d	68.86c	2.1a	33.0b	33.08a	35.23a	12.97b	39.3d
Mean	103	98.90	24.02	9.45	124.5	76.82	2.24	36.0	26.06	26.39	12.97	39.3
Stand. dev	4.76	10.14	2.24	2.06	27.59	9.58	0.46	0.03	4.73	7.16	13.98	42.35
Std. error	1.80	3.83	0.84	0.77	10.43	3.62	0.17	0.01	1.78	2.70	1.55	7.10
Coeff. var	4.63	10.25	9.33	21.82	22.16	12.47	20.61	8.37	18.15	27.13	0.58	2.68

DF = Days to 50% flowering, PH = Plant height (cm), PL = Panicle length (cm), FGN = Fertile grain number per panicle, F% = Fertility percentage, 100-GW = 100 grain weight (g), HI = Harvest index, FLL = Flag leaf length (cm), FLA = Flag leaf area (cm^2), GYP = grain yield per panicle, PY = Plot yield (qt ha^{-1}).

*Data pooled from a total of 3 replicas each of two plots. Mean values (within column) followed by different alphabets (superscript) are significantly different ($p < 0.05$; Duncan's Multiple Range Test [DMRT]).

Correlation coefficient analysis among different attributes showed very interesting results. Days of 50% flowering found significant correlation with panicle length, panicle number, fertile grain number, fertility percentage, 100-grain weight, flag leaf length, and grain yield per plant (Table 2). Plant height was found high correlation with harvest index. 100-grain weight, and grain yield per plant. Plot yield and harvest index found very high significant correlation with panicle length (r=0.82 & r=0.70), panicle number (r = 0.86 &r = 0.67), fertility % (r = 0.78 & r = 0.63). Fertility % found significant correlation with harvest index (r = 0.63), grain yield per plant (r = 0.99) and plot yield (r = 0.78). Flag leaf length (r = 0.93), flag leaf area (r = 0.50), and grain per plant (r = 0.56) showed significant correlation with plot yield.

Table-2

Correlation coefficient values among different yield attributing characters of late-duration varieties of rice.

	PH	PL	PN	FGN	F%	100-GW	HI	FLL	FLA	GYP	PY
DF	0.34	0.91	0.94	0.50	0.78	0.50	0.28	0.89	0.45	0.96	0.19
PH		0.22	0.08	0.05	0.25	0.90	0.24	0.14	0.01	0.70	0.14
PL			0.10	0.05	0.03	0.45	0.70	0.72	0.39	0.80	0.82
PN				0.40	0.01	0.13	0.67	0.10	0.05	0.37	0.86
FGN					0.31	0.50	0.82	0.83	0.29	0.48	0.06
F%						0.12	0.63	0.48	0.33	0.99	0.78
100-GW							0.55	0.39	0.68	0.44	0.03
HI								0.47	0.25	0.06	0.33
FLL									0.01	0.32	0.93
FLA										0.40	0.50
GYP											0.56

3.2 Cluster analysis of morphological attributes and PCA analysis

Cluster analysis, based on the morphological attributes, revealed that, Bahadur, Bahadur Sub1 and TDK Sub1 formed a single group forming Cluster-I where Bahadur formed a out group and Swarna, Swarna Sub1, TDK along with check variety Mrunalini formed Cluster-II where Mrunalini remained out group (Fig. 1). However, all the varieties formed a single tree with genetically more distantly related varieties forming Cluster-II than Cluster-I having less genetic variability. The same type of result was also found in Principal Component Analysis (PCA) as evident in Figure 2. TDK Sub1 and TDK although found in Cluster-I and Cluster-II respectively, but they found in different quadrant. Swarna and Swarna Sub1 found genetically more closer as compared to Bahadur Sub1 and Bahadur. The most distant relation was found between TDK and TDK Sub1 where Mrunalini shared a same quadrant with TDK.

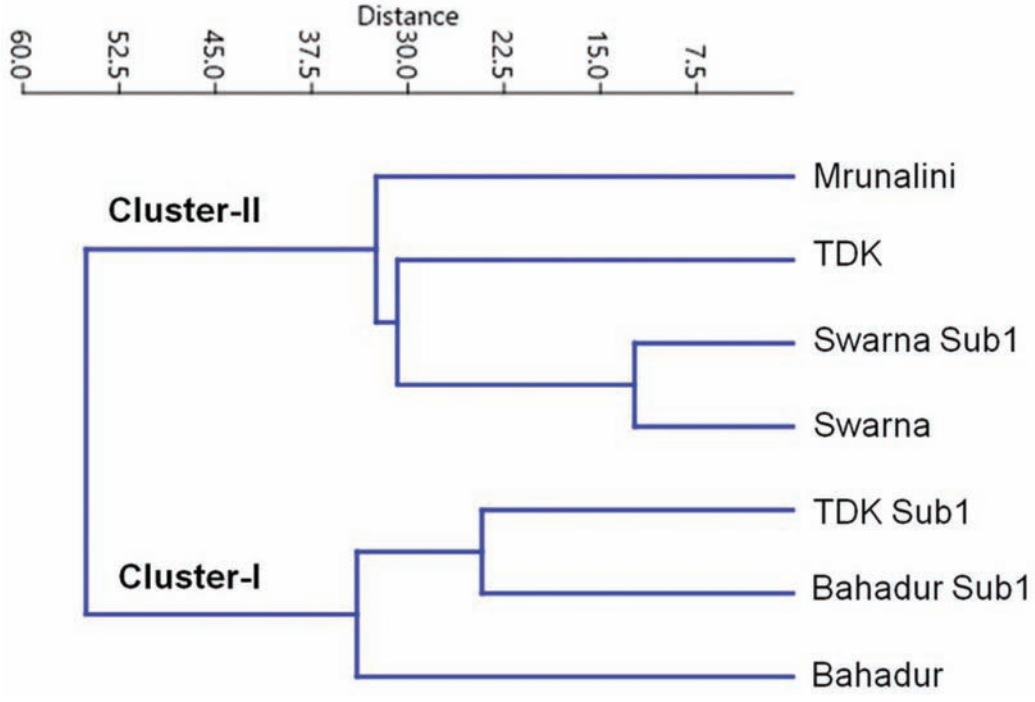

Fig. 1: Cluster analysis showing genetic relationships among different late-duration varieties of rice based on yield attributing characters.

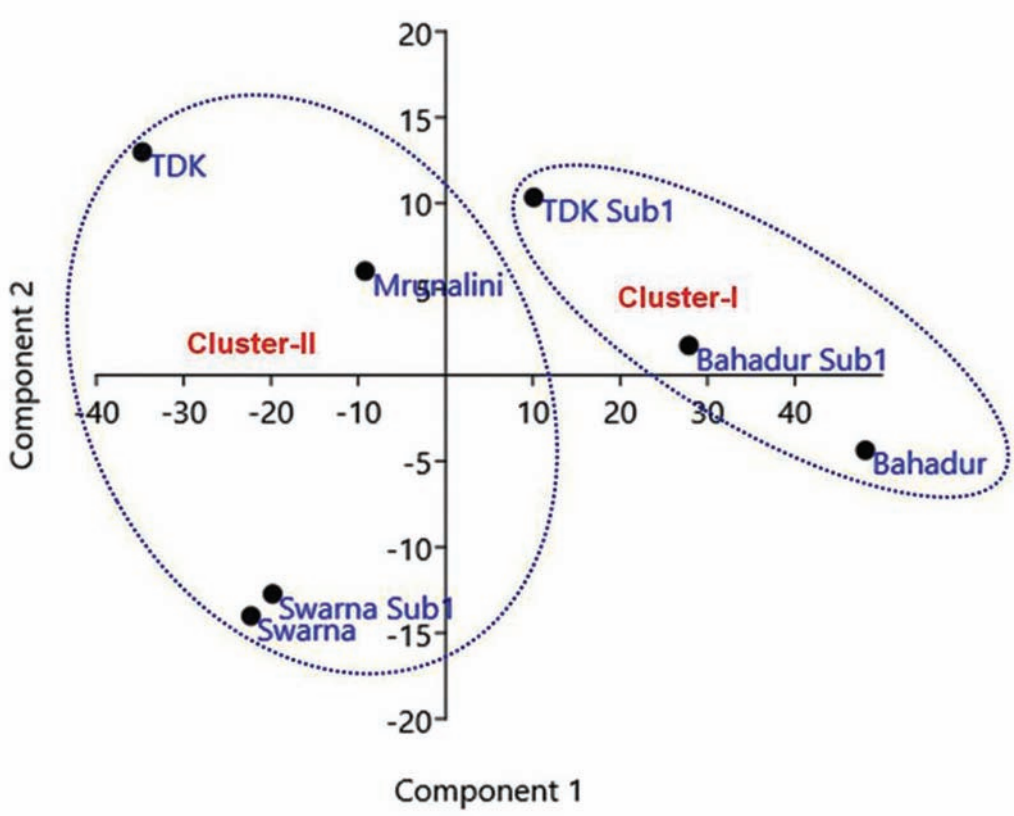

Fig. 2: Principle component analysis showing genetic relationships on the basis of phenotypic variability among the seven varieties of rice.

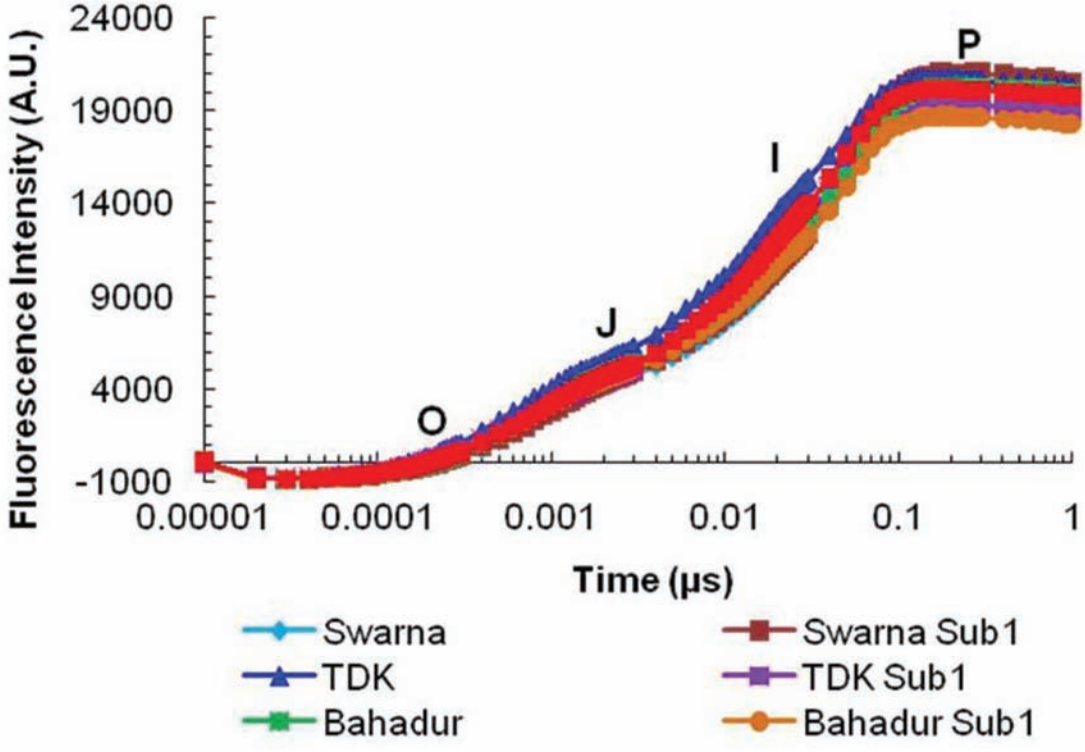

Fig. 3: OJIP graphs of PSII chlorophyll *a* fluorescence of flag leaf of seven late-duration varieties of rice.

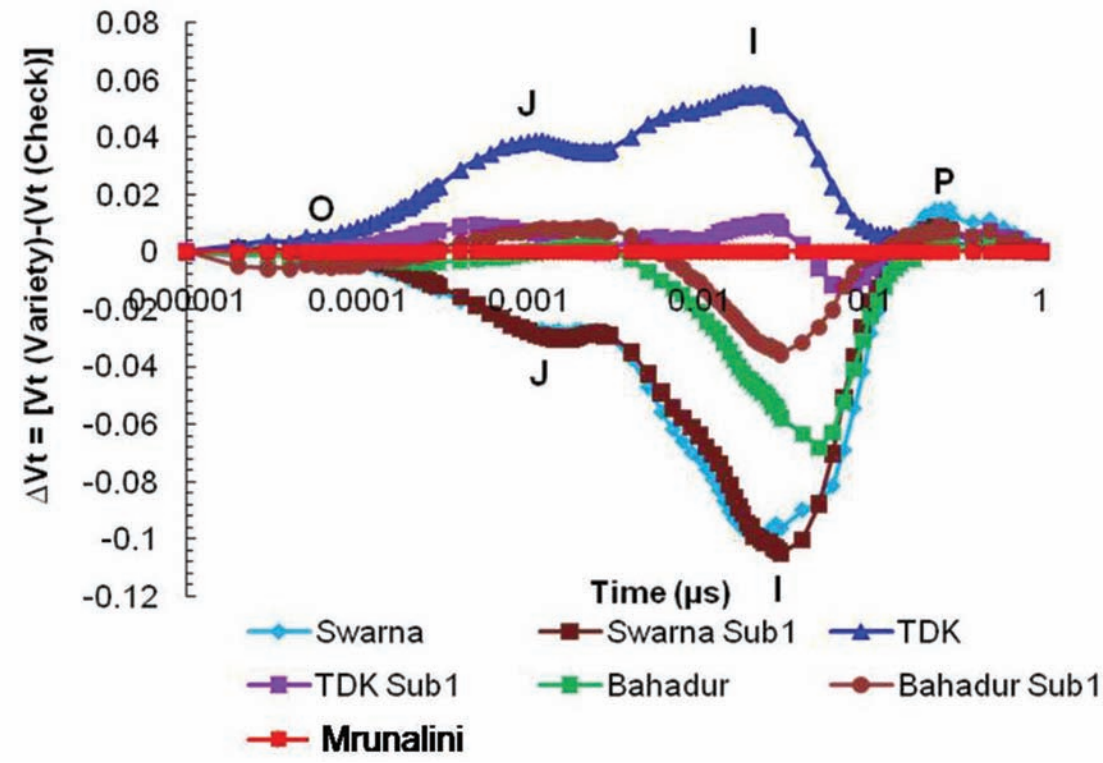

Fig. 4: Changes in prompt fluorescence (PF) curves in seven late-duration varieties of rice. PF curves plotted on a logarithmic time scale from 20 µs to 1 s (JIP time). The steps O (at 20 µs), J (at 2 ms), I (at 30 ms), and P (peak) are marked. Each curve is the average of 3 replicate measurements. Variable fluorescence curves (ÄV = Ä[(Ft—Fo)/(Fm—Fo)]), which were constructed by subtracting the normalized (between the O step and P step) values of the PF recorded in check variety (Mrunalini).

3.3 OJIP analysis

From the OJIP graph, it was observed that there are variable significant differences of different steps of OJIP curves among different varieties (Fig. 3). Chl *a* fluorescence transient shows normal OJIP curves in all the studied seven varieties of rice (Fig. 3). Comparatively high PS-II fluorescence activity was found in Swarna Sub1 followed by Swarna in 'P' transient point. TDK and TDK Sub1 showed significantly lower value at 'I' transient. The higher values of energy flow was noted in 'J' point in Swanra Sub1 and Swarna followed by Bahadur, Bahadur Sub1, TDK Sub1 showed at per with check variety Mrunalini. TDK showed remarkable low energy flow at 'J' transient point as compared to check variety (Fig. 4). Among the studied varieties, plot yield was significantly high in Sub1 introgressed Bahadur (36.36 qt ha^{-1}) as compared to its normal (32.58 qt ha^{-1}) which was significantly lower than check variety (39.30 qt ha^{-1}). In contrary, rest of the varieties showed higher yield in respective varieties without Sub1 introgression.

3.4 Chlorophyll fluorescence studies

Quantum yield (at t=0) of energy dissipation i.e.Fo/Fm could not show much significant variation (Fig. 5). Relative variable fluorescence at J step (Vj) was highest in TDK and lowest in Swarna Sub1 as compared to check. Relative fluorescence in and I step (Vi) also significantly varied among the varieties. The same type of result was also found in ABS/RC (Absorption per Reaction Centre), Dissipation per Active Reaction Centre (DI_0/RC), Trapping per Active Centre (TR_0/RC). Electron Transport per Active Reaction Centre (ET_0/RC) found lowest in Bahadur and highest in TDK. The flux of electrons transferred from QA to final PSI acceptors per active PSII (RE_0/RC) was maximum in Swarna, Swarna Sub1 followed by Bahadur Sub1, Bahadur. No variation was noticed in the φ(Po) whereas probability of trapped excitation which was used in electron transport beyond QA i.e.$^{\Psi}(E_0)$ and the maximum quantum yield for primary photochemistry (at t=0) i.e. Quantum yield of the electron transport beyond QA Õ(E_0) recorded TDK while highest noted in Swarna Sub1. Quantum yield for reduction of end electron acceptors at the PSI acceptor side (φR_0) showed a maximum in Swarna and Swarna Sub1. The electron transfer efficiencies to PSI acceptor (ΔR_0) was found similar trend (Fig. 5). The performance indices, Absorption Basis (PIabs) found lowest in TDK and highest in Swarna and Swarna Sub1.

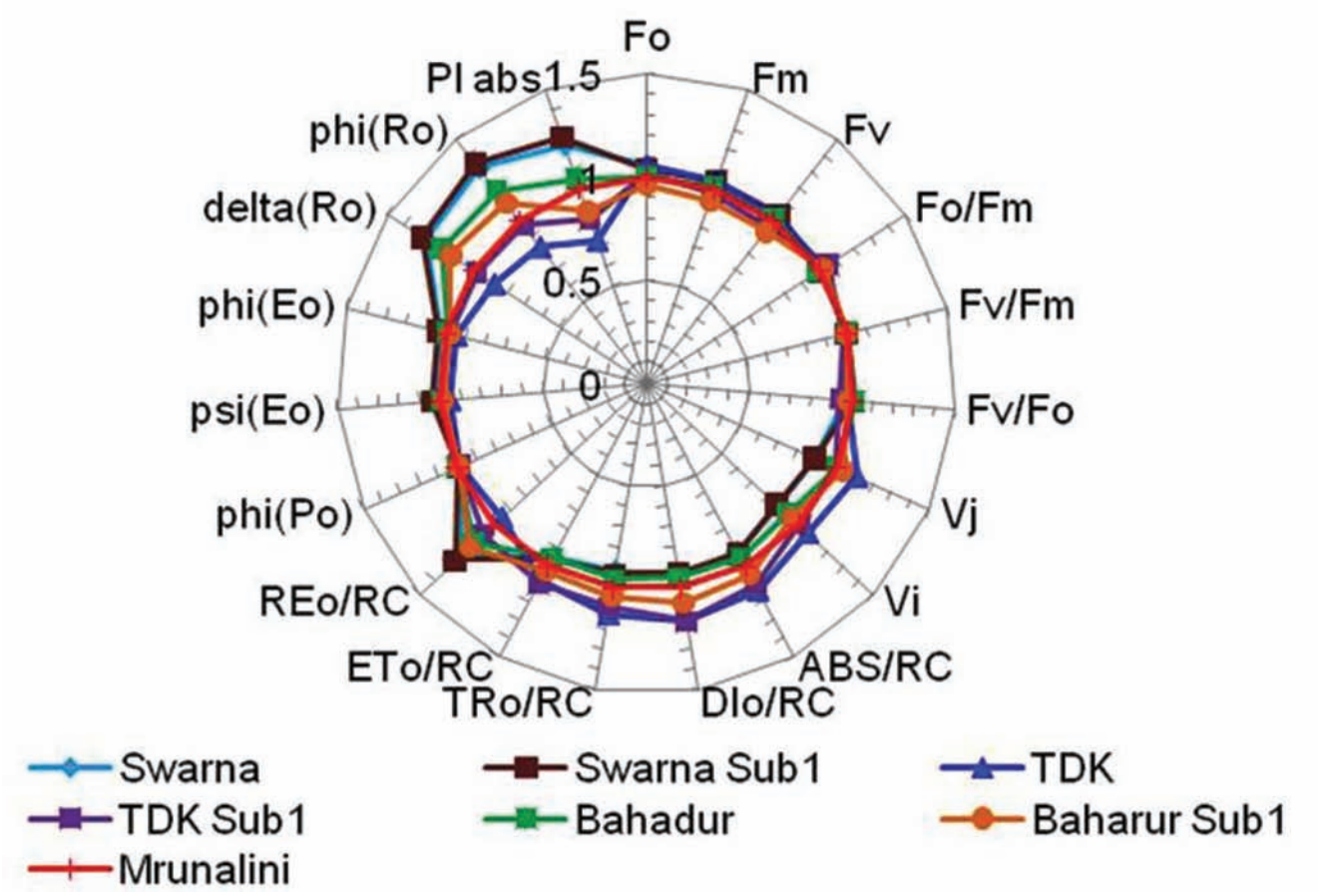

Fig. 5: Comparative analysis of radar graphs of different OJIP transient parameters in seven late-duration seven varieties of rice.

