PHYSICO CHEMICAL PROPERTIES AND THEIR DPPH ACTIVITIES OF MANGROVE MYCOFLORA, KARANKADU, TAMIL NADU

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Abstract

The study aimed to investigate the physico chemical properties of mycofloral soil in karankadu mangrove forest, Tamil Nadu, India and to evaluate the DPPH anti-oxidant activity of isolated fungal groups from the same soil. Fungal diversity and distribution were correlated with physico – chemical properties of soil. Salinity ($r = 0.860$; $P < 0.05$) and organic matter ($r = 0.921$; $P < 0.01$) showed positive correlation in the year of 2013. The commonly isolated soil mycoflora namely *D. nanum*, *P. cyaneum* and *T. polysporum* were subjected to in vitro antioxidant assays. In vitro antioxidant activity of ethyl acetate extract of *D. nanum*, *P. cyaneum* and *T. polysporum* was studied by DPPH free radical scavenging assay, the tested extracts produced considerable antioxidant activity. The maximum inhibition of DPPH free radical was exhibited *P. cyaneum* followed by *T. polysporum* and *D. nanum* showed lowest inhibition at 120 µg/mL concentration.

Key words: Physico- chemical properties, Mycoflora, Mangrove, DPPH activity.

1.Introduction

Mangroves are salt-tolerant forest ecosystems confined to intertidal zones of sheltered shores, estuaries, tidal creeks, backwaters, lagoons, marshes, and mudflats of the tropical and subtropical latitudes. These important ecosystems are a dynamic ecotone between terrestrial and marine habitats and of great ecological, economic, and social significance (Gopal and Chauhan, 2006). World's coastline is dominated by mangroves that are distributed in 123 countries and territories comprising a total area of about 150,000 km2. Among the marine ecosystems, mangroves constitute the second most important ecosystem in productivity and sustained tertiary yield after coral reefs (Spalding *et al*., 2010).

Free radicals or Reactive oxygen species (ROS) might be labeled as molecular crooks that harm molecules in mitochondria, cell membranes, DNA and are very unstable, be apt to stripe electrons from the molecules in the instantaneous surroundings in order to substitute their own losses. Reactive oxygen species (ROS) is a combined term, which comprises not only the oxygen radicals (O_2•−, and •OH) but also certain non-radical byproducts of oxygen. These include hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2) and ozone (O_3) (Bandhopadhyay *et al*., 1999). ROS mediated reactions are ascertained to play multiple roles in degenerative or pathological events such as aging, cancer, heart dysfunction and Alzheimer's disease (Finkel and Holbrook, 2000). Almost 100 syndromes like rheumatoid arthritis, cardiovascular disorders, hemorrhagic shock, cystic fibrosis, gastrointestinal ulcerogenesis, metabolic disorders, AIDS and neurodegenerative diseases have been reported as ROS intervened disorders(Aher *et al*., 2011).

Different *in vitro* chemical-based assays have been developed to determine the antioxidant capacity of natural products, including the popular DPPH free radical scavenging method, ferric reducing capacity, *etc*. and most recently the use of nanoprobes to assess the metal-reducing capacity of antioxidants. These assays are based on different strategies and endow different information about the ROS/RNS-sample interaction. DPPH (2,2-Diphenyl-1-picrylhydrazyl) and ABTS (2,2′-Azinobis-(3-ethylbenzothiazole-6-sulphonate) radical cation, $ABTS^{•+}$) are two stable and colored free radicals that have been widely employed to determine antioxidant capacity (Miller *et al*., 1993; Brand-Williams *et al*., 1995; López-Alarcóna and Denicolab, 2013). This study focused the following aim (a) To study the physico - chemical parameters of soil samples from Karankadu mangroves. (b)To study the relationship between the mycoflora and physico – chemical parameters of soils. (c) To select the mangrove fungi based on the abundance for further investigation. (d) To find out the antioxidant potential of selected mangrove fungi by 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

Materials and method

2.1 Analysis of physico-chemical characteristics of the soil

The physico-chemical parameters of the soil samples of Karankadu mangrove environment were analyzed at Soil Testing Laboratory, Department of Agriculture, Government of Tamil Nadu, Tiruchirappalli - 20. Soil samples suspended in distilled water after removing the debris (1:2 w/v) and kept it to settle down the sand particles. The pH of the suspension was read using pH meter (Systronics, India), to find out the soil pH.

Electrical conductivity, salinity, organic Carbon, organic matter, available Nitrogen, available Phosphorus, available Potassium, available Zinc, available Copper, available Iron, cation exchange capacity, available Manganese, Calcium, Magnesium, Sodium and Potassium were analyzed by APHA method (1989).

2.2. Statistical analysis

Pearson's correlation analysis was used to assess the relationship between physico-chemical parameters and total fungal colonies. The data were computed and analyzed using Statistical Package for Social Sciences (SPSS) software.