4. Discussion

4.1 Genetic analysis of yield attributing characters

The variations of morphological characters of late duration rice was significant.The grain yield and plot yield properties of rice were influenced directly by some of the characters while some other characters are indirectly responsible for the yield. The coefficient of variation (CV) is an important tool to analyze relative variability of genotype parameters for the biological experiment is <10% (Acquaah 2012). Most of the traits like days to 50% flowering, plant height, panicle length, harvest index showed CV < 10%. Such type variations were observed in different varieties of rice which is according to our result (Yaqoob *et al*., 2012; Ikmal *et al*., 2019). The CV values were ranged from ~4.63% to ~20.6% in other morphological characters. Thus, high CV in different characters among the varieties might be due to background variation of genotypes having Sub1 gene which have been established as normal varieties with distinct morphological characters during long cultural practices.The variability as observed in plant height as recorded from 86.65 cm in Swarna to 111.50 in Bahadur might be due to genetically controlled ethylene-responsive transcription factors beside other physiological factors. SNORKEL1 (SK1) and SNORKEL2 (SK2) gene activation due to low oxygen level during submergence (Colmer and Voesenek, 2009; Bailey-Serres *et al*., 2010) may not be active in the case of Sub1 introgressed varieties in normal environmental conditions. Thus, the varieties with the Sub1 gene showed their efficiency in normal condition which might be the alteration of accessory position effect of genes which affect plant growth and grain yield and stable during selection procedure.

It is interesting to note that flag leaf length and flag leaf area played a big role among the late-duration varieties. The highest yield was found in TDK (52.65 qt ha^{-1}) followed by TDK Sub1 (49.39 qt ha^{-1}) showed more flag leaf length and area. The second highest plot yield recorded in Swarna

(44.77 qt ha^{-1}) and Swarna Sub1 (41.44 qt ha^{-1}) showed less leaf length and leaf area as compared to TDK varieties of Cambodia origin. The lowest plot yield was recorded in Bahadur and Bahadur Sub1 (Table 1) with higher leaf area as compared to Swarna as well as check variety Mrunalini (39.30 qt ha^{-1}). The variation of leaf area is thus, an important characteristic for higher light-harvesting capacity with the generation of more photosynthates towards the yield attributes which can be considered for breeding purpose. Although, ethylene mediated activation of α-amylase and activation of GA and ABA on growth in rice with the activation of genes like CIPK15, MYBS1, and SnRK1 in low oxygen conditions might be cause of genetic control of leaf growth (Nghi *et al*., 2019; Pucciariello, 2020). Thus, the length of rice plant and leaf length is the genetically controlled mechanism and all the Sub1 introgressed varieties which might have not expressed that gene in the normal non-submergence conditions in this experiment.

The morphological variation was found significant among different characters as depicted in Duncan multiple range test. A significant correlation was observed between fertile grain number with days to 50% flowering. Plant height showed a significant positive correlation with flag leaf length, area and plot yield. Fertile grain number showed a significant correlation with grain yield per plan which had a correlation with plot yield. The morphological variation of rice agronomic characters was also reported earlier by us (Dikshit *et al*., 2013, Das *et al*., 2021). The varieties with long grain having good morphological attributes could be used for crop improvement through conventional breeding approach. Although, considerable crop genetic diversity continues to be maintained in farms in traditional varieties (Jarvis *et al*., 2008).

Variation of panicle length was observed in Bahadur (25.08 cm) with highest fertile grain number of 169 per panicle. However, lowest fertile grain number (89.5 per panicle) having maximum plot yield of 52.65 qt ha^{-1} might be due to highest 100-grain weight in TDK which could be a very good agronomic character to choose a breeding partner in rice improvement programme. The introgression of Sub1 gene in TDK although increase fertile grain number significantly without much variation of 100-grain weight also failed to perform equal to TDK in non-submergence condition. The productive tiller producing genotypes could produce higher grain yield which was in accordance to our findings as reported earlier in other varieties of rice (Dutta *et al*., 2002). The positive correlation of number of filled grains panicle with yield could be a critical in yield improvement program of rice (Samonte *et al*., 1998; Mahto *et al*., 2003). Although panicle development and immergenceare important additional factors to have the greater yield in rice (Hori *et al*., 2012).Thus, the improvement of primary branch number could be made by transferring this genetic trait from a stabilized potential donor. The fertile grain number showed significant variation among the studied varieties. All these yield attributing characters are directly related to grain yield and plot yield which need to be carefully observed and an integrated approach of considering all these yield characters needs to be addressed in parent selection of rice during breeding. However, SUS (starch synthesizing enzymes sucrose synthase) and AGPase (ADP Glucose Pyrophosphorylase) activation in endosperm which was found to be blocked with high ethylene synthesis upon activation of Sub1 gene in Swarna Sub1 in submerged condition (Kuanar *et al.*, 2019) causing low starch and carbohydrate accumulation in grains in the developing spikelets. This is similar to the higher accumulation of sugars in inferior spikelets of rice panicles as reported by Naik and Mohapatra, (2000) in addition to the activities of SUS and AGPase in grain-filling of rice (Tang *et al*., 2009; Zhu *et al*., 2011) which are responsible for starch synthesis *vis a vis* sink strength of the grain (Panda *et al*., 2015). In addition, the not used sugar during starch conversion accumulate in caryopsis (Patel and Mohapatra, 1996). These are the limiting factors for production of high quality grains in poorly-developed spikelets in rice.

4.2 Cluster analysis on the basis of morphological attributes

All the varieties formed two clusters *i.e.* Cluster-I and II having Bahadur, Bahadur Sub1, TDK Sub1 in one cluster and rest are in another cluster. The genetic closeness was recorded in Bahabur and Swarna along with their Sub1 introgressed varieties. The check variety Mrunalini formed an out group and a substantial genetic variability was recorded between TDK and TDK Sub1. The use of more genotypes could yield better genetic relations among the studied rice varieties. Principal component analysis (PCA) is used in exploratory data analysis and for making predictive models. PCA data also confirmed Distinct genetic distance between TDK and TDK Sub1 which might have happened due to the ingrogression of Sub1 gene. A similar type of study in morphological and submergence-related traits in different land rice supports our result (Kumar *et al*., 2016). However, cluster formation and grouping of genotypes are also greatly influenced by environmental and soil factors in the landraces of indigenous rice (Wangpan *et al*., 2018). PCA analysis for different attributes interactions found useful for intensive selection procedures could be used for the improvement of submergence tolerance rice.

4.3 Photosynthetic activity and chlorophyll a fluorescence analysis

High PS-II fluorescence activity was found in TDK, TDK Sub1, Swarna and Swarna Sub1 among the late-duration varieties.The lowest was observed at 'P' transient point in Bahadur Sub1. TDK found the highest PSII activity having more plot yield (52.65 qt ha^{-1}). The variations in the 'J' transient point were no significantly noticeable in TDK Sub1, Bahadur, Bahadur, Sub1 as compared to check variety Mrunalini. Significant high 'J' transient and 'I' transient was noticed in TDK with higher productivity. Fv/Fm ratio found no significant variation among the studied varieties which is the maximum quantum yield of primary PSII photochemical reactions, that is mainly used to state the primary health as reported in various plant (Kalaji *et al*., 2017;Stirbet*et al.*, 2018). The presence of stress signature resulting due to either inactivation of PSII or inhibition of quenching might be the causes of low Fv/Fm (Long *et al*., 1994).

Relative variable fluorescence at 'J' step (Vj) and 'I' step (Vi) high in TDK and TDK Sub1. ABS/RC, DI_0/RC, TR_0/RC also showed the same type of result. ET_0/RC was more in TDK which was quite similar with the flux of electrons transferred from QA to final PSI acceptors per active PSII (RE_0/RC). The lowest $^{\Psi}(E_0)$ was recorded in TDK and TDK Sub1 *i.e*. the quantum yield of the electron transport beyond QA. However, quantum yield for reduction of PSI acceptor side (φR_0) showed highest in Swarna and Swarna Sub1 among the studied varieties which were also noted the inefficiency of electron transfer to final PSI acceptors.The performance indices reflected in PIabs for although showedhighest in Swarna and Swarna Sub1, followed by Bahadur, as compared to Mrunalini and lowest in TDK followed by Swarna Sub1, Bahadur Sub1 which might be related to some other factors which are genetically controlled. Radar Plot of different OJIP parameters showed significant variation in RE_0/RC, $\Delta R0$, φR_0 which was reflected in PIabs. Thus, comparatively high plot yielded TDK and TDK Sub1 with distinct genetic background could be used as breeding partner to improve grain yield per panicle and panicle number in other rice variety through conventional breeding methods. However, delayed senescence of flag leaf and sty green character could ensure prolonged photosynthesis without significant yield improvementexcept better grain filling. Furthermore, survival of genotypes with Sub1 gene during flooding by triggering stem elongation and conserving carbohydrates (nonstructural) for fast regeneration (Bailey-Serres *et al*., 2010). Although, semi-dwarf genotypes were reported to perform better growth over Sub1-introgressed varieties (Kuanar *et al*., 2019) with less growth and delayed flowering and maturity as evident in our study.

5. Conclusion

The genotypic variations of late-duration varieties of indica rice were noticeably variety-specific with divergent clusters on the basis of measured agro-morphological traits for selection of a breeding partner in future crop improvement programs in rice. Selection of the traits based on plant height, leaf area, number of spikelets per panicle, leaf area, grain length, breadth, 100-grain weight in the promising TDK and TDK Sub1 could be used as genetic resources for rice breeding programme. Clustering patterns and PCA could also suggest the above varieties for breeders about the suitability of different yield attributes of rice for future accomplishments. Genotype specific OJIP parameters confirms better photosynthetic activity of high yield varieties like TDK, TDK Sub1, Swarna, Swarna Sub1. The yield attributes can be supplemented with a large number of PSII activity-related parameters like photosynthetic efficiencies, electron transfer from PSII to PSI acceptor for selection of better yield attributing rice varieties for rice breeding purpose.

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Isolation and characterization of Zinc solubilizing bacteria from Bhitarkanika mangrove forest of Odisha, India

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ABSTRACT

Zinc is a crucial element for optimal plant growth. Some bacteria that solubilize zinc are potential substitutes for zinc supplements because they transform applied inorganic zinc into usable forms. As zinc cannot be produced by the plants by themselves, bacteria play important role in growth increasing of plants by providing zinc. This investigation was done to examine the potential of zinc-solubilizing bacteria isolated from mangrove forests of Odisha. In this study, seven zinc solubilizing bacteria (ZSB) were isolated, of which ZSB2 was found to have maximum zinc solubilizing efficiency of 111.51% on solid medium and solubilizes 6.55 mg/L of Zn in liquid medium. The bacterium was identified as *Enterobacter kobei* by 16S r-RNA sequencing. Along with zinc solubilization, some ZSB isolates showed positive response towards siderophore production. The study bears significance for application in plant growth promotion.

1. Introduction

Zinc is an essential micronutrient required in small but critical amount for optimum plant growth. It acts as a cofactor for many enzymes, thus have a key role for plant growth, development and defense mechanism. Plants can absorb zinc as a divalent cation, but the amount of soluble zinc in soil solution is relatively less (Kabata and Pendias, 2001). Zinc is mainly present as ore, minerals and insoluble compounds in the soil (Alloway, 2008). Due to unavailability of zinc in soil, many plants show zinc deficiency, one of the most common micronutrient deficiencies. There are many techniques that have been used for a long time to treat zinc deficiency. Zinc fertilizers have been used in the form of zinc sulphate (White and Broadly, 2005) or Zn-EDTA (Karak *et al.*, 2005), but their use affect the economy and environment, and these fertilizers change into insoluble complex forms within 7 days of application (Rattan and Shukla, 1991).

Zinc-solubilizing bacteria can serve as an alternative to zinc supplements by converting applied inorganic zinc into useable forms. They solubilize zinc by various mechanisms such as acidification and chelation. The microbes secrete organic acids to the soil which sequester the zinc cations by lowering the pH of the soil (Alexander, 1997). Further the anions can also chelate zinc to enhance its solubility via zinc chloride, zinc phosphate, zinc carbonate etc. (Jones and Darrah, 1994). Other mechanisms include oxido-reduction reactions on cell membrane with chelated ligands (Chang *et al.*, 2005), production of protons or siderophores (Saravanan *et al.*, 2011). *Azotobacter, Azospirillum, Bacillus, Serratia, Rhizobium, Gluconacetobacter, Pseudomonas* and facultative thermophilic iron oxidizers are a few Zn-solubilizing bacterium genera that have been identified so far (Deepak *et al.*, 2013; Saravanan *et al.*, 2007). Many PGPR have been reported to be efficient zinc solubilizers. By populating the rhizosphere and converting complicated zinc compounds into simpler

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ones, these bacteria aid in the improvement of plant growth and development. Moreover, these zinc solubilizing bacteria with PGPR activity were successfully utilized for enhanced nutrition and zinc biofortification in maize, soyabean, wheat (Khande *et al.*, 2017; Kamran *et al.*, 2017) and rice (Vaid *et al.*, 2014).

Mangroves are typical ecological niches found in the inter-tidal zones of river deltas with waterlogged saline conditions which facilitates the growth of diverse bacterial. Further, the delta being the slowest stretch of river flow accumulates the major portion of effluents and nutrient carried over by the river system and thus impacts the soil physico-chemical parameters and microbial diversity. The microbial diversity is the backbone of nutrient recycling and have significant role in the sustainability of such ecosystem. Most of the Indian soil are deficient of micronutrients which results in to low crop productivity and reduced nutritional quality (Sukla *et al.*, 2021). Therefore, the present study focused on the isolation and characterization of zinc solubilizing bacteria from the diverse habitat of mangrove forest of Odisha with an aim for future utilization of bioresource for agricultural yield.

2. Materials and methodology

The soil samples were collected from Bhitarkanika mangrove forests of Kendrapara district, Odisha. Various physicochemical parameters were measured to determine the soil properties following standard methods (Banerjee, 2018).

2.1. Isolation of Zn solubilizing bacteria (ZSB) from soil samples

The spread plate method was used to count and isolate Zn-solubilizing bacteria from soil samples on Bunt and Rovira medium (Bunt and Rovira, 1955) containing 0.1% insoluble zinc oxide (ZnO). The zinc solubilizing bacteria displayed a clear hollow zone on the plate, and the bacteria were isolated as zinc solubilizers and purified further to make pure cultures. The bacteria showing distinct solubilization zone were named as ZSB1 to 7 respectively and selected for further study.

2.2. Growth and morphological characteristics of the ZSB isolates

The population of the ZSB isolates were evaluated by counting their colony abundance from the master plate and was expressed in colony forming units (CFU) as follows. All the selected isolates were examined morphologically through their colony morphology, cell shape and gram staining reaction (Barthalomew and Mittewer, 1950).

Colony forming unit/ml =

$$\frac{No\ of\ colonies\ x\ dilution\ factor}{Volume\ of\ inoculum}$$

2.3. Determination of zinc solubilizing efficiency and biochemical characterization of ZSB isolates

All the 7 isolates were tested for solubilization efficiency by plate assay using modified Bunt and Rovira agar medium containing 0.1% of ZnO as insoluble source. After inoculation, the plates were incubated for 48hrs at 37^0C. The hollow zone on the plates indicated solubilizing efficiency of bacteria. The diameter of the colony growth and clear zone created by it was measured and then the solubilization efficiency was calculated as per Khanghahi *et al.* (2018). Indole test and Siderophore production tests were examined for the bacteria by using Kovac's reagent and $FeCl_3$ test (Dave *et al*., 2006).

Solubilizing efficiency (%) =

$$\frac{Diameter\ of\ holozone\ -\ diameter\ of\ colony\ growth}{Diameter\ of\ colony\ growth}\ x\ 100$$

Zinc solubilization capacity was also tested under liquid culture conditions using Bunt and Rovira medium containing 0.1% ZnO as the sole source of Zn. To determine the effect of ZSB on pH of growth media, samples were withdrawn at regular intervals and pH of the broth were measured. An uninoculated media sample was also kept as control. The bacterial culture and control were centrifuged and filtered. To measure the titrable acidity of the broth, 4ml of supernatant was titrated against 0.01% NaOH using phenopthalene as indicator (Nenwani *et al.*, 2010). The measurement of soluble Zinc ion in the culture was done through AAS. The sample was prepared by digestion with triacid mixture (prepared by nitric acid, sulphuric acid and perchloric acid in the ratio of 4:2:1) in a glass bottle. Five ml of triacid mixture was added to 2ml of filtered broth and heated for digestion until the solution became transparent. Then the volume was made up to 20ml (White *et al.*, 1997) and Zn concentration was measured by using an AAS (ISE 3000AA, Thermo Fisher).

2.4. Molecular characterization of ZSB isolate

The bacteria showing maximum solubilization efficiency was further identified by 16S rRNA sequencing. Bacterial DNA was extracted from a loop full of overnight grown cells on nutrient agar by using a QIAmp DNA mini kit (Qiagen, Duesseldorf, Germany) as per manufacturers' protocol. The purity and concentration of DNA were examined by electrophoresis on agarose gel (1%) and quantified with a

Nanodrop 2000c spectrophotometer (Thermo Fischer Scientific Inc, Walthum, MA, USA). Two universal primers 27f and 907r were used for PCR amplification using a thermocycler (Bio-rad T100, USA) with initial 5 min denaturation at 95°C, followed by 35 cycles of 1 min denaturation, 1 min annealing at 55°C, 2 min extension at 72°C, and a final extension of 10 min at 72°C. The PCR product was resolved in 1.2% agarose gel along with ethidium bromide staining followed by visualized using a gel documentation system (Bio-rad Gel Doc XR+, USA).

The PCR product was purified using a DNA purification kit (Illustra GFX PCR DNA and Gel Band purification kit, GE Healthcare, UK) as described in the manufacturer's protocol. Purified amplicon then sequenced by outsourcing. The DNA sequences thus obtained were compared with known sequences through BLAST (http://www.ncbi.nlm.nih.gov/blast). Phylogenetic analysis was conducted based on neighbor joining method by using MEGA-X.

3. Results

In this study attempts were made to isolate zinc solubilizing bacteria from soil samples of Bhitarkanika mangrove soil. After examining the physicochemical analysis of collected soil samples the pH was found in the range of 7.2 to 7.8. The soil samples were clay type and found to have high water holding capacity as well as moisture content. The water holding capacity was observed between 38 % to 49 % and the moisture content were recorded between 33.86 to 34.2%.

Seven zinc solubilizing bacteria were isolated from the mangrove soils showing distinct solubilization zone and having morphologically different colonies. The selected isolates were characterized based on morphological and biochemical properties as presented in Table 1. The population density of the ZSB isolates in the mangrove soil ranged from $16x10^5$ to $28x10^6$ CFU/g soil. Three ZSBs showed siderophore production ability, whereas all bacteria showed negative response to indole test (Table 1). The ZSB isolates were tested for their efficiency to solubilize the insoluble zinc on solid medium and ZSB2 showed the highest zinc solubilization efficiency i.e 111.51% whereas, ZSB7 showed the least solubilization efficiency of 22.21% (Fig. 1).

A gradual decrease in the pH of the culture broth was observed under liquid culture conditions up to 6 days of incubation, after which it remained constant (Fig. 2). Maximum decrease of pH was observed for ZSB2 on 5^{th} days of incubation, whereas ZSB7 showed least change in culture pH. With decreasing pH, the titrable acidity of the culture medium was found to be increased gradually for each ZSB isolates. The maximum titrable acidity of 3.34 ml was recorded by ZSB2 on 6^{th} day of incubation followed by ZSB4 and minimum acidity was observed by ZSB7 (Fig. 3). ZSB2 showing maximum solubilization efficiency and titrable acidity was tested for zinc solubilization in liquid medium containing zinc oxide as a sole source. Fig. 4 showed a significant ($P<0.05$) increase in zinc ion concentration in each day (except 6^{th} day) in the culture medium with a maximum value of 6.55 mg/L on 5^{th} day of incubation. The value showed 2.5 times higher available zinc ion concentration in the ZSB2 inoculated culture on 5^{th} day of incubation than the control (uninoculated) medium. Further the ZSB2 bacterial isolate was identified as *Enterobacter kobei* by 16SrRNA sequencing showing 95% similarities with the data available in the NCBI gene bank (Fig 5).

Table-1

Morphological characterization of ZSB isolates

Sl. No	Isolates	Gram staining Type	Population density (CFU/g soil)	Nature	Colony Forms	Elevation	Margins	Optical Density	Colony colour	Sidero-phore
1	ZSB1	-ve	$17x10^6$	Dull	Irregular	Flat	Undulated	Opaque	Yellow	+
2	ZSB2	+ve	$19x10^6$	Sticky	Circular	Raised	Round	Opaque	White	+
3	ZSB3	+ve	$22x10^6$	Sticky	Irregular	Flat	Round	Opaque	White	-
4	ZSB4	+ve	$16x10^6$	Dull	Circular	Raised	Undulated	Opaque	Off white	++
5	ZSB5	-ve	$28x10^6$	Dull	Circular	Raised	Undulated	Opaque	Pale Yellow	-
6	ZSB6	+ve	$24x10^6$	Sticky	Circular	Flat	Round	Opaque	Pale Yellow	-
7	ZSB7	-ve	$22x10^6$	Dull	Irregular	Raised	Undulated	Transparent	White	-

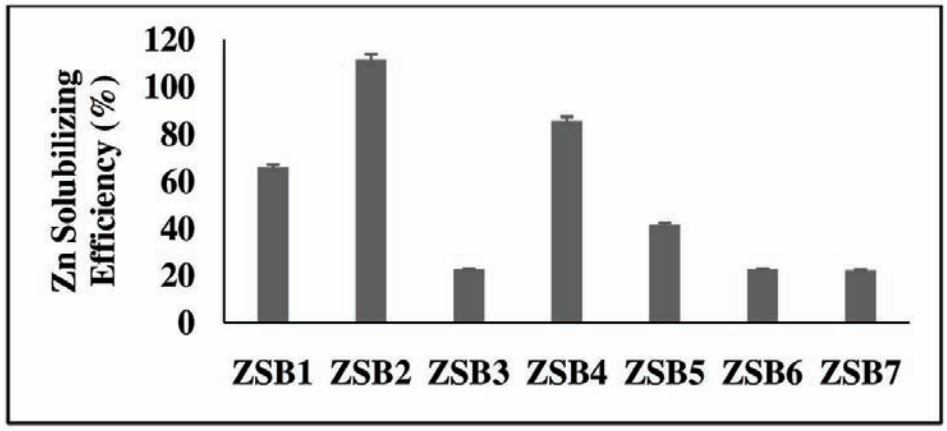

Fig 1. Solubilizing efficiency of ZSB isolates on solid media.

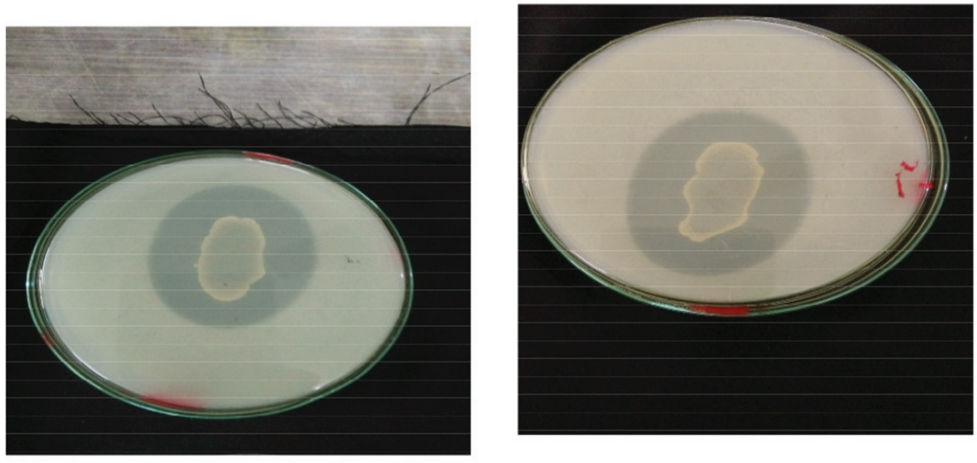

Plate 1: Solubilization zone produced by ZSB isolates (A) ZSB2 (B) ZSB4 on Bunt and Rovira solid medium.

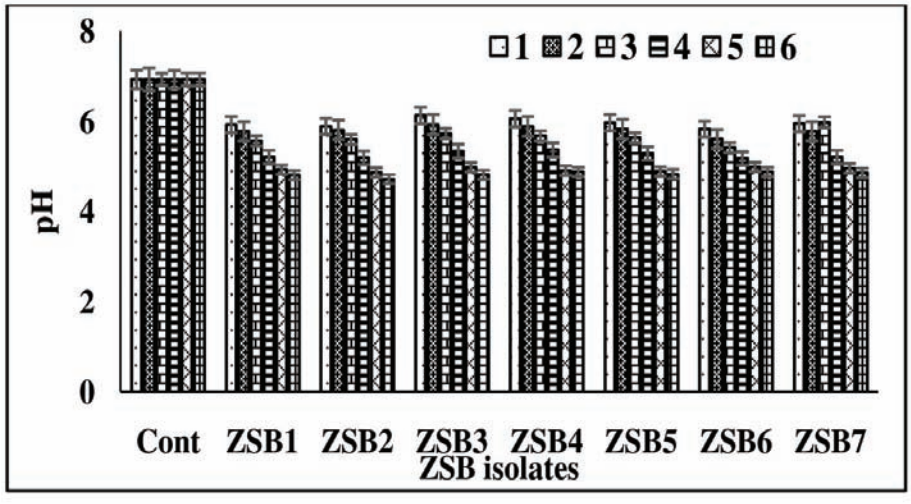

Fig 2. Change in pH of culture broth by ZSB isolates.

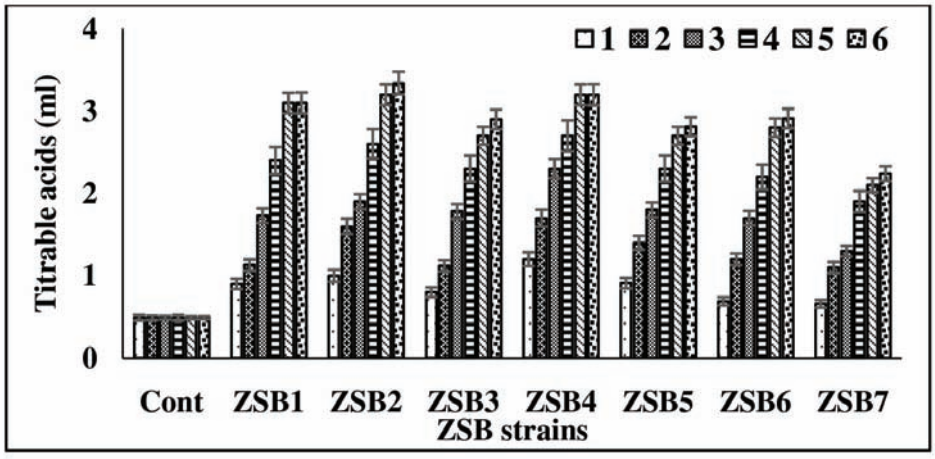

Fig 3: Amount of titrable acids produced by ZSB isolates on different days of incubation.

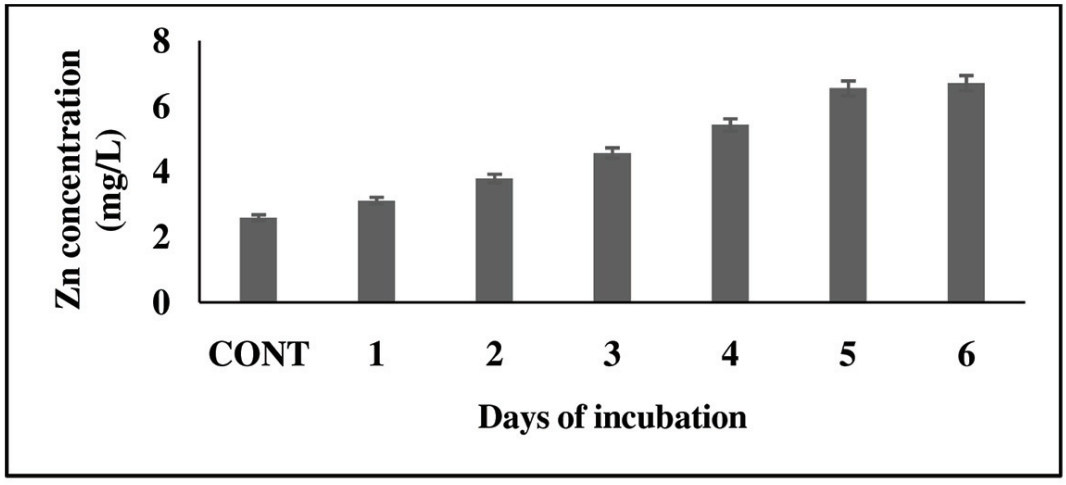

Fig 4: Concentration of soluble Zn ion in the culture broth of ZSB2.

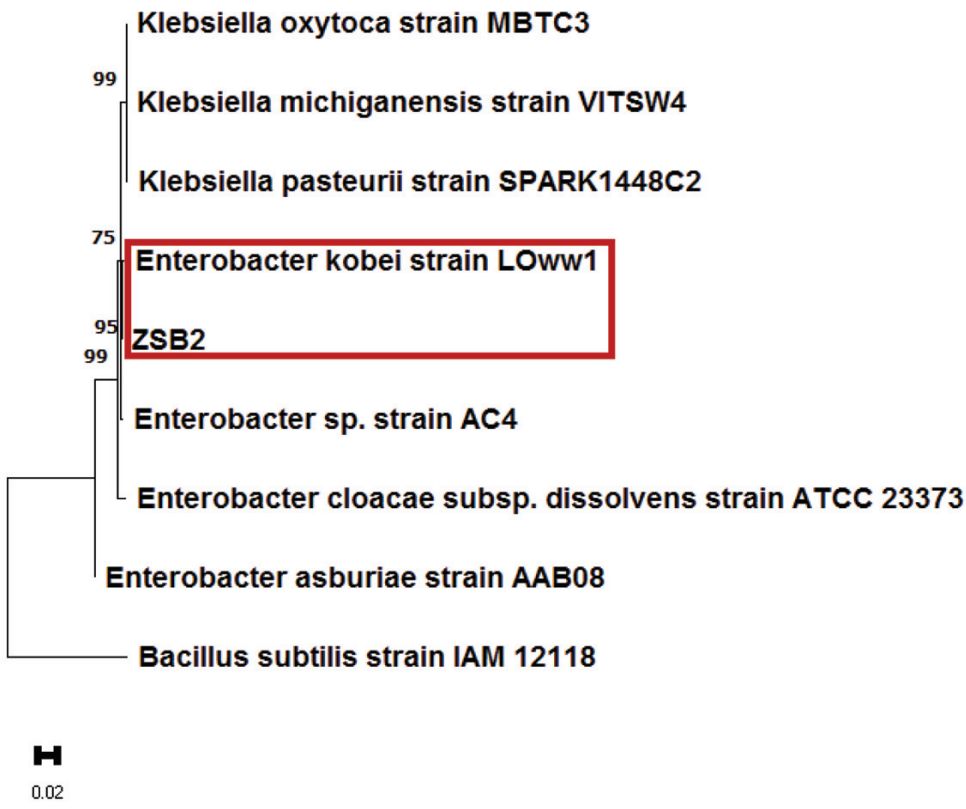

Fig 5: 16S rRNA sequencing of ZSB2 identified as *Enterobacter kobei.* Phylogenetic tree showing the relationships among the isolated bacteria and between representatives of other related taxa. The tree was constructed by using the software package MEGAX.0 and the distance matrix inferred by the neighbour-joining method using Jukes–Cantor model. The number at the branching points indicate the levels of bootstrap support based on data for 1000 replicates; values greater than 50% are only presented. The scale bar indicates 0.02 substitutions per nucleotide position.

Discussion

Bhitarkanika mangrove is located in the east coast is a second largest tropical mangrove ecosystem in India. A diverse range of microorganisms are present in mangrove areas due to its estuary environment to maintain the nutrient cycling and ecological balances. On the basis of physicochemical properties of soil, the microbial diversity varies from place to place. In this study, the soil samples were clay type and the pH ranges from 7.2 to 7.8. Our results corroborate with the reports of Mishra *et al.* (2012) where a pH variation of 6-8 was mentioned due to seasonal fluctuation. Banerjee *et al.* (2018) reported that silt and clay soil have a positive correlation whereas, sandy soil has negative correlation with the organic carbon content of the soil. High organic carbon content leads to high microbial diversity and population than that of sandy soil as evident from our result (Table 1). This variation in population size might be due to other soil factors such as soil nutrients, pH, moisture and salinity (Vikram *et al.*, 2007).

There is several literature suggesting a variety of soil microbes which can solubilize zinc from insoluble mineral sources to available form, thereby increasing availability to crop and produce plant growth promoting substances like auxin which is known to stimulate the growth of crop plants (Sedhegi *et al.*, 2012). In our study out of several colonies grew on the Bunt and Rovira medium, 7 best isolates were selected on the basis of their zinc solubilizing efficiency and colony morphology on solid medium. Among the 7 isolates, ZSB2 showed maximum solubilization efficiency (Fig 1, Plate 1). In our study with increase in incubation period, a decrease in pH and increase in titrable acid of the culture medium by all the seven ZSB isolates was observed (Fig. 2 and 3). It might be due to production of organic acid. A drop in pH of the broth with insoluble zinc compounds has been argued by many authors. Fasim *et al.* (2002) reported a decrease in the pH due to bacterial growth was associated with the secretion of gluconic acid, which was attributed to release Zn ions from Zn metal. Production of H^+ and organic acids (gluconic acid and 2-keto gluconic acid) was found to be

the most important mechanism for heterotrophic metal (Zn) solubilization (Bennett *et al.,* 1978) however, few bacteria solubilize Zn by secreting siderophores (Saravanan *et al.*, 2011). In this study, ZSB1, ZSB2 and ZSB4 showed better solubilization efficiency than other strains, this could be due to the dual mechanism of organic acid production and siderophore production by the isolates as evident from Table 1 and Figure 2,3. Among them ZSB2 showed maximum efficiency both in solid as well as liquid medium (Fig. 1, 5) and identified as *Enterobacter kobei* by 16SrRNA sequencing with 95% similarities with *Enterobacter kobei* strain LOww1 by neighbour-hood joining method (Fig 5).

4. Conclusion

From this study it was concluded that the mangrove soil has a rich diversity of zinc solubilizing bacteria. Among the isolates, highest Zn solubilization efficiency was observed by ZSB2 which was further identified as *Enterobacter kobei* by 16 S rRNA sequencing. A 2.5-fold rise in available Zn concentration was detected from the culture broth of *E. kobei* as compared to the uninoculated culture. Along with Zinc solubilization the isolate also showed siderophore production, thus may be considered as a suitable candidate for plant growth promotion after evaluating its actual potential.

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In vitro free radical scavenging activities of leaves and rhizomes of *Maranta arundinacea* L.

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ABSTRACT

Maranta arundinacea L. is a very useful and valuable medicinal plant belongs tothe family Marantaceae. This plant is commonly known as arrowroot plant. The present study was aimed to investigate scavenging activities of leaf and rhizome extracts of *M. arundinacea*. The radical scavenging activity of leaf and rhizome was investigated by DPPH, H_2O_2, reducing power, metalchelating activity and nitric oxide scavenging activity. All the four different extracts exhibit concentration dependent scavenging activity. Out of the 4 solvent extracts ethanol was found to have the maximum scavenging potential followed by methanol, aqueous and hexane extracts. The IC_{50} value (*µ*g/ml) of the extracts for this activity was in the order of ethanol< methanol< aqueous<hexane.Ethanol extract of rhizome exhibited the lowest IC_{50}(37.84±1.27, 72.66±0.78, 16.33 ±0.64, 45.66 ±0.75 and 68.33±0.56 *µ*g/ml) indicating the highest scavenging activity of DPPH, H_2O_2, reducing power, metal chelating activity, nitric oxide scavenging activity followed by methanol, hexane and aqueous extract. The present study reveals that the leaf and rhizome possess significant antioxidant activities and also can be used in treatment of chronic diseases. *M. arundinacea* plant may serve as a good pharmaceutical agent to ameliorate oxidative stress.

1. Introduction

Plant kingdom is the valuable and precious store house of potential drugs. Medicinal plants are generally known as “Chemical Gold Mines” as they contain natural chemicals which are acceptable to human and animal system. A new era of health and its management has evolved with the discovery of free radicals and antioxidants. A free radical is a molecule which consists of one or more unpaired electron in its outermost shell. Free radical includes reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Naskar *et al*., 2011). Oxygen is the most essential element for survival on the earth, but about 5% of it reduce to active free radicals. Generation of oxidants due to endogenous factors (metabolism, infections, exercise and ageing) or exogenous factor (exposure to radiation, metal catalyzed reaction and oxygen free radicals as pollutants in the atmosphere). The antioxidant capacity of living system is less active than the external factors. Every biological system has a antioxidant capacity which are beyond the capacity of antioxidant of biological system of body which results in oxidative stress (Zima *et al*., 2001). Oxidative stress causes lipid peroxidation and this leads to the membrane intensity, protein denaturation including enzymes and ion channels of the membranes and this also leads to damage of DNA. These antioxidants prevent the reaction of reactive O_2 species by donating hydrogen or electron. Therefore, the antioxidants play a vital role in inhibition and act as free radical scavengers and this provide protection to human beings to fight against the infections and degenerative diseases. Synthetic additives are now being used in food item to increase color and

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flavor. Two most commonly used synthetic antioxidants are BHA and BHT but due to their toxic nature and role in DNA damage, their usage in almost prohibited (Sasaki *et al*., 2002). For this reason, plant derived antioxidants are gaining importance for their safety measures. There is a great demand on the later and more investigation is required for screening of plant species for their antioxidant properties. Modern consumers are more health conscious, more dependent on natural antioxidants either in raw or pure form. Termination and prevention of the formation of free radicals are carried out by antioxidants both enzymatic and non-enzymatic. Antioxidants derived from plants have the capacity to scavenge harmful free radicals generated in human body due to various external and internal factors. So, it isessential to increase the antioxidants in dietary products. Depending on the mechanisms, the two major antioxidant capacity methods can be classified as (i). Hydrogen atom transfer (HAT) method and (ii) Single electron transfer (SET) method. They have also theability to chelate transition metals. Various methods have been adopted to estimate antioxidant in plants including 2,2-azinobis 3-ethyl–benzothiazoline-6- sulfonic acid (ABTS) (Leong and Shui, 2002), 2,2-diphenyl 1-picrylhydrazyl (DPPH) (Gil *et al*., 2000) and Ferric reducing antioxidant power (FRAP).

2. Materials and Methods

Maranta arundinacea, a medicinal and food plant, was selected and the rhizomes of this plant were collected from P. G. Department of Botany, Utkal University, Bhubaneswar. Washing of fresh rhizomes and leaves were done in running tap water and the healthy rhizomes and leaves were cut in to pieces. The rhizomes were allowed to sundry for 5 hours followed by shade drying. The leaves were allowed to dry under shade. After shade drying, the leaves were kept in oven at 50^0C for 6 hours and ground to semi powdered form. 50g of plant material was extracted with 450ml of different solvents like methanol, ethanol, aqueous and hexane for 24 hours in Soxhlet apparatus. The extract was filtered and condensed in a rotary evaporator. Final sample was prepared by reducing the volume in a desiccator.

2.1. DPPH radical scavenging assay

DPPH assay was carried out according to the protocol of Blois (1958). DPPH 1mM (0.1 m) was thoroughly mixed with 3 ml of plant extracts of different concentrations (50-250 ìg/ml) followed by incubation in dark for half an hour. Absorbance was recorded at 517nm for the reduced DPPH. Absorbance of DPPH without extract were taken as control. An equal amount of ascorbic acid solution of various concentration in distilled water was used as standard. DPPH radical scavenging was calculated as the antioxidant activity of sample and was denoted interms of IC_{50} values essential to scavenge 50% of DPPH radicals.

2.2. Hydroxyl radical scavenging activity

This assay of *M. arundinacea* extracts were quantified following the procedure of Halliwell (1987). The reaction mixture consisted of 0.1 ml of 1 mM $FeCl_3$, 0.1 ml of 20 mM H_2O_2, 0.1 ml of 1 mM ascorbic acid, 0.1ml of 1 mM EDTA, 0.1 ml of 30 mM deoxyribose and plant extract at various concentrations (50-250 ìg/ml) incubated for 1 hour at 37^0C. Deoxyribose degradation was measured by adding 1 ml of 2.8% TCA and1 ml of 1% TBA to the reaction mixture. Subsequently the tubes were kept at 100^0C for 20 minutes. After cooling O.D. was recorded at 532 nm with Quercetin (flavonoid) as the standard. The antioxidant capacity of each sample was expressed in terms of IC_{50}values which scavange 50% of radical formation.

2.3. Nitric oxide scavenging activity

According to the protocol of Wong *et al*. (2012), NO_2 scavenging activity was calculated. 0.2 ml of 5 mM sodium nitroprusside was gently mixed with 0.8 ml of plant extracts at different concentrations (50- 250 ìg/ml). Incubation was carried out at 25^0C for150 minutes under a light source (24 Watt). After incubation, 0.6 ml was transferred into a test tube containing 0.6ml of Griess reagent and incubated for 30 minutes. Pinkcolour chromatophore was formed and absorbance was recorded at 546 nm. Control consists of only plant extracts. IC_{50} value was calculated from curve of concentration plotted against inhibition percentage.

2.4. Metal chelating activity

Chelation of Fe^{2+}ions has been quantified by the protocol of Faran *et al.*, 2012. 0.5 ml of different (50- 250 ìg/ml) extracts thoroughly mixed with $FeSO_4$(0.5 ml) and 0.5 ml of 0.6 mM ferrozine and incubated for 10 minutes at RT.O.D of the resultant red coloration was taken at 562 nm. Reaction mixture with ultrapure water in place of sample solution was used as control and ultrapure water instead of ferrozine was used as blank. EDTA was taken as standard. The potential of a sample was calculated, how much Fe^{2+} ion has been chelated in comparison to a control.

2.5. Reducing power

The extracts have the potential to reduce Fe^{+3} ions to Fe^{2+}, was assayed following the protocol of Oyaizu (1986). Extracts of different concentration *i.e.*, from (50- 250 ìg/ml) were taken for analysis. One ml of *M. arundinacea* extract was added thoroughly with 2.5 ml of 0.5 mM phosphate buffer (pH 6.6), 2.5 ml of 1% potassium ferricyanide and incubated at 50^0C for 20 minutes. Further 2.5 ml of 10% TCA

was added and centrifuged and 2.5 ml of top layer was drawn out and gently placed over the tube with 2.5 ml sterile water and 0.5 ml of 0.1% ferric chloride, mixed thoroughly and after 5 minutes O.D. was recorded at 700 nm against a blank. Control contains all reagents except plant extracts. Ascorbic acid in various concentrations was taken as the standard. Increase in O.D. indicates enhancement in reducing power.

2.6. Estimation of IC_{50} value

The IC_{50} value is the amount of the sample required to scavenge 50% of the radical generated in the reaction mixture. The inhibition percentage was plotted against corresponding concentration and IC_{50} values were drawn out from the curve by calculating the concentration corresponding to 50% inhibition.