***2.3. In vitro* antioxidant activities of selected mangrove fungi**

2.3.1. DPPH (1, 1-Diphenyl-2-picrylhydrazyl) free radical scavenging activity

Sample preparation

Different concentrations (20, 40, 60, 80, 100 and 120 µg/ml) of ethyl acetate extracts of *D. nanum*, *P. cyaneum*, *T. polysporum* and standard L-ascorbic acid were prepared.

Assay

Assay for DPPH free radical scavenging potential is based on the scavenging activity of stable DPPH free radicals. The scavenging of DPPH free radicals was used for measuring the antioxidant activity of the fungal extracts, according to the method of Brand-Williams *et al*. (1995). DPPH (150 µm in ethanol) was used as free radical, about 10 µl of each concentration of test extracts were mixed with 190 µl of DPPH in clean and labeled test tubes separately. Same procedure was followed with standard L-ascorbic acid, and the standard test tubes set up were maintained separately.

After vortexing, the tubes with mixture were incubated at 37 °C in dark for 30 minutes. The decrease in absorbance of the test mixture (due to quenching of DPPH free radicals) was measured at 517nm using UV-Visible spectrophotometer, and the inhibition percentage was calculated. The degree of stable DPPH decolourization to DPPH (reduced form of DPPH) yellow indicates the scavenging efficiency of the extracts tested. The scavenging activity of the fungal extracts against the stable DPPH was calculated by using the following formula:

$$\% \text{ of DPPH Inhibition} = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

Where A control = Absorbance of control reaction set up.

(Containing all reagents except the test extract)

A Test = Absorbance of sample extracts set up.

3.Results:

3.1. Physical properties of soils in mangrove environment

Physical properties of the soils samples collected from mangrove environment of Karankadu, Ramanathapuram Dt. were given in table 1. Mangroves soils are grey in colour. The soil texture was clay loam in Karankadu mangrove environment soils. The soils have less than 37, 34 and 41% of sand, silt and clay particles, respectively in different months in Karankadu mangrove environment.

Table 1. Physical properties of the soils samples collected from mangrove environment of Karankadu, Ramanathapuram Dt.

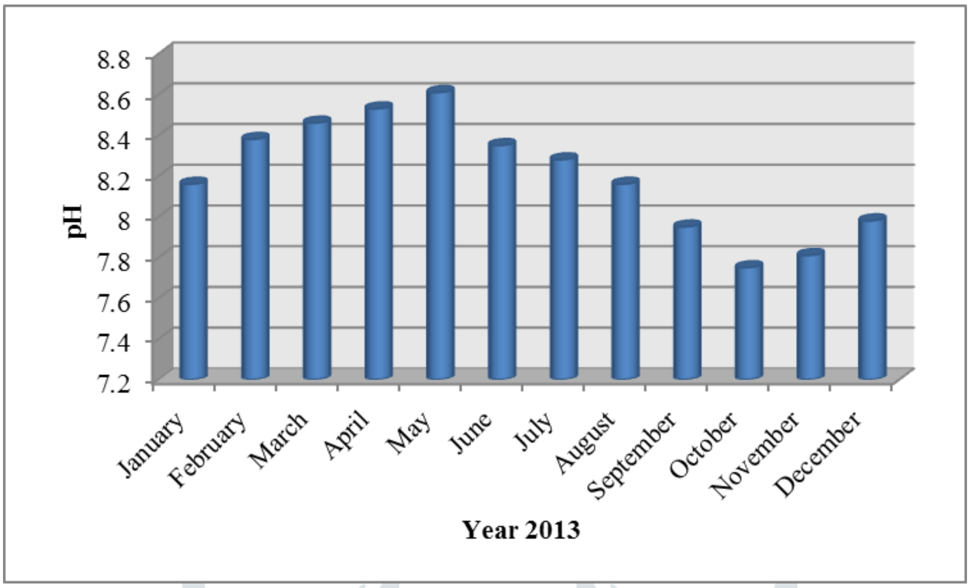
S. No.	Description	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
1.	Soil Texture	Clay Loam	Clay Loam	Clay Loam	Clay Loam	Clay Loam	Clay Loam	Clay Loam	Clay Loam	Clay Loam	Clay Loam	Clay Loam	Clay Loam
2.	Sand (%)	32.18	36.54	32.58	34.16	35.16	32.23	33.45	35.21	33.27	35.62	35.13	36.26
3.	Silt (%)	27.37	29.08	32.64	26.94	27.07	26.89	27.02	31.3	33.8	28.87	28.17	28.61
4.	Clay (%)	40.45	34.38	34.78	38.90	37.77	40.88	39.53	33.49	32.93	35.51	36.70	35.13

3.2. Chemical properties of soils in mangrove environment

pH

pH was alkaline in all the soil samples collected during all the months from Karankadu Mangroves. The pH of soil was ranged from 7.75 to 8.61. The minimum of 7.75 was recorded in the sample was collected during the month of October 2013 and the maximum of 8.61 was observed in the sample collected during the month of May in the year of 2013 (Figure 1).