3. Result and Discussion

3.1. DPPH scavenging assay

The radical scavenging activity of *M.arundinacea* and standard ascorbic acid based on DPPH assay is depicted in table1 and figure 1. Aqueous, methanol, ethanol and hexane extracts exhibit IC_{50}53.52±1.59, 51.66±1.56, 45.66±1.47 and 66.03±1.67µg/ml in leaf 50.67±1.49,49.31±1.34, 37.84±1.27, 69.84±1.67 µg/ml. IC_{50} of ascorbic acid was found to be 70.32±1.69 µg/ml, as low value indicates higher scavenging activity. Out of the 4 solvent extracts ethanol was found to have the maximum scavenging potential followed by methanol, aqueous and hexane extracts.

According to Rai, 2006, DPPH reduced by oxidative stress busters of the plant extract. á,á- diphenyl- â-

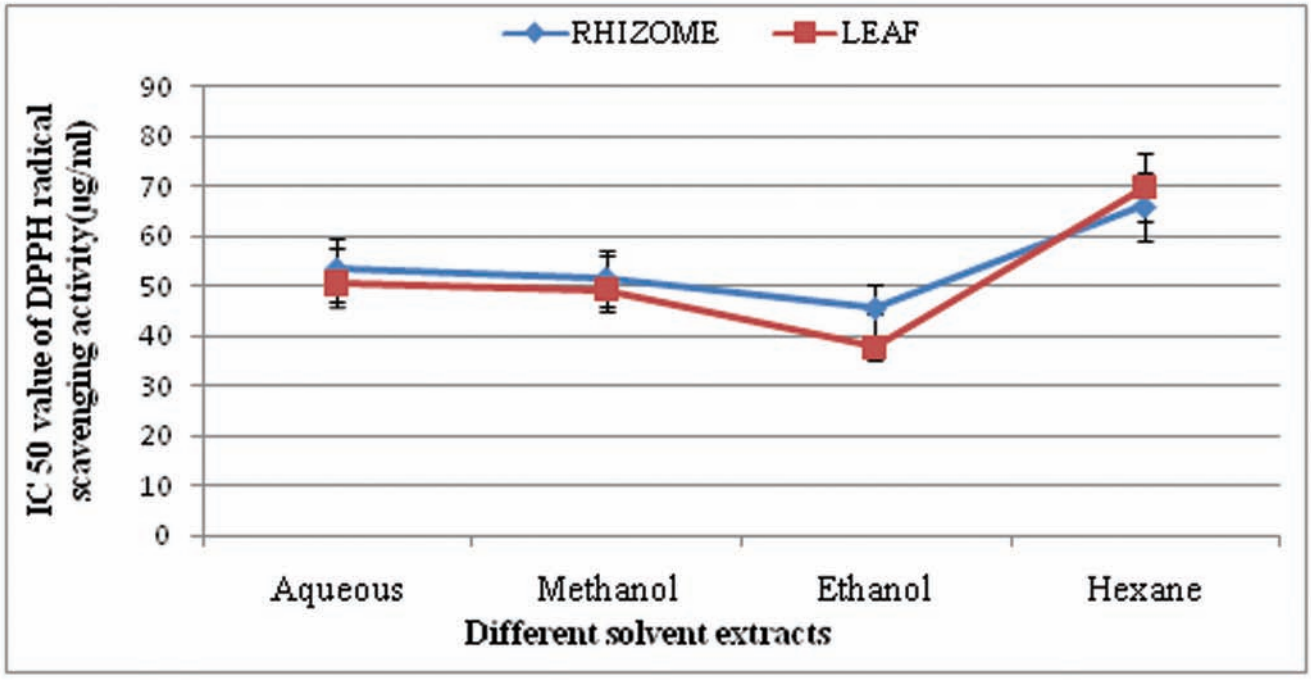

Fig. 1: DPPH radical scavenging activity of *M.arundinacea*

picrylhydrazine Yellow colour is an indication of formation of active oxygen radicals by 2,2- diphenyl -1- picrylhydrazyl DPPH (purple colour) (Akowuah *et al*., 2005). Comparing among various solvent extracts of *M. arundinacea*, it has been observed that ethanol extract with minimum IC_{50}(37.84±1.27 ìg/ml) which indicates higher scavenging activity. Similar studies were carried out by Barua *et al*., 2014, with *A. calamus* with higher scavenging potential by aqueous extract.

3.2. Nitric oxide scavenging activity

Nitric oxide scavenging activity of leaf and rhizome of *M. arundinacea* was depicted in the Fig. 2 evidenced that rhizome powder extract effectively reduce the generation of NO radical where the activity was highest in ethanolic extract (76.33±0.82 µg/ml) and lowest in aqueous extract (85.8±0.903 µg/ml). The ethanolic extract of *M. arundinacea* has better nitric oxide radical scavenging activity. IC_{50} was 85.8±0.903µg/ml, 82.66±0.911µg/ml, 76.33±0.82µg/ml and 83.33±0.827 µg/ml in leaf and 137.67±0.78µg/ml,78.35±0.56µg/ml, 68.33±0.56µg/ml and 121.89±0.34µg/ml in rhizome for aqueous, methanol, ethanol and hexane extract respectively, suggesting higher efficiency of ethanol extract for 50% inhibition to NO radical.

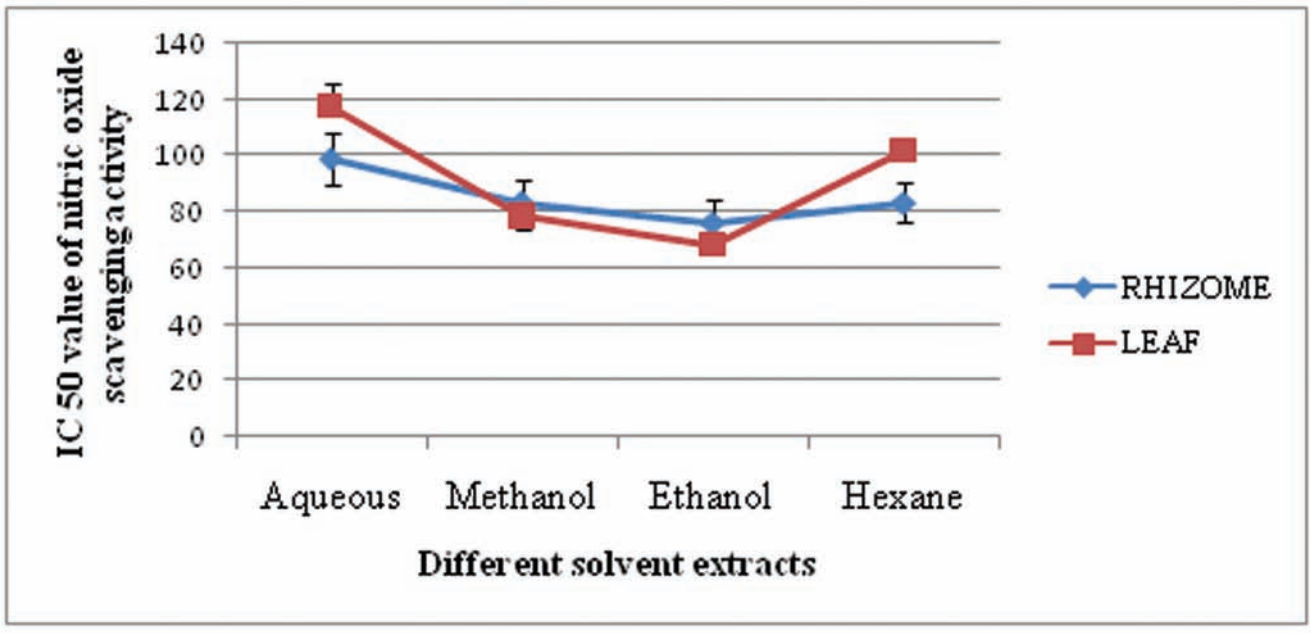

Fig. 2: Nitric oxide scavenging activity of *M.arundinacea*

Balakrishnan *et al*. (2009) stated that scavengers of nitric oxide lead to a decreased generation of NO by competing with oxygen, as nitrite can be produced from spontaneous formation of nitric acid from sodium nitroprusside which when reacts with oxygen produce nitrite. Similarly, Barua *et al*., (2014) indicated that the ethanolic extract of *A. calamus* with highest NO scavenging activity and had an IC_{50} comparable to the reference standard. The workers also specified that the generation of NO gets suppressed in a concentrated dependent manner.

3.3. Metal chelating activity

When the concentrations of plant extracts were increased, the chelating activity was also increased. The extract of *M. arundinacea* has chelating activity with an $IC_{50}$of 71.66±0.75µg/ml, 51.26±0.41µg/ml, 50.33±0.64 µg/ml,61.66±0.64 µg/ml in leaf and 71.63±0.64 µg/ml, 55.45±0.52 µg/ml, 45.66±0.75 µg/ml, 69.33±0.89µg/ml in rhizome in aqueous, methanol, ethanol and hexane respectively.

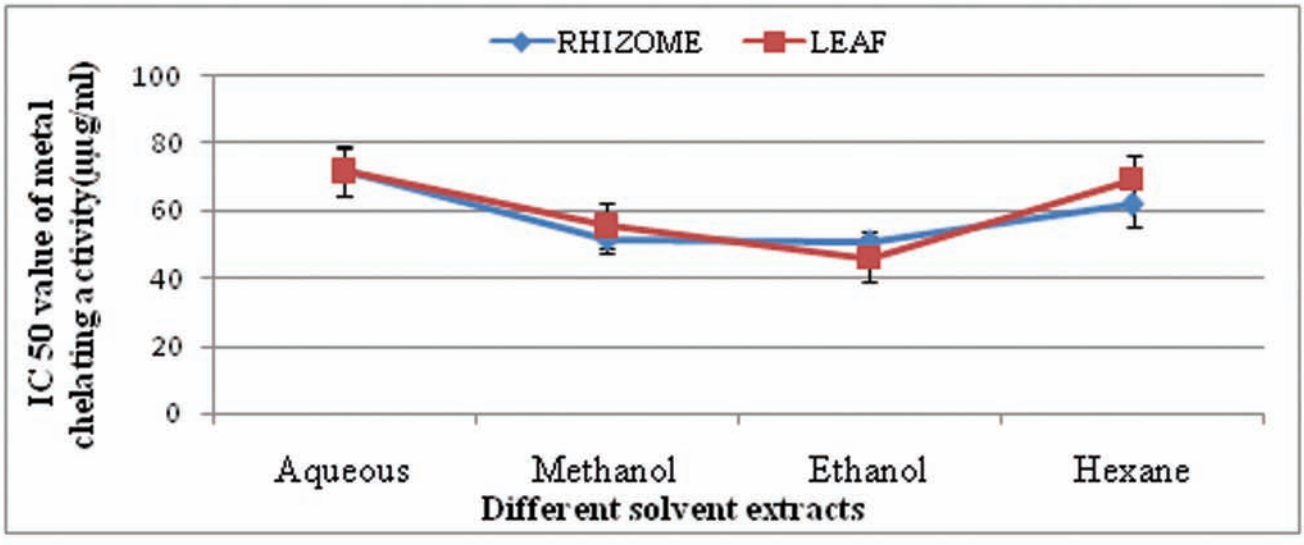

Fig. 3: Metal chelating activity of *M. arundinacea*

Elmastas *et al*. (2006) indicated that natural plant extracts have antioxidant activity as they have the ability to chelate transition elements like Fe+2 and Cu+2. Fenton reaction is the medium by which Ferrous ions starts lipid peroxidation by denaturing lipid hydroperoxides into peroxyl and alkoxyl radicals (Fridovich, 1995 and Halliwell, 1991). If the addition of chelating agent is stopped colour will remain the same. The chelation can be determined by the chelating agent and the metal ion. Chelating agents function as secondary antioxidant as these metabolites decrease the redox potential keep intact the oxidative status of the metal (Gordon and Hudson, 1990). Jayasri *et al*. (2009) evidenced metal chelating effect of aqueous, methanol and ethanol extract of leaf and rhizome of *Costus pictus*. The chelating effect of extracts of rhizome and leaf were in the order of methanol > aqueous > ethanol, among which methanolic extract exhibited higher chelating activity,whereas minimum chelating activity was observed in case of ethanolic extract of both rhizome and leaf. In addition to that the chelating capacity of the extracts were also observed to be increased with increasing concentration.

3.4. Reducing power

The reducing powerof different solvent extracts of *M. arundinacea* increased with increase in concentration ofplant extracts. All the concentration evidenced significant activity than the control. Aqueous, methanol, ethanol and hexane extracts came up with IC_{50} of 33.78±0.75µg/ml, 24.66±0.82µg/ml, 21.66±0.76µg/ml, 32.23±0.82 µg/ml in leaf and 33.66±0.64µg/ml, 19.33±0.64µg/ml, 16.33±0.64µg/ml, 47.52±0.73 µg/ml as compared to standard ascorbic acid with IC_{50}42.33±0.82 µg/ml.

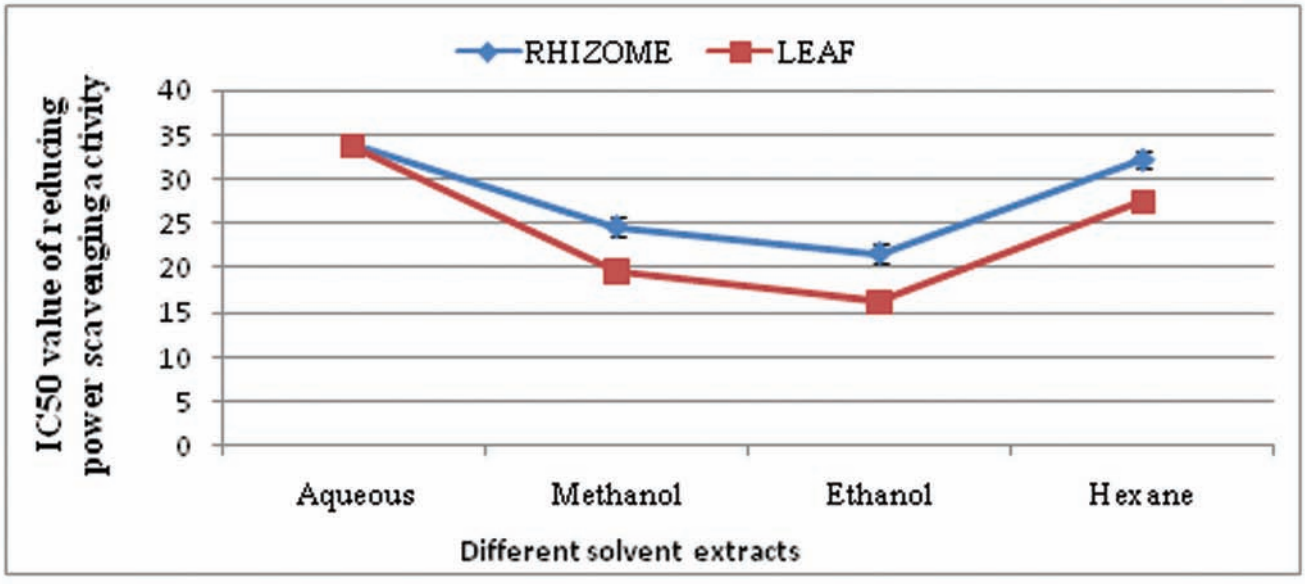

Fig. 4: Reducing power scavenging activity of *M.arundinacea*

The antioxidative potential of compounds is determined by its reducing power. The conversion of Fe^{3+} or ferric cyanide complex to ferrous by reductants like H_2O_2 takes place with the evolution of O_2 and the colour change from yellow to green can be recorded at 700nm. The greater the reducing capacity of a compound the greater is the antioxidant activity. The antioxidants prevent chain initiation, metal chelation, decomposition of peroxides and radical scavenging (Subashini *et al*., 2007). The reducing activity is due to the donation of hydrogen atom to disturb the radical chain reaction (Saeed *et al*., 2012). It was reported that ethanol extract of *M. arundinacea* has minimum IC_{50} (16.33±0.64 ìg/ml).

3.5. Hydroxyl radical scavenging

The antioxidant activity of *M. arundinacea* based on hydroxyl radical scavenging activity is illustrated in the Fig. 5. Ethanol extract of rhizome exhibited the lowest IC_{50}(72.66±0.78µg/ml) indicating the highest scavenging activity of hydroxyl radical followed by methanol, hexane and aqueous extract. However, IC_{50} of ascorbic acid was found to be higher than the ethanol extract with 147.67±0.27 µg/ml.

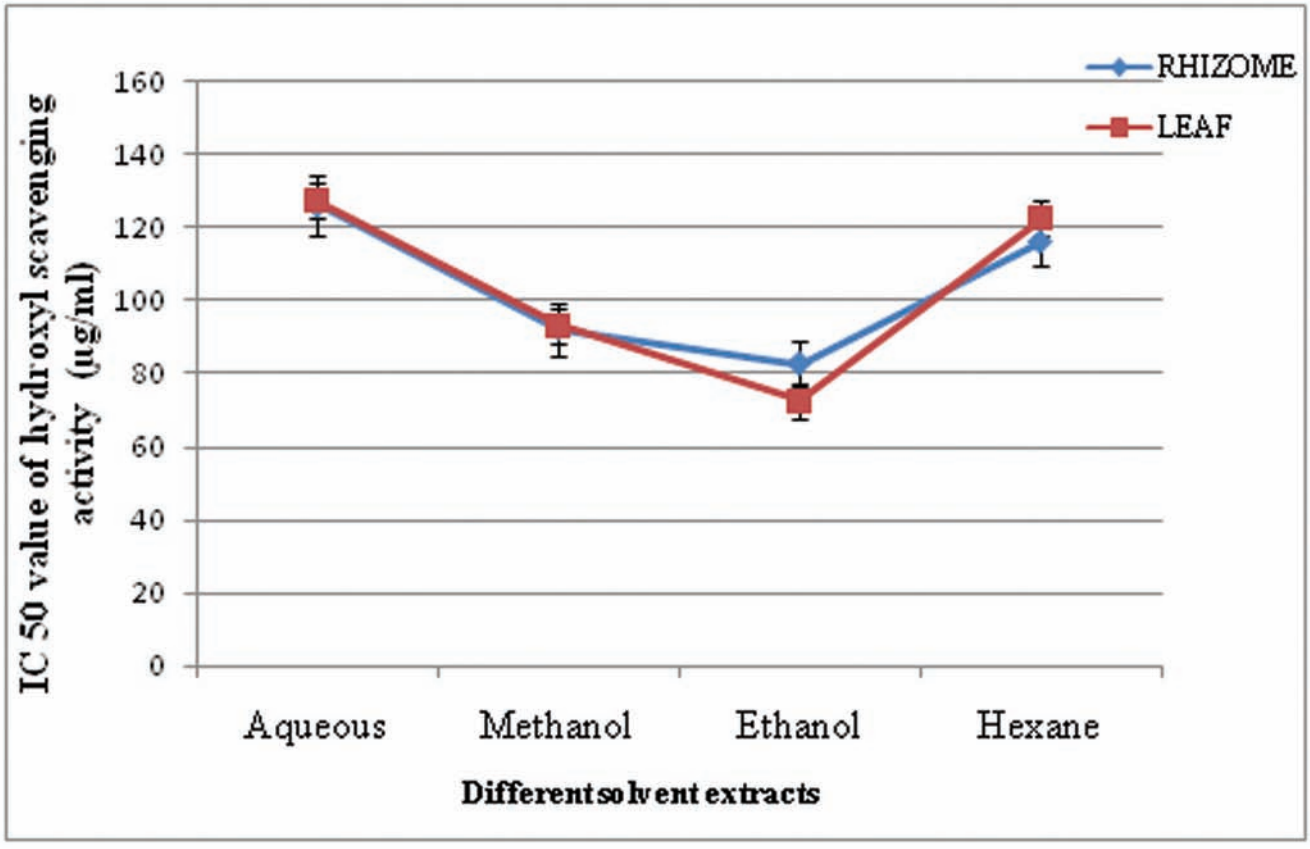

Fig. 5: Hydroxyl scavenging activity of *M. arundinacea*

Duan *et al*. (2007) stated that hydroxyl radicals are generated when H_2O_2 combines with iron commonly known as fenton reaction.H_2O_2 initiates cell damage as it is one of the most reactive dioxygen. Hydroxyl radicals bring about a lot of damage to almost all in the biomolecules like sugars, aminoacids, lipids and nucleotides (Wang *et al*., 2008). So scavenging of this radical is highly essential. Hydroperoxides when reacts with transition metals highly reactive hydroxyl radicals are formed which reacts with proteins, DNA, polyunsaturated fatty acids present in the membranes as well as other biomolecules thus inactivating their structural make up (Aruoma, 1999). In doing so they remove hydrogen atoms from membrane lipids and cause peroxidation of membrane lipids (Yen, 1994). OH- radical combine with nucleotide and cause bond break in DNA strands (Moskovitz and Yim, 2002). According to Fenton reaction, Ferric-ascorbate-H_2O_2-EDTA generates OH-radical which has higher affinity for deoxyribose to produce thiobarbituric acid reactive substance (TBARS). When heated a pink chromatogram was visualized. Barua *et al*. (2014) showed better scavenging activity with minimum IC_{50} value in the ethanolic extracts of *Acorus calamus*. Similar result was also observed in the present study *i.e*., in case of ethanolic extract of *M. arundinacea*.

The DPPH, hydroxyl radicals, superoxide scavenging, metal chelating and reducing power assay confirmed that each plant have antioxidant and free radical scavenging property. These plant extracts can be used as natural sources of antioxidants as they could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases. They also have potential application in industry as natural antioxidants, that could be used as food additives to prevent food quality deterioration due to addition of synthetic antioxidants which were associated with a lot of side effect.

Table 1

IC_{50} of radical scavenging activities of DPPH, hydroxyl radical, reducing power, metal chelatingandnitric oxide activities of leaf and rhizome of *Maranta arundinacea**

Sample	Solvent	DPPH radical scavenging activity IC_{50} (µg/ml)	Hydroxyl radical scavenging activity IC_{50} (µg/ml)	% reducing power activity IC_{50} (µg/ml)	% metal chelating activity IC_{50} (µg/ml)	Nitric oxide radical scavenging activity IC_{50} (µg/ml)
Leaf	Aqueous	53.52±1.59	116.33±0.82	33.78 ±0.75	71.66±0.75	85.8±0.903
	Methanol	51.66±1.56	92.33±0.82	24.66±0.82	51.26 ±0.41	82.66±0.91
	Ethanol	45.66±1.47	82.66±0.91	21.66±0.76	50.33±0.64	76.33±0.82
	Hexane	66.03±1.67	140.33±0.64	32.23±0.82	61.66±0.644	83.33±0.82
Rhizome	Aqueous	50.67±1.49	127.32±0.45	33.66 ±0.64	71.63±0.64	137.67±0.78
	Methanol	49.31±1.34	93.23±0.46	19.66±0.64	55.45±0.52	78.35±0.56
	Ethanol	37.84±1.27	72.66±0.78	16.33 ±0.64	45.66 ±0.75	68.33±0.56
	Hexane	69.84±1.67	132.67±0.34	47.52 ±0.73	69.33 ±0.89	121.89±0.34
Standard	70.32±1.69	147.67±0.27	42.33±0.82	85.33±0.82	141.95±0.67	

*The data represent mean±SEM of replicates (n=3)

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Notes on the occurrence and distribution of *Ipomoea rumicifolia* Choisy (Convolvulaceae) in Gujarat, Western India

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ABSTRACT

During the taxonomic inventory of flora of the Indian Desert, a rare plant species namely, *Ipomoea rumicifolia* Choisy (Convolvulaceae) has been recorded after a long time from the Kachchh district of Gujarat state, Western India. The present paper deals witha detailed taxonomic description of the species along with its phenology, ecology, range of distribution and citations of specimens studied. Further, a colour photoplate displaying different vegetative and floral parts has been provided for easy identification of the species in the field.

1. Introduction

Kachchh district of Gujarat state is recognized for its unique geographical location, rich plant biodiversity and diversified ecosystems. As per the forest classification of Champion and Seth (1968), the area is classified under 'Northern Tropical Thorn Forest'.The district harbours quite a good number of rare, threatened and endangered plants for the flora of Gujarat state.

The genus *Ipomoea* is one of the dominant and diverse genera of the family Convolvulaceae represented by about 633 species in the world (https://powo.science.kew.org). In India, the genus is comprised of 57 taxa including 49 species, 2 subspecies, 4 varieties, and 2 forma (Kattee, 2019). During the post-monsoon exploration in some unexplored Westernand Eastern parts of the Kachchh district, the first author collected a few specimens of the genus *Ipomoea* L. After critical laboratory investigations and consultation of relevant literature (Clarke, 1883; Woodrow, 1898; Cooke, 1905; Patel, 2013; Kattee, 2019), the specimens were identified as *Ipomoea rumicifolia* Choisy. Earlier the species was reported from five states of India such as Gujarat, Tamil Nadu, Karnataka, Andhra Pradesh, and Rajasthan. In Gujarat state, this species was earlier reported by some workers (Jain & Deshpande, 1960; Raghvan *et al.*,1981; Sabnis & Rao, 1983; Bhatt, 1993) from Kachchh district, but there is no recent record of occurrence of this less known taxon from Gujarat. Since in earlier published literature, detailed botanical description and photoplates of the species were not available, a detailed description, color photographs, relevant notes on its distribution, ecology and habitats are provided in the present communication to facilitate easy identification of the species. The herbarium specimens have been deposited in the Herbarium of Vande Vasundhara Research Laboratory (VVRL), Bhuj, Kachchh, Gujarat.

2. Taxonomic treatment

Ipomoea rumicifolia Choisy, Mem. Soc. Phys. Genève. 6: 447. 1833; Hook. f., Fl. Brit. Ind. 4: 207. 1883; Woodrow in J. Bombay Nat. Hist. Soc.12: 171. 1898; T. Cooke, Fl. Bombay 2: 244. 1905; Gamble, Fl. Madras 2: 643. 1923; Jain & Desh., Furt. Con. Fl. Kutch. Bull. Bot. Surv. Ind. 2: 291. 1960. (Fig. 1 and 2)

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Fig. 1. Herbarium specimen of *Ipomoea rumicifolia* Choisy

Fig. 2. ***Ipomoea rumicitolia*** Choisy **a**. Habit; **b**. Adaxial surface of leaf; **c**. Abaxial surface of leaf; **d**. & **e**. Leaf apex, **f**. Side view of flower; **g**. Top view of a flower; **h**. Androecium; **i**. Gynoecium; **j**. & **k**. young capsule vs. dried capsule; **l**. fruiting twig (up to 5 capsules) **m**. Side view of the mature calyx; **n**. Seeds

Annual; stem herbaceous,erect when young, prostrate at maturity, 1 – 2 m long, hirsute throughout. Leaves2 – 4×2 – 2.5 cm long, ovate-oblong, subreniform, apex obtuse or apiculate, sometimes emarginate, margin entire, mostly undulate, irregularly lobulate, surface glabrous above, hirsute below, hairs present only onveins, base cordate with a rounded lobe; petioles up to 5 cm long, sparsely hirsute. Inflorescence axillary, usually 1 – 2 flowered, sometimes 4 – 6 flowered. Flowers bracteate, pedicellate; pedicel short, 0.5 – 1 cm long, hairy, slightly thickened towards the apex in fruit, deflexed; bracts 3 – 4 mm long, linear, hairy. Calyx 5, 5 – 6 × 2 – 4 mm, subequal, ovate, acute to acuminate, compactly clothed with spreading hairs at the base, margin ciliate, green when young, light reddish at maturity. Corolla 5 lobed, tubular-campanulate, tube 0.5cm long, pure white, 1 – 1.5 cm wide at the mouth, glabrous throughout or somewhat hairy on the outer side of each lobe, slightly apiculate. Stamens 5; filament 2 – 3 mm, subequal, included, glabrous at the base. Style 4 – 5 mm long; stigma bilobed, capitate. Fruit 0.5 – 1 × 0.3 – 1.1 cm, ovoid, apiculate, glabrous, slightly reddish at maturity, and light brown at the dispersal stage. Seeds usually 4, 5.5 – 6 mm long, velvety.

Flowering & Fruiting: *August – December*

Distribution:

India **(**Rajasthan, Gujarat, Karnataka, Tamil Nadu, Andhra Pradesh), Ethiopia, Egypt, Yemen, Malaysia, Australia and Sudan.

Specimens examined:

INDIA, Rajasthan: Pali district, Guru Pratap Singka Guda, 01.09.1975, *B. V. Shetty*, 1985 (CAL); Jodhpur dist., Tolesar, 21.08.1977, *A. N. Singh*, 4352 (CAL). Karnataka: Bellary, 17.11.1979, *B. R. Ramesh*, 10500 (CAL). Tamil Nadu: Ramanathapuram dist., Chithrangud, 11.11.1989; *V. Balsubramaniam*, 2142 (CAL); Pullanthai, Sayalgudi, 25.12.1989, *V. Balsubramaniam*, 2315 (CAL). Gujarat: Kachchh district, Nakhtrana, Dhinodhar, *Jain* 46905 (BSI); Adesar, 12.09.1968, *R. S Raghvan*, 95193 (BSI); Nakhtrana, *Bhatt J. B.* 811 (MSU).

Additional specimens examined*:*

Gujarat, Kachchh district, Bhuj Taluka, Mokhana, N 23^0 17’11", E 70^0 01’18", 68 m, 2.8.2021, *K. I. Prajapati*, KP-017; Zikdi-Habay Rakhal, N 23^0 20' 17", E 69^0 47' 36", 124 m, 11.9.2021, *K. I. Prajapati*, KP-018 (VVRL); Anjar Taluka, Chandrani, N 23^0 17' 39", E 70^0 03' 57", 53 m, 19.8.2021, *K.I. Prajapati*, KP-019 (VVRL); Sataper, N 23^0 08’46", E 70^0 02’29", 59 m, 19.8.2021, *K. I. Prajapati*, KP-020 (VVRL); Abdasa Taluka, Naliya, Dhufi Nani, 15.9.2021, N 23^0 14’30.3", E 69^0 00' 28.4", 68 m, *K. I. Prajapati*, KP-021 (VVRL); Raper Taluka, Adesar, 25.11.2021, N 23^0 36’46", E 71^0 02' 23", 10 m, *K. I. Prajapati*, KP-035 (VVRL).

Habitat & Ecology:

During the extensive field trips in the Kachchh district, the species was found wild in five localities and all the locations had different ecological habitats and associated species. In Mokhana field, the specimen was collected from dried loamy soil of the annual canal and associated species were *Achyranthes aspera* L., *Fagonia cretica* L., *Chloris barbata* Sw., *Aristida adscensionis* L., *Cyperus* sp. However, at Chandrani field, the plant was collected from dried soil of pond margins and *Merremia emarginata* (Burm. f.) Hall. f., *Trianthema portulacastrum* L., and one rare species *Hibiscus obtusilobus* Garcke were the close associates. In Habay Rakhal, the habitat was a hilly track and *Oropetium thomaeum* (L.f.) Trin. was the dominant associated species. Similarly, in Abdasa field, the habitat was dominated by agricultural fields and open grasslands and *Convolvulus stocksii* Boiss., a threatened plant, was observed as the close associate in such habitats. In last locality Adesar, the specimen was collected from highly saline soil of Little Rann of Kachchh and *Aeluropus lagopoides* (L.) Trin. ex Thw was a strongly associated species.

Note:

During the literature survey, the authors noted that the species reported in the Flora of the Indian Desert (Bhandari, 1990) and Flora of Rajasthan (Shetty & Singh, 1987) are misidentified as *Ipomoea verticillata* Forsk. [Syn. *Ipomoea biflora* (L.) Pers.].

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Phycoremediation of paper mill effluents by waste grown microalgae

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ABSTRACT

Microalgae gained a lot of attention in recent years because of their possible use in pollution management. They can be cultivated in unproductive habitats like nutrient rich wastewater as microalgae require high amount of nutrients for their growth that helps to reduce the pollution load from the environment. During this study six waste grown microalgal species, viz. *Limnothrix planctonica, Hapalosiphon hibernicus, Scytonema hoffmanii, Dolichospermum affine, Oocystis polymorpha* and *Tetradesmus obliquus* sourced from different wastewater habitats were utilized for remediation of wastewater collected from effluent treatment pond (ETP) of Emami Paper Mill, Balasore. To measure efficiency of wastewater utilization, the physico-chemical parameters of standardised wastewater were ascertained before and after utilization by all the tested species of algae under controlled culture conditions in laboratory. The phycoremediation results revealed that *H. hibernicus, O. polymorpha* and *L. planctonica* played better role for removal of pollutants from wastewater followed by *T. obliquus* and *S. hoffmanii. H. hibernicus* showed better performance by completely removing (100%) colour, turbidity, iron and phosphate from the wastewater. pH was corrected to normal after treatment by all the test algal species except *S. hoffmanii.* Moreover, the cyanobacterial species were found to be more efficient in terms of removal of nutrients like nitrate, silica, potassium and sodium as compared to green algae.

1. Introduction

Pollution is a man-made phenomenon which become a major concern of today's society due to increase in both natural and artificial compounds in the environment. The release of industrial and municipality wastewater to various water bodies makes them fully contaminated by depositing huge organic and inorganic pollutants such as heavy metals, xenobiotics, microplastics, phosphates, nitrates, carbon compounds etc. (Chowdhury *et al*., 2016; Mencio *et al*., 2016; Sausa *et al*., 2018; Eerkes-Medrano *et al*., 2019). The treatment of wastewater can not be done by a single methodology due to its extremely variable scales, types of pollutants and regional conditions. But this global problem can be solved by using microalgae as they are capable to perform phototrophic, mixotrophic or heterotrophic metabolism (Hu *et al*., 2018; Subashchandrabose *et al*., 2013) that represent a biological system for remediation of various types of wastewaters. Presence of nitrate and phosphate in wastewater make it a good feed stock for microalgae as both are essential for their growth. On the other hand, with growth it can remove maximum quantity of toxic materials from wastewater. Various reports showed that microalgae (cyanobacteria and green algae) can remove organic and inorganic nutrients from domestic and industrial wastewater up to 96% (Oswarld and Gotaas, 1957; Olguin, 2003; Chinnasamy *et al*., 2010; Kong Q-x *et al*., 2010; Wang *et al*., 2010) and efficiency of this technique is promising. However, very few researches have been carried out on treatment of paper mill effluents by using microalgae (Shruthi *et al*., 2012; Sasi *et al*., 2020). Being eco-friendly and cost effective, in the present investigation an attempt has been made to remediate wastewater of paper industry by using

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microalgae (cyanobacteria and green algae) sourced from wastewater.

2. Materials and methods

2.1. Sampling of paper mill wastewater; isolation and culture of dominant waste grown microalgae as test organisms

Field visits were made to the Baripada municipality area, Mayurnhanj and Emami Paper Mill, Balasore for the collection of algal samples along with wastewater from Emami Paper Mill during the period March, 2016 to May, 2017 (Plate-1). Six dominant microalgal species isolated from wastewater habitats viz. *Limnothrix planctonica, Dolichospermum affine, Hapalosiphon hibernicus, Scytonema hoffmanii, Oocystis polymorpha* and *Tetradesmus obliquus* were selected for remediation of industrial wastewater collected from effluent treatment ponds of Emami Paper Mill (ETP). The standardised wastewaters in which the test species showed maximum growth in comparison to inorganic medium were used as medium for phycoremediation process. The remediation experiment was carried out in culture vessels under cool fluorescent light (7.5 W/m^2) at 26 ±1°C temperature up to 20 days of inoculation.

2.2. Analysis of wastewater

The physicochemical parameters of wastewaters were analysed before and after utilization using the standard procedure of APHA (1992), IS: 3025 (1984, 1986, 1988 and 2003), testing kit and spectrophotometer.

3. Results

3.1. Physico-chemical properties of paper mill wastewater

The partially treated effluent (ETP) of Emami Paper Mill was analysed for its physico-chemical parameters (Table 1). Due to organic load in paper industry, the colour of the effluent was orange and up to 32.4 HU. At the time of wastewater collection, the temperature was recorded to be 29°C and the nature of effluent was alkaline having pH 8.68. BOD and conductivity of the effluent were very high, approximately 230 mg/L and 2199µS/cm respectively. The effluent was little turbid with turbidity 10.9 NTU, total hardness 720 mg/L and alkalinity 40 mg/L. TSS and TDS were noted to be 78 and 195 mg/L respectively. The nutrient load in the wastewater found higher than the normal value and maximum value recorded for sulphate (147.9 mg/L) followed by calcium (125.63 mg/L), silica (57.18 mg/L), sodium (56.9 mg/L), magnesium (45.56 mg/L), nitrate (20.52 mg/L) and potassium (15.4 mg/L) but minimum phosphate (1.81 mg/L) and iron (1.23 mg/L).

3.2. Utilization of wastewater by test organisms

For nutrient utilization, *H. hibernicus* showed better removal of calcium (80%) than *L. planctonica* (63%) but less than that of *D. affine* (44%), *S. hoffmanii* (16%), *O. polymorpha* (12%) and *T. obliquus* (10%). Maximum reduction of magnesium was brought about by *L. planctonica* (58%) followed by *O. polymorpha* (57%), *S. hoffmanii* (41%) and less in *T. obliquus* (10%), *H. hibernicus* (17%) and *D. affine* (14%). Except *T. obliquus* (26%), all the test algal species showed better performance in terms of removal of sodium as in *D. affine* (88%) followed by *T. obliquus* (87%), *O. polymorpha* (60%), *H. hibernicus* (55%) and *S. hoffmanii* (51%). Potassium reduction was maximum (88%) in *L. planctonica* followed by *D. affine* (84%) and *O. polymorpha* (56%), *S. hoffmanii* (46%), *T. obliquus* (34%) and *H. hibernicus* (33%). Complete utilization (100%) of iron was observed in *L. planctonica*, *H. hibernicus*, *O. polymorpha* and *T. obliquus* followed by *D. affine* (70%) and *S. hoffmanii* (66%). Regarding sulphate, *L. planctonica*, *H. hibernicus*, *T. obliquus* and *D. affine* showed less utilization (percentage of reduction) than *S. hoffmanii* (60%) and *O. polymorpha* (56%). *L. planctonica*, *H. hibernicus*, *O. polymorpha* and *T. obliquus* completely utilized (100%) phosphate from wastewater than *S. hoffmanii* (88%) and *D. affine* (48%). However, maximum nitrate usage was executed by cyanobacterial species viz. *L. planctonica* (95%) followed by *H. hibernicus* (77%), *S. hoffmanii* (60%), *D. affine* (47%). But green alga showed less reduction of nitrate i,e 25% in *T. obliquus* and 15% in *O. polymorpha*. For silica, *S. hoffmanii* showed maximum utilization (81%) followed by *D. affine* (62%), *H. hibernicus* and *O. polymorpha* (60% in both), whereas less in *L. planctonica* (41%) and *T. obliquus* (18%) (Table 2, Plate 2).

4. Discussion

The above findings emphasized that all the test alga species are effective agents for amelioration of polluted habitats. They have potential to remove physical, chemical and nutritional loads from wastewaters (Rajasulochana *et al*., 2009; Rao *et al*., 2011; Umamaheswari & Shanthakumar, 2017). This highlighted the possibility and prospects of an alternative, cost effective and eco-friendly approach for wastewater utilization. The physico-chemical analysis of paper mill industry revealed that most of the parameters examined in assessing quality of wastewater are below permissible limit except colour, conductivity, turbidity, hardness, BOD and silica. All the waste grown test microalgal species could effectively remove all the physical and chemical impurities, remediate to permissible limit as reported earlier (Pathak *et al*., 2014; Brar *et al*., 2017; Das and Deka, 2019). Moreover, higher values of nitrates, phosphates and

BOD in the effluent favour growth of cyanobacteria (Palmer, 1969; Boominathan, 2005; Sanjaya *et al*., 2011) than green algae.

In Emami Paper Mill wastewater, iron and phosphates were fully removed (100%) by both the green algae, *O. polymorpha* and *T. obliquus* (Sengar *et al*., 2011) followed by cyanobacterial species like *L. planctonica* and *H. hibernicus.* Cyanobacterial species were proved to be more efficient in removal of nutrients like nitrate, silica, potassium and sodium as compared to green algae. *S. hoffmanii* and *T. obliquus* showed better performance inremoval of TSS, TDS and BOD from wastewater followed by *H. hibernicus* and *L. planctonica.* Except *S. hoffmanii,* other algal species were found effective for removal of turbidity from wastewater with satisfactory performance (100%) in *H. hibernicus*. Colour reduction and removal of hardness from wastewater was more in *H. hibernicus, L. planctonica.* pH was corrected to normal after treatment by all the test algal species except *S. hoffmanii.*

This establishes the versatility of microalgae as an important agent for remediation of industrial wastewater and as an important biological tool for assessment vis-a-vis monitoring of environmental toxicants (Chu, 2012). Assertion of their phycoremediation potential will definitely boost future research on development of algae-based technologies for better exploitation of their bioremediation potential with production of value-added by-products.

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Plate 1 (Figs. 1-6): Collection of waste grown algae and wastewater from Emami paper mill, Balasore, 1-3: Industrial area of the paper mill with effluent treated pond, 4-5: Surrounding rice fields of the paper mill, 6: Collection of waste grown algae from municipal sewage wastewater, Baripada.

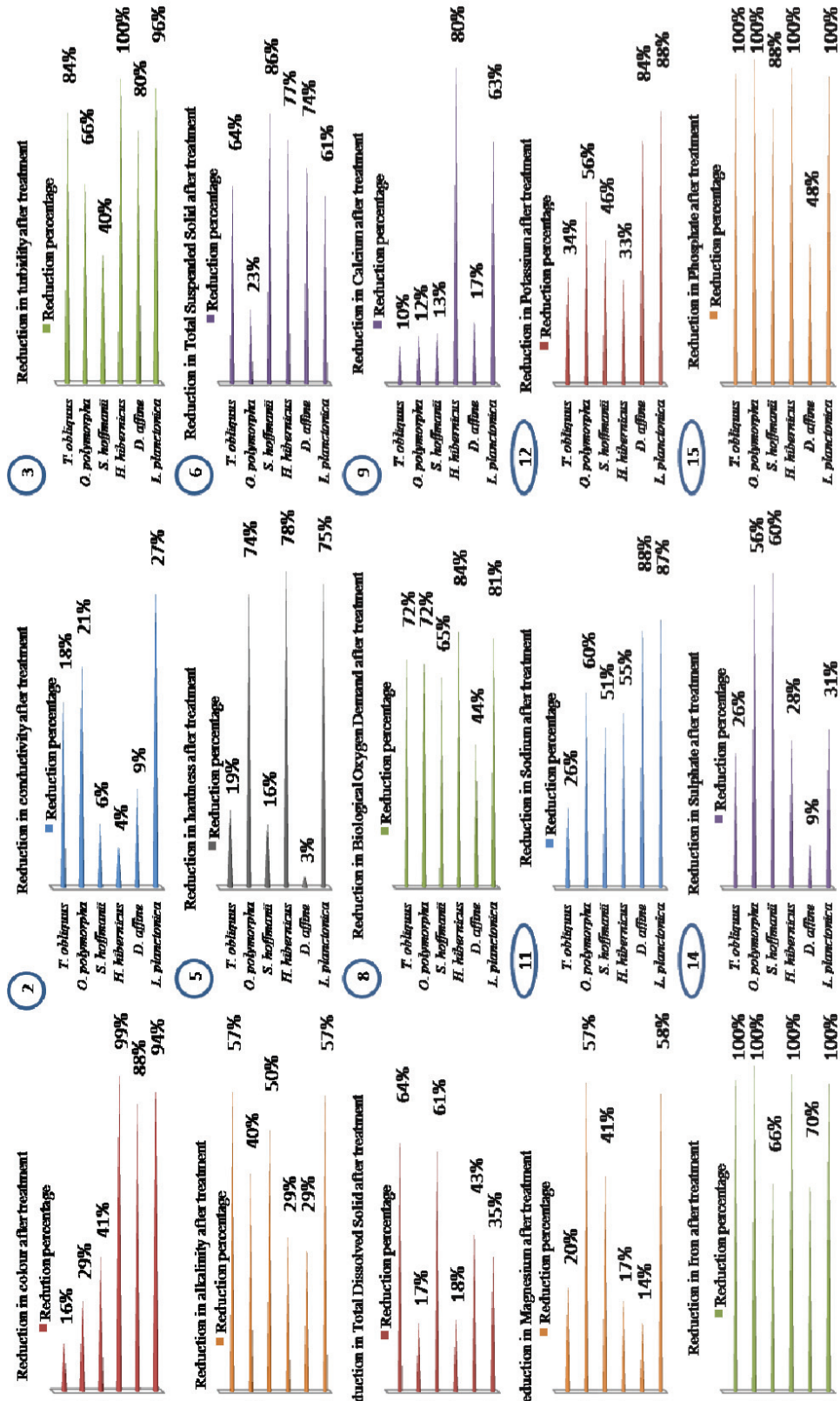

Plate 2 (Figs. 1-17): Comparative utilization (%) of different physicochemical parameters (colour, conductivity, turbidity, alkalinity, hardness, TSS, TDS, BOD, calcium, magnesium, sodium, potassium, iron, sulphate, phosphate, nitrate and silica) of selected concentrations of Emami paper mill wastewater by test microalgae.

Table-1

Physico-chemical characteristics of wastewater collected from effluent treatment pond (ETP) of Emami Paper Mill, Balasore.

Water Parameter																	
Colour (HU)	pH	Conductivity (µS/cm)	Turbidity (NTU)	Total alkalinity (as $CaCO_3$) (mg/L)	Total hardness (as CaCO3) (mg/L)	TSS (mg/L)	TDS (mg/L)	BOD (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	Sodium (mg/L)	Potassium (mg/L)	Iron (mg/L)	Sulphate (mg/L)	Total phosphate (mg/L)	Nitrate (mg/L)	Silica (mg/L)
32.4	8.68	2199	10.9	40.0	720.0	78	195	230	125.63	45.56	56.9	15.4	1.23	147.9	1.81	20.52	57.18

Table-2

Physico-chemical characteristics of selected concentrations of Emami Paper Mill wastewater (before and after utilization) collected from effluent treatment plant (ETP) suitably remediated by specific test algal species incubated at 26±1°C under continuous light of 7.5 W/m^2 up to 20 days.

Test Algal species		Water Parameter																	
		Colour (HU)	pH	Conductivity (µS/cm)	Turbidity (NTU)	Total alkalinity (as $CaCO_3$) (mg/L)	Total hardness (as CaCO3) (mg/L)	TSS (mg/L)	TDS (mg/L)	BOD (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	Sodium (mg/L)	Potassium (mg/L)	Iron (mg/L)	Sulphate (mg/L)	Total phosphate (mg/L)	Nitrate (mg/L)	Silica (mg/L)
Limnothrix planctonica (75% effluent)	Before	24.2	7.6	1649.3	7.8	30.0	540.0	58.5	146.3	172.5	94.2	34.2	42.7	11.6	0.9	110.9	1.4	15.4	42.9
	After	1.5	7.8 (-)	1209.3	0.34	13.0	135.0	23.0	95.0	32.2	35.3	14.2	5.7	1.4	0	76.5	0	0.7	25.3
	(% reduc.)	**(94%)**		(27%)	**(96%)**	**(57%)**	**(75%)**	**(61%)**	(35%)	**(81%)**	**(63%)**	**(58%)**	**(87%)**	**(88%)**	**(100%)**	(31%)	**(100%)**	**(95%)**	(41%)
Dolichospermum affine (75% effluent)	Before	24.2	7.6	1649.3	7.8	30.0	540.0	58.5	146.3	172.5	94.2	34.2	42.7	11.6	0.92	110.9	1.4	15.4	42.9
	After	3.0	7.7 (-)	1506.9	1.6	21.3	526.5	15.0	83.0	96.6	78.6	29.5	5.3	1.9	0.28	101.0	0.7	8.2	16.5
	(% reduc.)	**(88%)**		(9%)	(80%)	(29%)	(3%)	**(74%)**	(43%)	(44%)	(17%)	(14%)	**(88%)**	**(84%)**	**(70%)**	(9%)	(48%)	(47%)	**(62%)**
Hapalosiphon hibernicus (50% effluent)	Before	16.2	7.2	1099.5	5.5	20.0	360.0	39.0	97.5	115.0	62.8	22.8	28.5	7.7	0.61	73.9	0.91	10.3	28.6
	After	0.2	7.3 (-)	1055.6	0	14.2	81	9.0	80.0	18.4	12.4	19.0	12.9	5.2	0	53.3	0	2.3	11.5
	(% reduc.)	**(99%)**		(4%)	**(100%)**	(29%)	**(78%)**	**(77%)**	(18%)	**(84%)**	**(80%)**	(17%)	**(55%)**	(33%)	**(100%)**	(28%)	**(100%)**	**(77%)**	**(60%)**
Scytonema hoffmanii (100% effluent)	Before	32.4	8.7	2199.0	10.9	40.0	720.0	78.0	195.0	230.0	125.6	45.6	56.9	15.4	1.2	147.9	1.8	20.5	57.2
	After	18.9	7.4	2061.0	6.5	20.0	608.0	11.0	75.0	80.5	108.9	26.8	27.9	8.3	0.42	59.3	0.21	8.3	10.9
	(% reduc.)	(41%)	**(15%)**	(6%)	(40%)	**(50%)**	(16%)	**(86%)**	**(61%)**	**(65%)**	(13%)	(41%)	**(51%)**	(46%)	**(66%)**	**(60%)**	**(88%)**	**(60%)**	**(81%)**
Oocystis polymorpha (50% effluent)	Before	16.2	7.2	1099.5	5.5	20.0	360.0	39.0	97.5	115.0	62.8	22.8	28.5	7.7	0.6	73.9	0.9	10.3	28.6
	After	11.5	7.8 (-)	867.0	1.9	12.0	93	30.0	81.0	32.5	55.3	9.8	11.3	3.4	0	32.4	0	8.8	11.5
	(% reduc.)	(29%)		(21%)	**(66%)**	(40%)	**(74%)**	(23%)	(17%)	**(72%)**	(12%)	**(57%)**	**(60%)**	**(56%)**	**(100%)**	**(56%)**	**(100%)**	(15%)	**(60%)**
Tetradesmus obliquus (75% effluent)	Before	24.2	7.6	1649.3	7.9	30.0	540.0	58.5	146.3	172.5	94.2	34.2	42.7	11.6	0.9	110.9	1.4	15.4	42.9
	After	20.3	7.8 (-)	1354.4	1.2	13.0	435.0	21.0	53.0	48.3	84.5	27.3	31.5	7.6	0	82.1	0	11.5	35.3
	(% reduc.)	(16%)		(18%)	**(84%)**	**(57%)**	(19%)	**(64%)**	**(64%)**	**(72%)**	(10%)	(20%)	(26%)	(34%)	**(100%)**	(26%)	**(100%)**	(25%)	(18%)

(-) = No reduction; (reduc.)= Reduction

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New distributional records of three Naviculales (Naviculaceae) from the Bhitarakanika National Park of Odisha, India

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ABSTRACT

Bhitarkanika Wildlife Sanctuary, situated in the north-eastern part of Kendrapada district of Odisha, India, spreads over an area of about 672 sq. km. Within the sanctuary, an area of approximately 145 sq.km. is designated as Bhitarkanika National Park and obtained the status of a Ramsar site on 19th August 2002. It is the second largest mangrove ecosystem of India consisting of huge mangrove forests, river deltas, estuaries, backwaters and mud flats etc. They serve as the base of an elaborate and productive food web in this extraordinarily diverse estuarine ecosystem. The present investigation aims at enumeration and ultrastructural examination of certain diatom taxa collected as a part of our ongoing studies on diatom diversity of major areas of Bhitarkanika national park *viz*. Dangmal, Kalibhanjdian, Khola, Bhitarkanika and Ragarapatia. The present paper reports the occurrence of three diatom species namely, *Navicula subrostellata* Hustedt, *Navicula torneensis* Cleve and *Caloneis oregonica* (Ehrenberg) R.M. Patrick belonging to order Naviculales under the family Naviculaceae from Bhitarkanika National Park as the new distributional records from India based on examination of morphological features and ultrastructure through scanning electron microscopy (SEM). Occurrence of these 3 diatom species in Bhitarkanika Wildlife Sanctuary is of considerable phytogeographical significance and important addition to the checklist of least-explored diatom flora of this important mangrove habitat.

1. Introduction

Mangroves are one of the most extraordinarily diverse evergreen estuarine open ecosystems, formed by a variety of salt tolerant species growing in the inter-tidal areas and estuary mouth, can provide critical habitat for a diverse marine, brackish and terrestrial flora and fauna. They act as nutrient sinks and protect offshore ecosystems and often referred to as bio-shields or natural sea defence (Roy *et al.*, 2009). Nearly 60-70% of the world's tropical and subtropical coastlines are covered with mangroves, which are known to be world's most productive ecosystems of remarkable ecological value (Thatoi *et al.*, 2013). The dynamic mangrove ecosystem supports the growth of many phosphate solubilizing, nitrogen fixing, sulphate reducing and methanogenic bacteria, wood degrading fungi as well as photosynthetic algae like diatoms, cyanobacteria, green-algae and other microalgae, which play significant role in maintaining nutrient cycling and ecological balance in this unique ecosystem. One of the major groups of microalgae in mangroves is diatoms, which is a very valuable environment indicator. About one-fifth of the photosynthesis on earth is carried out by these fabulous photosynthetic workhouses. Therefore, exploration of these bioresources from different ecosystems will definitely help us to understand their role in ecosystem functioning and show the path for bioprospecting for commercial applications.

Bhitarkanika, the second largest mangrove ecosystem of India, consists of mangrove forests, river deltas, creeks, estuaries, backwater, accreted land and mud flats. Most of the published literature on this sanctuary pertains to documentation of flora and fauna. Due to inaccessible forest cover and vast expanse of water, the microbes of thisnational park have remained poorly studied. Though scanty reports are available on phytoplankton and other microalgal diversity (Rath & Adhikary, 2006; Chakraborty *et al*., 2012; Thatoi *et al*., 2012; Dash *et al.*, 2019), not much work has been done

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on the taxonomy and diversity of diatoms of Bhitarkanika. In the present investigation, attempt has been made, for the first time, to examine the ultrastructure of three brackish water diatoms through scanning electron microscopy (SEM).

2. Materials and methods

Bhitarkanika, a mangrove wetland in Odisha is surrounded by Bay of Bengal on east, the villages of Kendrapara district on west, Dhamra estuary on north and the Mahanadi estuary on south. Field visits were made to Bhitarkanika and diatom samples were collected randomly from different niches of mangrove sediments, mud surfaces, attached to pneumatophores of some selected mangrove species. Sample collections were made from sites such as Khola, Dangmal, Kalibhanjdian, Bhitarkanika and Ragarapatia of Bhitarkanika Wildlife Sanctuary and National Park during November-December, 2018. Samples were collected in sterile specimen tubes (Tarson) of 25 × 50 mm size in duplicate of which one set was stored in ethanol. Ethanol was added to reach final concentration of 20% by volume (Kolbe, 1948; Krammer & Lange-Bertalot, 2000; Krammer, 2002; Karthic *et al*., 2010). Diatoms were cleaned by acid wash method using HCL and pre-oxidant potassium permanganate (Hasle, 1978; Round *et al*., 1990; Karthic *et al.*, 2010) and hot hydrogen peroxide method (Van Der Werff, 1955; Karthic *et al*., 2010). After that, cleaned diatoms were stored in 70% of ethanol for further study. For identification, the permanent slides of cleaned diatoms were prepared and observed under the microscope (Hund Wetzlar Trinocular Compound Microscope with Canon-EOS 550D Camera attachment), followed by photomicrography. The processed diatoms were diagnosed under a Scanning Electron Microscope (Zeiss EVO 18 special edition). The diatom species were identified through morphometrical analysis following standard monographs and literature (Cleve, 1894; Hustedt, 1930; Cleve-Euler, 1953; Desikachary, 1988). Taxonomy of each species and the protologues were verified as cited in algaebase.org (https://www.google.com) (Guiry & Guiry, 2019) and diatombase.org (https://www.diatombase.org).

3. Enumeration of species

3.1. ***Navicula subrostellata*** Hustedt 1955: 27 (Plate 1, Fig. 1-2)

Lanceolate to linear-lanceolate valves with protracted apices, length of valve (AA) 29-31.5 µm, width of valve (TA) 6.5 -7 µm. The axial area is narrow and straight. The central area is round to elliptical. External proximal raphe ends are straight and very close to each other. Striae are radiated around the centre, parallel then gently convergent at the apices, transapically elongated areolae, number of striae 14 in 10 µm.

Place of collection: Bhitarkanika.

Mode of occurrence: Benthic and epiphytic.

3.2. ***Navicula torneensis*** Cleve 1891: 33 (Plate 1, Fig. 3)

Synonym: *Schizonema torneense* (Cleve) Kuntze

Valve wide lanceolate with cuneate to slightly rostrate ends, length of the valve 35-36.9 µm, width of the valve 16-17 µm. Striae slightly curved around central area, radiate throughout the valve, no of striae 12-13 in 10µm, some short striae are found around the central area. The first group of areolae is slightly more elongated, slits like areolae are present surrounding the central area. Axial area narrow and linear. Small central area, circular to transversely elliptical due to position of striae. Raphe straight, thin with simple drop like central endings, apical endings are simple and slightly deflected to opposite side of the valve.

Place of collection: Bhitarkanika.

Mode of occurrence: Benthic.

3.3. ***Caloneis oregonica*** (Ehrenberg) R.M. Patrick 1966: 581 (Plate 2, Fig. 1-7)

Basionym: *Pinnularia oregonica* Ehrenberg

Synonyms: *Navicula oregonica* (Ehrenberg) Kützing; *Navicula liburnica* Grunow; *Caloneis liburnica* Grunow; *Navicula amphisbaena* var. *liburnica* (Grunow) M. Peragallo

Valve elliptic to linear-elliptic, flat valve external surface with rounded apices, length of valve (AA) 40-75 µm, width of valve (TA) 13-17 µm. Axial area slightly irregular, lanceolate, comparatively wider at centre, tapering towards the valve apices and situated more closely to the raphe on one side than the other. Central area distinctly separated from axial area and almost circular to transversally elliptical to slightly rhombic due to the position of striae. Central nodule slightly depressed externally. External raphe branches thin, straight, central raphe fissures end in pore-like expansions; large, hooked, sickle-shaped terminal raphe endings extending up to the end mantle area, both deflected to same side of the valve. A single longitudinal line runs parallel with the valve margin at about two-third of the distance from the raphe to the valve rim which is quite distinct (Fig.1). Internally the axial area quite distinct, at the centre a slightly siliceous thickening occurs on one side of the central raphe ends. Internally raphe straight with simple central raphe endings, slightly bent towards siliceous thickening and terminal endings with simple coaxial pore, small compressed and horseshoe-shaped helictoglossa (Fig.6). Striae slightly radiate to parallel, number of stria density: 15-16.

Place of collection: Bhitarkanika.

Mode of occurrence: Benthic.

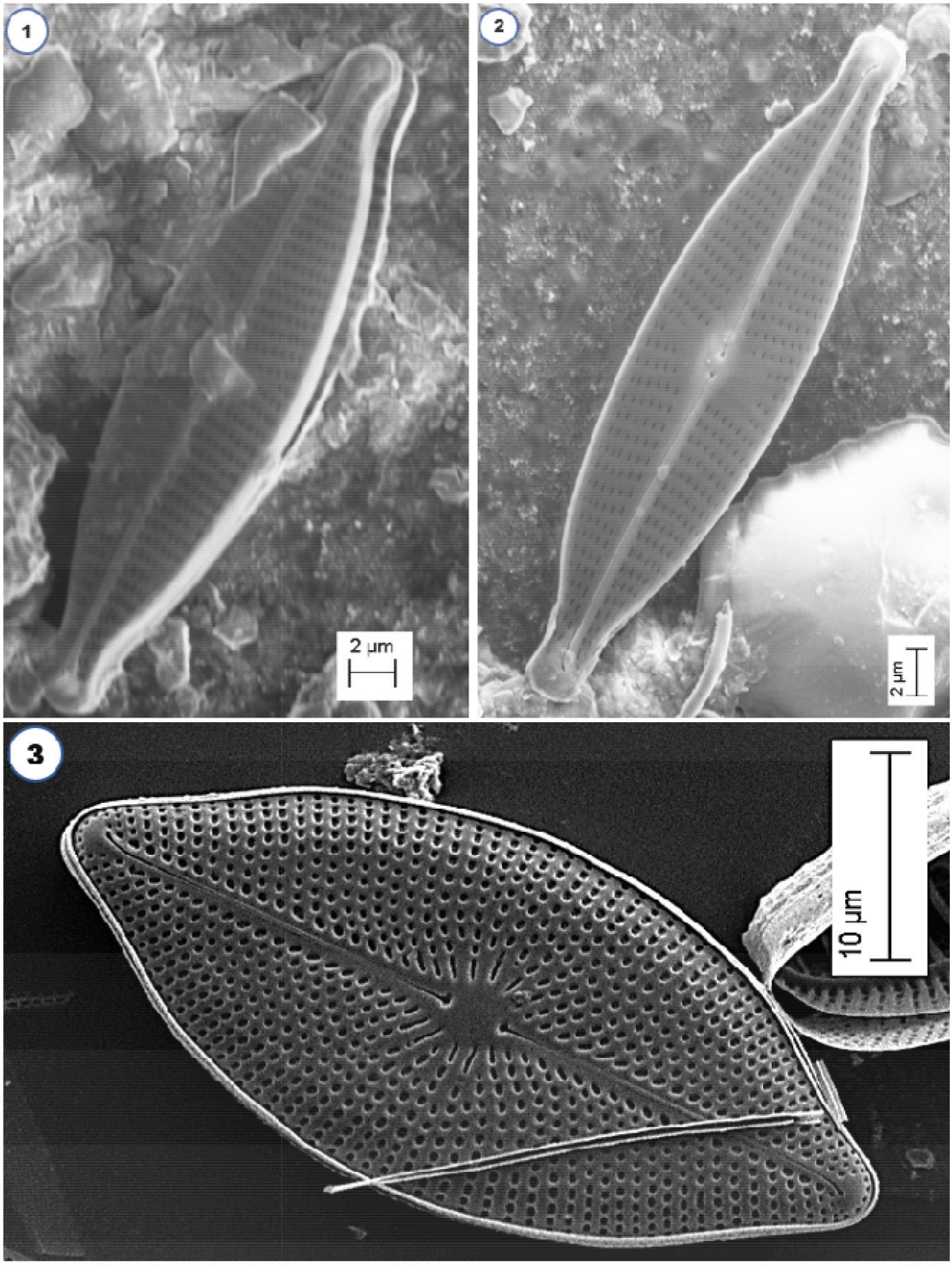

Plate 1 (Figs. 1-3): Fig. 1-2 *Navicula subrostellata*; **Fig. 3** *Navicula torneensis*

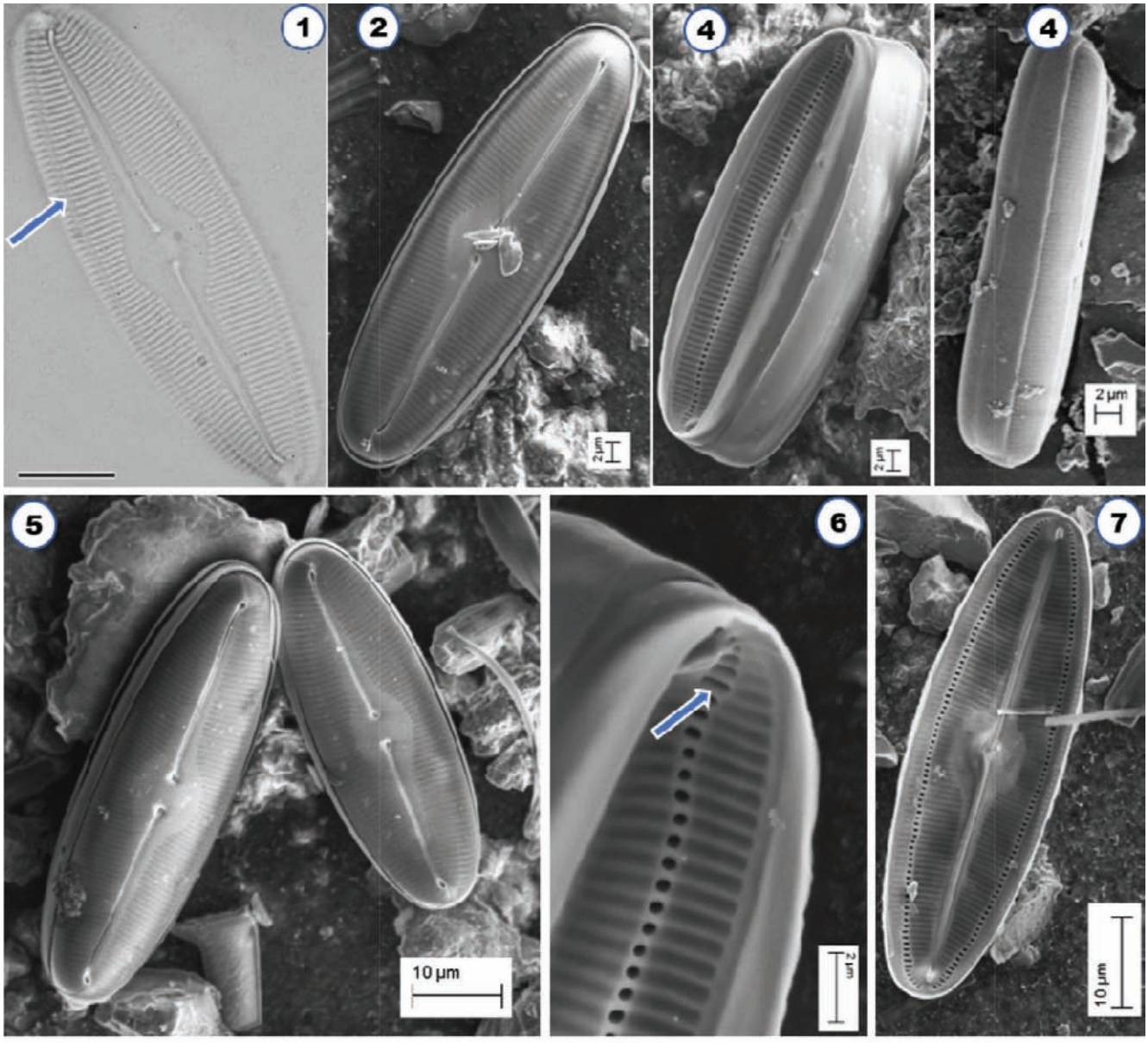

Plate 2 (Figs. 1-7): *Coloneis oregonica*, LM = 10X

4. Discussion

The present investigation discloses the occurrence of three Naviculales, namely, *Navicula subrostellata* Hustedt, *Navicula torneensis* Cleve, *Caloneis oregonica* (Ehrenberg) R.M. Patrick belonging to family Naviculaceae from Bhitarkanika, this being the first report from India. According to earlier distributional records, *Caloneis oregonica* was reported to be mostly confined to brackish water habitat worldwide (Kociolek, 2005; Maulood *et al*., 2013), but *Navicula subrostellata* was a marine form (Hafner *et al*., 2018). *Navicula torneensis* Cleve was the most common species of different estuaries of Korea (Joh, 2013) and mostly found as epipssamic forms. Exploring diatom diversity from this unexplored area will not only provide an opportunity for exploring of different endemic taxa and new distributional records from those unique habitats but also unlatch the opportunity for obtaining different novel organisms for various commercial purposes.

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Notes on the occurrence of *Spigelia anthelmia* L. (Loganiaceae) in Odisha, India

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ABSTRACT

The present communication reports the occurrence of *Spigelia anthelmia* L. (Loganiaceae), a native plant of South America, as a new distributional record for the state of Odisha. With this, the range of distribution of this species is extended further to the east of India. The nomenclature, botanical description, notes on phenology, ecology and distribution have been provided in this paper along with field photographs to facilitate easy identification.

1. Introduction

Due to peculiar geographical location, topography, varied climatic and edaphic conditions, the flora of Odisha is interesting in terms of species content, vegetation diversity and phyto-geographical considerations. The flora of the state is an admixture of north and south Indian elements; and it is often postulated that the higher hill tops are considered as the meeting grounds for the Himalayan and the South Indian plant species (Gamble, 1892; Meher-Homji, 2001). The flora and vegetation of Odisha have been studied by several workers in the past (Gamble & Fischer, 1915-36; Haines, 1921-25; Mooney, 1950; Saxena & Brahmam, 1994-96) and subsequently, a good number of wild and naturalized species have been added to the state flora.

In connection with a recent study of diversity and distribution of plant resources of Balasore district, Odisha, an interesting plant was collected from Mitrapur, Balasore. After critical examination of morphological characters and consultation of available literature, the specimen could be identified as *Spigelia anthelmia* L. (Loganiaceae). Updated nomenclature, botanical description, photographs of plant and plant parts, notes on ecology, phenology, distribution etc. of the taxon have been provided below.

The genus *Spigelia* L. (Loganiaceae) is native to Central and South East America to tropical and sub-tropical America and is represented by 96 species in the world (POWO, 2022). Though *Spigelia anthelmia* L. is native to tropical and sub-tropical America, the species has been reported to occur as an alien in different states of India. In India, it was first reported from Jabalpur, Madhya Pradesh (Oomachan& Srivastava, 1987). Subsequently, the occurrence of *S. anthelmia* has been recorded from Tamil Nadu (Umamaheswari *et al*., 1995), Maharashtra (Pardeshi & Srinivasu, 2006; Kamble & Chaturvedi, 2010), Rajasthan (Meena & Yadav, 2010), Karnataka (Hegde *et al*., 2013), Gujarat (Desai & Raole, 2013; Meena *et al.*, 2014), Kerala (Sasidharan & Dantus, 2014) and Telangana (Swamy & Jalander, 2021). However, the species is not reported from any state of Eastern India. The present report on occurrence of *Spigelia anthelmia* L. in Odisha extends the range of

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distribution of the species to eastern part of the country and the taxon is expected to have invaded in adjoining states too. The extended distribution of this American alien may be due to seed dispersal through different means and subsequent establishment and naturalization in suitable habitats. The species turned out to be a new generic addition to the flora of the Odisha.

Spigelia anthelmia L. Sp. Pl. 149. 1753; Oomachan & Srivastava, J. Bomb. Nat. Hist. Soc. 84 (3): 730-732.1987; Umamaheshwari *et al*., Nelumbo. 37 (1-4): 133-137. 1995; Meena & Yadav, J. Indian Bot. Soc. 89 (3 & 4): 258-261.2010; Hegde *et al*., J. Bot. 2 (2): 9. 2013. (Fig. 1.)

Herbs, upto 50cm.; roots wiry, spreading; stems unbranched or few branches towards the base; erect, hollow, glabrous, green, terete; leaves simple, sessile, pseudo-whorl of two opposite pairs, anisophyllous, upper pair larger than lower, ovate-lanceolate, 3-11×1-5 cm, basal leaves smaller than apical leaves; blade sparsely pubescent to glabrous adaxially and puberulous along the veins abaxially, margin ciliate with simple trichomes, acute-acuminate apex, base equal to unequal, decurrent, mid vein broad basally, narrowed towards apex, secondary nerves prominent anastomising at margin, parallel from origin; stipules interpetiolar, membranaceous, broad and triangular, 2-3mm long; inflorescence monochasial scorpoid cyme, trichotomous, terminal, secund with 10-30 flowers; flowers linear-lanceolate, bisexual, 1.2-1.5 cm long, pedicel 1mm long; calyx 5 lobed, linear-lanceolate, 2-3.5 mm long, ciliate at margin; corolla lobes 5, lilac to white, tubulate-funnel shaped, tube 6-10 mm long, 2-2.2 mm broad at throat, lobes ovate-deltoid, acute, with two purple lines on middle surface; stamens epipetalous, adhering to lower part of the tube, filaments 2-3 mm long; stigma pubescent, style elongated with slightly swollen at middle portion, 5-6.2 mm long,ovary superior, 0.5 mm long, sub-globose, capsule green muricate, bi-lobed with persistent portion of style upto 2mm long, 5-5.5 mm long and 5-5-6 mm wide, receptacle boat shaped 3.3-4×0.5 mm, seeds many per locule, curved, brown, 1.7×1 mm.

Flowering and Fruiting: September-January.

Specimen examined:

Odisha**:** Nuapadhi, Balasore, A. K. Biswal and P. B. Sahoo, 1574, Dt. 07.09.2022, Herbarium, North Orissa University, Baripada.

World distribution:

Native to South America; introduced and naturalized in Tropical West Africa, South East Asia and India.

Distribution in India:

Madhya Pradesh, Maharashtra, Gujarat, Rajasthan, Karnataka, Kerala, Tamilnadu, Telangana and Odisha.

Ecology:

The taxon is sporadically found in wastelands, along roads and scrub jungles under shade. A total of 112 individuals of *Spigelia anthelmia* were found in the population in association with species like *Achyranthes aspera* L., *Alloteropsis cimicina* (L.) Stapf, *Alysicarpus vaginalis* (L.) DC., *Chamaecrista mimosoides* (L.) Greene, *Digitaria longiflora* (Retz.) Pers., *Evolvulus nummularius* (L.) L., *Grona triflora* (L.) H. Ohashi & K. Ohashi and *Physalis angulata* L.

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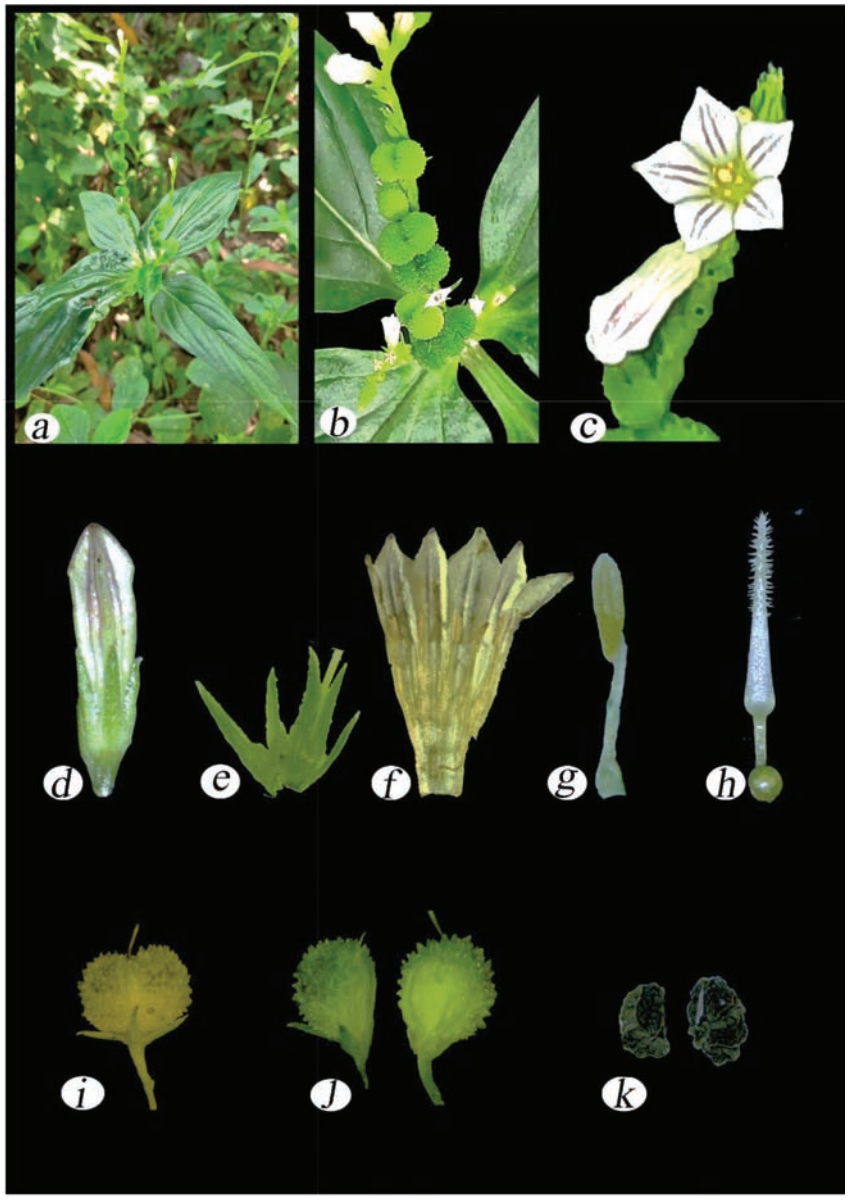

Fig. 1 *Spigelia anthelmia* L.: A. habit, B. inflorescence, C. flower, D. flower bud. E. calyx, F. opened corolla, G. stamen, H. carpel, I. bi-lobed capsule, J. longitudinal section of capsule, K. seed

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Secondary metabolites in *Uvaria hamiltonii* Hook. f. & Thoms. (Annonaceae) and their pharmacological properties: A review

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ABSTRACT

Uvaria hamiltonii, commonly known as "Eastern Uvaria", "Lakankoli" or "Latkan", is one of the valuable medicinal plants of Annonaceae family. It is native to India, Bangladesh, Myanmar, Nepal, Bhutan, Cambodia, Thailand, Vietnam Nepal, Bhutan, Bangladesh and Burma. Different parts of the plant are reported to contain a number of alkaloids, steroids, flavonoids and terpenoids, which are responsible for antibacterial, anticancer and á-glucosidase inhibitory and various other pharmacological properties.This paper presents a comprehensive overview of traditional uses, current knowledge on the phytochemistry and biological activities of *U. hamiltonii,* which may open up avenues for its utilization in traditional and modern medicine in future.

1. Introduction

Natural products and traditional medicines have always been in the forefront of all cultures and civilisations despite the predominance of modern medicine. The therapeutic properties of medicinal plants are attributed to the presence of various secondary metabolites such as alkaloids, flavonoids, steroids, phenolics, saponins, tannins, terpenoids, iridoids and cardiac glycosides. They are potential sources of phytochemicals for drug formulation and the examples are: quinine from *Cinchona*, insulin from roots of *Dahlia*, morphine from poppy and digoxin from *Digitalis* (Padmavathi, 2013). WHO endorses and promotes the application of herbal drugs in national health care programmes of the states as they are cost-effective, eco-friendly and without minimal or no side effects in comparison to synthetic drugs (Singh & Singh, 1981).

2. Botany, distribution and traditional uses of *U. hamiltonii*

The family Annonaceae is comprised of 108 accepted genera and about 2400 species globally (Chatrou *et al.*, 2012). The species of the family are used in the tropics in traditional medicines for the treatment of various illness (Frausin, 2014). *Uvaria hamiltonii* Hook. f. & Thoms., a rare woody climber of the family Annonaceae is well-known for its anti-cancerous properties. Taxonomically, *Uvaria hamiltonii* belongs to the family Annonaceae, order Magnoliales, subclass Magnoliidae, class Magnoliopsida and phylum Tracheophyta (Mabberley, 2008). The plant is known by several vernacular names such as Latkan in West Bengal, Zawl-thei in Mizoram, Taipak in Tripura and Lakankoli/ Lakhankoli in Odisha (POWO, 2022) (https://www.flowersofindia.net).

Uvaria hamiltonii Hook. f. & Thoms. is a large scandent shrub with rusty tomentose branches and the leaves are simple, elliptic to oblong, obovate, tomentose beneath. The flowers are deep red or scarlet, bisexual, solitary or 2-3 fascicles; flower stalks are woolly; sepals 3, petals 6; stamens many. Carpels are many, about 10, orange, tomentose; style short, thick; seeds many, flat and shining (Saxena & Brahmam, 1994) (Fig. 1 a & b).

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Fig. 1. (a) *Uvaria hamiltonii* plant (b) Flower of *Uvaria hamiltonii*

Turner (2015) has described the range of distribution of the species in the world. It is native to South Asian countries like Nepal, Thailand, Vietnam, Bhutan, India, Bangladesh and Burma. In India, it is reported to occur wild in Uttar Pradesh, Bihar, Mizoram, Sikkim, West Bengal, Assam, Meghalaya, Tripura, Odisha and Andhra Pradesh.

Though no published report on the mode of propagation of *U. hamiltonii* is available, as per the field observation by the authors and local inhabitants, it multiplies by seeds. In Odisha, the species mostly grow wild along streams and rivers and the seeds are dispersed by water and get established at suitable habitats. Majority of the ripe fruits are consumed by the local tribals and frugivorous birds and the fallen seeds in stream water perish and do not germinate, resulting in rarity of the species in forests.

In traditional medicine, the extracts of different parts of this plant are being used for treatment of diabetes mellitus, malaria, gonorrhea, jaundice, bronchitis and minor infections (Asha *et al*., 2003; Rahmatullah *et al*., 2010).

3. Phytochemical constituents

The phytochemical analysis of different parts of *U. hamiltonii* carried out by previous workers revealed the presence of alkaloids, steroids, flavonoids, terpenoids and carbohydrates. List of phytochemicals found in the plant is provided in Table 1. Besides, a total of five aristolactams have been isolated from the stem bark of *U. hamiltonii* by Hasan *et al*. (2001). While four of these were isolated from the dichloromethane extract, the fifth alkaloid was obtained from the methanolic extract. Two flavanones, hamiltones A and B, an aurone, hamiltrone, a chalcone, hamilcone and a tetrahydroxanthene, hamilxanthene have been isolated from *U. hamiltonii* extracts guided initially by fractionation based on DNA strand-scission and/or 9KB cytotoxicity assays (Huang *et al*., 1998). Further, Huong *et al.* (2022) analysed the leaf essential oil of *U. hamiltonii* and identified germacrene D (22.9%), â-caryophyllene (21.1%), bicyclogermacrene (11.2%) and caryophyllene oxide (8.6%) as the main constituents. Two known steroids, stigmasterol and 6-hydroxystigmasta-4, 22-dien-3-one and two unusual polyketides, *cis*-4-hydroxymellein and *trans*-4-hydroxymellein were isolated from the stem bark of *Uvaria hamiltonii* (Asha *et al*., 2004). They elucidated the structures of the compounds by high-resolution 2D-NMR techniques and confirmed the same by comparison with previously reported values.

Barman *et al*. (2021) identified specialized metabolites contributing to colour and scent volatiles in *Uvaria hamiltonii* flowers. Among the 34 compounds identified, they found sesquiterpenoids as the dominant constituents in the floral volatiles. The anthocyanin pigment responsible for the flower colour was also explored and it was revealed that a single anthocyanin compound, cyanidin-3-O-glucoside, was principally responsible for petal colour.

4. Pharmacological properties

Different plant parts of *U. hamiltonii* have been investigated for their pharmacological properties and biological activities as presented in Table 1. The bioactivities of different plant parts are described below, which may facilitate exploration of the species for various pharmaceutical applications.

Table 1

Chemical compounds with medicinal value isolated from plant parts of *U. hamiltonii*

Pharmacological Activity	Plant parts used	Solvent used	Specific compounds involved	Figures	References
Anticancer	Leaf and stem	Ethanol	Hamiltone, Hamiltrone, Hamilcone and Hamilxanthene	Fig.2. (a, b, c, e)	Huang *et al*., 1998
á-glucosidase inhibitory	Leaf	Ethyl acetate	Grandifloracin, Kaempferol and Benzoic acid	Fig.2. (d, f, g)	Meesakul *et al*., 2020
Antibacterial	Stem bark	Petroleum ether Dichloromethane Methanol	Piperolactum C	-	Asha *et al*., 2003
Anti-oxidant	Flower	Methanolic extract Aqueous extract	Anthocyanins	-	Barman *et al*., 2021

4.1. Anticancer activities

Huang *et al.* (1998) isolated compounds from the combined leaf and stem ethanolic extracts of *U. hamiltonii*, guided initially by fractionation based on DNA strand-scission or 9KB cytotoxicity assays.They found that two flavanones namely, hamiltones A & B and an aurone called hamiltronewere inactive in the 9KB cytotoxicity assay, while a chalcone, hamilcone and a tetrahydroxanthene, hamilxanthene exhibited weak cytotoxic activity. It was suggested that these compounds can be useful in cancer chemotherapy.

4.2. á-glucosidase inhibitory activities

A preliminary screening of á-glucosidase inhibitory activity of this plant indicated that ethylacetate extract of leaves have promising á-glucosidase inhibitory activities with an IC_{50}value of 78.4 µg/mLand compounds like grandifloracin, kaempferol and benzoic acid have potent á-glucosidase inhibitory activity (Meesakul *et al*.,2020).

4.3. Antibacterial activities

The antibacterial activity of *U. hamiltonii* has been assessed with standard antibiotic kanamycin by Asha *et al.* (2003). The work discussed aboutchemical compounds namely piperolactum C, goniopedaline, 6b-hydroxystigmasta-4,22-dien-3-one and a mixture of cis-and trans-4-hydroxymelleins obtained from *U. hamiltonii* stem bark, which are responsible for antibacterial properties. The leaf essential oil of *U. hamiltonii* demonstrated notable antimicrobial activity against *Enterococcus faecalis* ATCC299212 with minimum inhibitory concentration (MIC) value of 7.99 µg/mL and *Bacillus cereus* ATCC14579 (MIC 5.67 µg/mL) (Huong *et al*., 2022)

4.4. Antioxidant capacity

Total phenolic content was evaluated and antioxidant capacities of *U. hamiltonii* flowers were studied by Barman *et al.* (2021) with the help of DPPH, FRAP and ABTS assays. The total phenolic content and the antioxidant capacity were found to be higher in methanolic extract as compared to aqueous, petroleum ether and ethyl acetate extracts.

The structures of medicinally potent chemical compounds reported in this plant are depicted in Fig. 2.

5. Conclusion

U. hamiltonii is a unique source of various types of chemical compounds with diverse bioactivities. Since very less works have been performed on its biological activity, intensive investigation is necessary to identify and isolate other bioactive compounds from the plant which may help in drug formulations. The present review focuses on the antibacterial, anticancerous, á-glucosidase inhibitory and antioxidant activity of this plant.

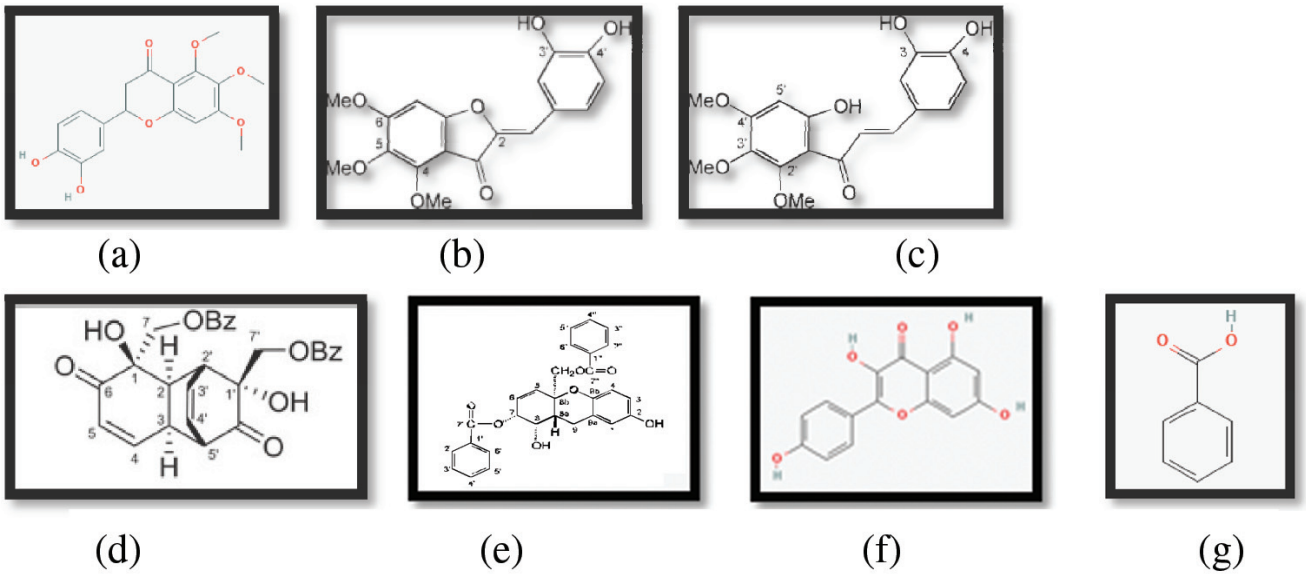

Fig. 2. Structures of compounds isolated from *Uvaria hamiltonii*: (a) Hamiltone (b) Hamiltrone (c) Hamilcone (d) Grandifloracin(e) Hamilxanthene (f) Kaempferol (g) Benzoic acid (Source: Huang *et al*., 1998; Asha, *et al*., 2003; Meesakul *et al*., 2020)

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Wild edible mushrooms of Similipal and their contribution to health, nutrition and economic security of tribals

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ABSTRACT

Similipal Biosphere Reserve, Odishais one of the most diverse ecosystems of the country harbouring rich flora and fauna including mushrooms. The fertile forest floors provide congenial habitat of a wide variety wild mushrooms. A survey was carried out during rainy season (June to October) of 2022 to collect information regarding different wild edible mushrooms occurring in Similipal and their collection by tribals for own consumption, medicine and sale in the nearby markets. The paper reports the occurrence of 12 wild edible mushrooms such as *Russula rosea, R. cyanoxantha, R. brevipes, R. nigricans, Astreatus hygrometicus, Bovista plumbea, Amanita vaginata, A. hemibapha, Termitomyce sheimii, T. eurrhizus, T. clypeatus* and *T. medius* commonly found in Sal forests of Similipal. Some of the mushrooms (*Russula rosea, R. cyanoxantha, R. brevipes, R. nigricans, Amanita vaginata, A. hemibapha*) are locally called 'Patra Chhatu, which are plentily available throughout the rainy season on forest floors rich in leaf litter. Another important group of mushrooms are *Termitomyces* species (*T. heimii, T. eurrhizus, T. clypeatus, T. medius*), which grow in termite nest and also in soft red soils in the Sal forests. These are tastier than that of '*Patra Chhatu'* and are available in bulk quantities in the forest. These mushrooms are also sold at a higher price but grow for a short period of time after rain. The market value of species belonging to genus *Russula* was cheaper in rural areas (Rs.10–30/kg) as compared to urban markets (Rs.60–90/kg) while that of *Termitomyces* species are sold at comparatively higher price in both rural (Rs.200-300/kg) and urban markets (Rs.350–400/kg). These mushrooms are regarded as highly nutritious containing large amounts of proteins, fibers, vitamins and minerals, which support the health of local tribals and boost their economy.

1. Introduction

Forest plays an important role in supporting the livelihood of rural and tribal communities and also in maintaining the ecological balance. Over 53 million tribals and about 60% of the rural communities in India directly rely on forests for their day-to-day requirements.The availability, quantity and pattern of utilization of both timber and non-timber forest products (NTFPs) are crucial to their livelihood sustainability and income generation. The forest dwellers and communities residing close to forests derive their day-to-day needs of food, firewood, shelter, medicine, fiberetc from forests. Some major NTFPs found in the Similipal Forest Division are Mahula flowers and seeds (*Madhuca longifolia* var. *latifolia*), Jhuna resin of *Shorea robusta*), Kurkuti (Red Weaver Ant), wild fruits like Kendu (*Diospyros melanoxylon*), Jamu (*Syzygium cumini*), medicinal plants like Mudika (*Cissampelos pareira*), Patala Garuda (*Rauvolfia serpentina*), Bhuin Nima (*Andrographis paniculata*), wild edible mushrooms etc. Among all NTFPs, though seasonal in habit, the wild growing mushrooms have long been used as a valuable source of food by the tribals.

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Wild edible mushrooms (WEM) are been collected and consumed by people for thousands of years. The edible mushrooms have different forms, some with gills and some with pores, some with stems and some without. Wild edible mushrooms are a valuable non-timber forest resource used by mycophilic societies and their use has been documented in many countries around the world (Chang and Lee, 2004, Roberto & Levesque, 2005). They substantially contribute to the diet and food habit of the local people. The collection, consumption and marketing of wild edible mushrooms is a practice of tribal and poor people in more than 80 countries in the world. The biological diversity of mushrooms is huge and they belong to different use-classes and morphological types.

The number of edible mushroom is only a small fraction of the total mushroom diversity. Of the 14,000 recognized mushroom species (Hawksworth *et al*., 2001; Kirk *et al*., 2008; Schmit *et al*., 2007), the recorded number of wild edible mushrooms is about 1154, which is only 8.24% of the estimated species (Boa *et al*., 2004). Only 15 of the 284 edible species are regularly eaten in Armenia (Boa, 2004). They are sold in traditional markets (Roberto & Levesque, 2005) or commercially exploited as food (Pilz *et al.*, 1999) or medicines (Chamberlain, 1996). The ethnomedicinal aspects of mushrooms have also been studied and documented by few workers in different parts of India and world (Harsh *et al.*, 1993; Didukh, 2001; Adhikary *et al*., 2005). In India, the tribal and rural communities traditionally use and possess ethnomedicinal knowledge on as many as 283 species of wild mushrooms out of approximately 2000 species known globally (Purkayastha and Chandra, 1985). Some of the wild edible mushrooms have also been reported from Manipur and Arunachal Pradesh of North East India (Sing and Sing, 1993; Sing *et al*., 2002). Sometimes edible mushrooms liked by one community may be considered unsuitable for others and often corelated with some health issues or adverse effects. The nutritional value of wild edible mushroom should not be underestimated as many of them are of comparable or nutritionally superior than conventionally consumed vegetables. However, the choice of mushrooms, edibility, pattern of consumption, market value etc. vary considerably among countries and localities.

Fungi are dependent on dead and living material for their growth. They obtain their nutrients in three basic ways: (i) Saprobic - growing on dead organic matter; (ii) Symbiotic - growing in association with other organisms and (iii) Pathogenic or Parasitic - causing harm to another organism. Mushrooms have extended relationship with men and are of profound economic and biological significance. Wild edible mushrooms have been eaten by human beings with delicacy possibly, for their pleasing flavour and taste (Das, 2010). They are rich sources of proteins and other nutrients and minerals including amino acids, sugar, and vitamins with low fat and cholesterol levels. In view of this, the developed countries are increasingly consuming wild edible mushrooms as a part of their daily diet (Barros *et al*., 2008; Kalac, 2009). According to Mattila *et al.* (1994), mushrooms are one of the natural sources of vitamin D, which is necessary for strong bones and teeth. Additionally, they are an excellent source of the B vitamins niacin (B3), pantothenic acid (B5), and riboflavin (B2) (Kalac, 2009; Caglarlrmak *et al.*,2002). Mushrooms are not only sources of nutrients but also have been known as therapeutic foods, useful in preventing diseases such as hypertension, hypercholesterolemia and cancer (Bobek and Galbavy, 1999; Kidd, 2000).

Mushrooms flourish universally under the warm and humid climates. They grow on a wide range of substrates. While most are saprophytic, some are highly habitat specific doing well on termite nest soil, sal leaf litter, decaying woods, rocks etc. Although mushrooms can be found year around, mostly they appear during monsoon rains after relatively dry spells of several weeks (Corner, 1935).

Research on wild edible mushrooms (WEM) is centred around a small number of species that are commercially cultivated. Until recently, WEMs have remained neglected by science, though amateur mycologists often documented species they found in field studies. Their biology and ecology have not been studied at greater detail though wild edible mushroom are used in all parts of the world. Consequently, the wild edible mushroom species used in tropical and developing countries are poorly known, though some information is available on species growing in temperate regions.

2. Materials and methods

Similipal, an important landscape in the entire peninsular India, is situated in Mayurbhanj district of the Odisha, India. This Biosphere Reserve spreads over an area of 5,569 km^2 and one of Odisha's most important watersheds with twelve perennial rivers originating from it.

The study area was located in and around the deep forest pockets of tribal villages situated in the transitional zone of Similipal Biosphere Reserve (SBR). Besides, surveying weekly markets in different areas of Similipal, field studies were undertaken and interviews were conducted among mushroom collectors about their edibility, time of collection, storage time and economic values. The fleshy mushrooms were collected from different habitats such as meadows, decaying woods, rotting plant parts, termite nest soils in the forest areas. Each of the collected sample was

wrapped in wax paper and brought to the laboratory for identification purposes. Local names and information on use values collected from the informants were of immense importance for this study. Sometimes, the local names for edible mushrooms were based on shape, taste and other properties that are distinctive or important to people. Field guides often disagree on which mushrooms are edible, either because they are cautious in recommending species that require pre-cooking or because the authors are unaware of local customs in different parts of the world. The edible mushrooms were morphologically described, identified and assigned a scientific name for use by scientific community. Besides, the scientific names were corroborated with local names to classify them to edible, non-edible and poisonous, medicinal uses etc. based of traditional knowledge of local people. Edible mushrooms occur in two major taxonomic groups: (i) the basidiomycetes containing bracket mushrooms and boletes and (ii) the ascomycetes including truffles and morels. There is no simple test for determining edibility. The scientific literature is the best objective source of advice, but local practices and preferences can also reveal useful information (Boa, 2004).

3. Results and Discussion

The wild edible mushrooms found in forests of Similipal Biosphere Reserve have medicinal, economic, and ecological importance. These mushrooms are high in protein and play an important role in tribal dietary supplements. Additionally, they provide seasonal augmentation of income contributing to the local economy through selling these mushrooms in local market places. A wide range of mushroom species were found to be sold in local markets around Similipal and in nearby cities and towns like Baripada, Karanjia, Jashipur, Thakurmunda etc. contributing to household income generation in lean periods. To encourage the sustainable use of these priceless food resources in the course of the country's economic growth, an effort has been undertaken to describe the economically significant wild edible mushrooms being collected by various communities in Similipal.

Indian tribal people are mostly uneducated and live primarily in forests and mountain ranges in rural areas. They are the poorest social groups having more than 50% of population living below the poverty line (Singh, 1993). The state of Odisha (India) has a multi-cultural and multi-lingual tribal populations. Most of the tribal communities such as Mankirdia, Kharia, Kolha, Santhal prefer wild edible mushrooms as their supplementary food source. Edible mushrooms belonging to genera *Astraeus*, *Russula* and *Termitomyces*, *Amanita*, *Tuber* etc. are consumed as food by many tribes of Mayurbhanj district, Odisha. Beside food, these ethnic groups also use wild mushrooms for ethnomedicinal purposes (Sachan *et al.*, 2013; Panda and Tayung, 2014).

A total of 12 wild edible mushroom species belonging to five families were recorded during the study period from different places of Similipal (Fig. 1; Table 1). Out of 12 wild edible mushroom species,seven species were found in Sal forest soil, two species inforest sandy soil, two species in termite nests and one species in decaying sal mixed soil. The genera with more number of species were *Russula* (four species), *Termitomyces* (four species) and *Amanita* (two species) (Table 2). *Russula* species were found abundantly in forest soils during the peak growing period in monsoon. This is one of the most widespread and commonly eaten group of mushrooms having several edible species. *Amanita* genus has both edible and deadly poisonous species.While *Termitomyces* species were abundant in the sandy forest soil around termite nests, several others were restricted to decomposing leaf litter in Sal forests. Many species of *Termitomyces* are widely eaten, known for their high nutritional value and are widely sold in markets, roadsides and sent to nearby townships. The favourable time for collection of wild edible mushrooms in the study area was just at the onset of rains, the period when the conditions were conducive for the mushroom growth. The wild edible mushroom species started growing in the month of June with the first shower of rains and spread gregariously in larger areas around August and then decreases gradually towards the end of October. Among these mushrooms, *T. heimii* is most popular and delicious, which is sold for more than Rs. 400/- per kg in local markets. Some of these mushrooms are also used as ethnomedicine by the tribals. This study also providedthe information on market values and nutrition properties of the mushroom. *Astreatus hygrometicus* and *Russula nigricans* have wide range of ethnomedicinal uses.

Wild edible mushrooms play an important role in ecosystem processes. Many of the leading species live symbiotically with trees and this mycorrhizal association sustains the growth of native forests and commercial plantations in temperate and tropical zones (Zotti *et al.*, 2013). The saprobic wild edible mushrooms are very crucial in nutrient recycling and also provide economic benefits to rural and tribal people by way of collecting and marketing in the local markets (Boa, 2004; Khulakpam *et al.*, 2018*).* From ancient times, mushrooms have been used as an important dietary supplement with high nutritional and medicinal value (Buyuk *et al.*, 1994). There is a strong emphasis on subsistence uses of wild edible mushrooms and their importance to rural people in developing countries, although this is an area where there are still significant gaps in information. Wild edible mushrooms are among the most

Fig. 1: 1. *Russula brevipes*, 2. *Russula cyanoxantha*, 3. *Termetomyces medius*, 4. *Rusula rosea*, 5. *Rusula nigrica*, 6. *Termitomyces eurrhizus*, 7. *Anamita hemibapha*, 8. *Termitomyces heimii*, 9. *Amanita vaginata (Mayurchatu)*, 10. *Termetomyces clypeatus*, 11. *Astreatus hygrometicus*, 12. *Amanita egregia.*

Table 1

Morphological characteristics of selected wild edible mushrooms.

Sl.No.	Name of the species	Morphological characters
1	*Russula brevipes*	It is laterite soil that is epigeous and dispersed. White, 5- to 7-cm-diameter piles with an inflexible border.The base of the central myceloid stipe is connected by a cylindrical stipe. A somewhat crooked, 3–4 cm long, attached decurrentgill with no volva is present.
2	*Russula cyanoxantha*	Solitary found in deciduous forest. Cap is convex to flat to upside with central depression, Pile is about 5-9cm diameter, dry green to purple green in colour. Gills attached to steam, adnexed, crowed, white to cream colour.Stem is cylindrical, about 3-7cm long, 2-3cm thick white in young towards maturity brownish sport no change in colour while handling, brushing or broken.
3	*Termetomyces medius*	On grassland, scattered. Pileus is attached to centre about 1-3cm diameter. No ring, Gill white, at maturity it turns light brown, crowed and free attached. Stipe is about 2-5cm long, cylindrical
4	*Russula rosea*	Scatter present on hill area, Cap 4-7cm, convex when young and slightly depressed at centre deep pink or red in colour, discolored with age,reflexed margin. Piles surface is fleshy covered by with fibrillose scale. No colour change by handling. No ring or volva. Gills are white in colour, serrate edges and adnexed.
5	*Russula nigrica*	It grows on hill top moist deciduous forest. Cap 5-8cm greyish white, and maturity it turned black, Stipe is 2-4cm long, cylindrical, firm and white. Gills are off white crowed, adnate, it turned black while handling or bushing within 20 mint.
6	*Termitomyces eurrhizus*	Mushroom symbiotic association with termites. Piles 3-10cm diameter, dry, convex, later expanded to flattened, scale present on surface, light brown and dark chocolate at centre, margin is irregular; Stipe is 20-cm long with 2-3cm thick, solid above the ground. No colour change while handling or brushing.
7	*Anamita hemibapha*	Epigeous, orange to yellow. Piles convex, smooth surface, inflexedmargin, fleshy. Stipe is at center cylindrical, ring, 3 cm long and 1-3cm thick. Gills crowed with free attachment
8	*Termitomyces heimii*	It is found in termite infested soil. Cap is about 5-8cm diameter after maturity, have scale fibrous on surface, white in colour. No colour change while handling or brushing. Centrally stipe is 10-20cm long, fibrous surface, annulus. Gills are crowed, free attached, separable white in colour.
9	*Amanita vaginata*	Initially cap is oval then it became convex or flattened, colour grey to light black, gills white and free, stem base is enclosed in loose volva, Ring may be present near the cap.
10	*Termetomyces clypeatus*	Solitary association, grow on grassland in slopes. Pilus is medium 10 cm diameter, Brownish in colour, fibrous scale on surface. White to deep brown centrally attached stipe, fibrous surface, stuffed. No ring no vulva; gills are white, turn brownish on maturity. Densely crowded, freely attachment.
11	*Astreatus hygrometicus*	Sporophores growing on ground scatter, at maturity up to 5cm diameter, globular two layers outer later is cremish white towards maturity is dark brown to black, hygroscopic and inner layer is white to grayish, opening by an ostiole in colour. Capillitium thread attached to the side of the endoperidium.
12	*Amanita hemibapha*	Cap is about 6-18cm, hemispherical to flat, orange red to yellow in colour. Stipe is white to cream yellow, narrow towords narrow, hallow with maturity.

Table 2

List of wild edible mushrooms from Similipal, their seasonality, ecology and selling price

Sl.No.	Scientific name	Local name	Seasonality	Ecology/Habitat	Price/ Kg (Rs.)
1	*Termitomyces heimii*	Parbanachatu, Bhiudachattu	August-October	Termite nest soil	300-400
2	*Termitomyces eurrhizus*	Bali chattu	June-September	Forest sandy soil	100-220
3	*Russula rosea*	NalichattuLali chattu	August-September	Sal forest soil	40-60
4	*Russula cyanoxantha*	Jammu chhatu, Patra chattu	August –September	Sal forest soil	30-50
5	*Termitomyces clypeatus*	Kalimundichhatu	June-Sept	Termite nest soil	80-150
6	*Russula brevipes*	Dhalachhatu, Patra chattu	June-Aug	Sal forest soil	60-100
7	*Amanita vaginata*	Mayur chhatu	June-Sept	Sal forest soil	30-40
8	*Bovista plumbea*	Tumbachhatu	June –September	Sal forest soil	30
9	*Astreatus hygrometicus*	Rutkachhatu, Patakachhattu.	June-August	Sal forest soil	200-250
10	*Russula nigricans*	Kali chatu, Angrachhatu,	June –September	Sal forest soil	50-70
11	*Amanita hemibapha*	Haladi mundi chhatu	June-September	Decaying sal leaf mixed soil	30-40
12	*Termitomyces medis*	Chotobalichhatu	August-September	Forest sandy soil	100-150

valuable (NWFP) with much potential for expansion of trade, but there are also challenges in the integration of their management and sustainable production as part of multiple use forests. There are concerns about the impact of excessive harvesting, which require better data on yields and productivity and a closer examination of collectors and local practices. Closer cooperation between forest managers and those who use wild edible mushrooms is needed.

Wild edible mushroom add flavour to bland staple foods but they are also valuable foods in their own right. Local names for termite mushrooms (*Termitomyces*) reflect local beliefs that they are a fair substitute for meat, a belief that is confirmed by nutritional analyses. Not all wild edible mushrooms have such a high protein content but they are of comparable nutritional value to many vegetables. The mushrooms produced more than 100 medicinal uses and antioxidant, anticancer, antidiabetic, antitumor, anti-inflammatory,antiallergic, immunomodulating, cardiovascular protector, anticholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxification hepatoprotective and other biological activities (Cang *et al.,* 2012; Panda *et al.*, 2014, 2017).

4. Conclusion

Wild useful mushrooms contribute towards diet, income and human health. Many mushrooms also play vital ecological role through the symbiotic relationships known as mycorrhizas that they form with trees. Simlipal is a vast area dominated by Sal forest and the forest floor is abundant with loose soil and leaf litter. When monsoon starts, the leaves start decomposing and mushrooms spores grow being attached to leaves, bark and sometime roots. Some species growing in leaf litter are species of *Russula, Termitomyces, Amanita,* etc. The tribals and other forest dependent people easily identify mushrooms that are edible, non-edible or poisonous. Income from wild edible mushrooms is an important source of revenue earning for rural communities, especially in developing countries.

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TABLE OF CONTENTS