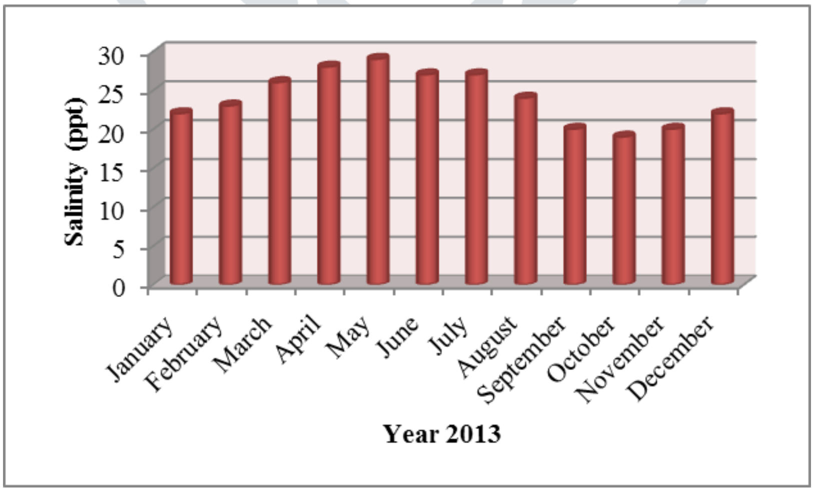
Figure 1. Monthly variation of pH recorded in soil samples collected from mangroves of Karankadu, Ramanathapuram Dt.



Salinity

The salinity showed considerable variations between the soil samples. The minimum of 19 ppt was recorded in the sample collected during the month of October 2013 and the maximum of 29 ppt was recorded in the sample collected during the month of May in the year of 2013 (Figure 2).

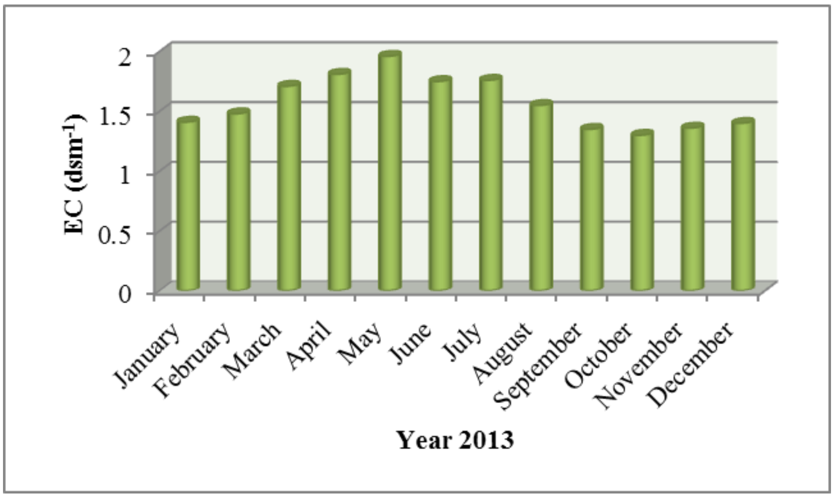
Figure 2. Monthly variation of Salinity recorded in soil samples collected from mangroves of Karankadu, Ramanathapuram Dt.



Electrical conductivity

The electrical conductivity of soils ranged from 1.30 to 1.96 %. The minimum of 1.30 dsm^{-1} was recorded in the sample collected during the month of October 2013 and the maximum of 1.96 dsm^{-1} was recorded in the sample collected during the month of May in the year of 2013 (Figure 3).

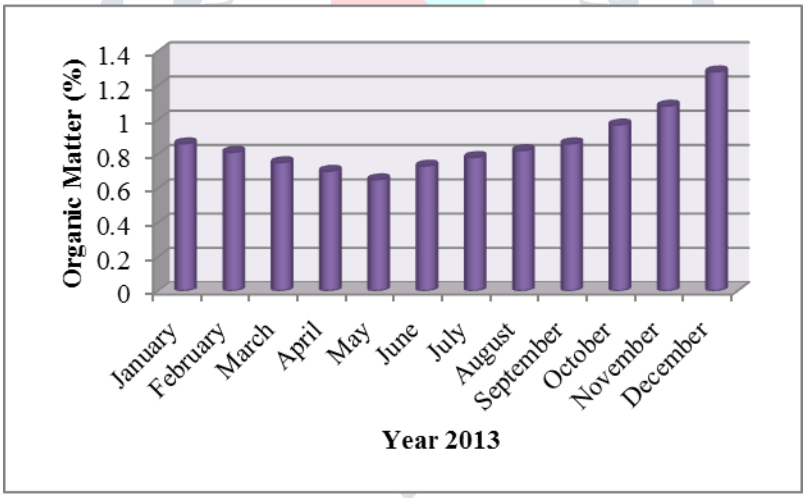
Figure 3. Monthly variation of Electrical conductivity recorded in soil samples collected from mangrove environment of Karankadu, Ramanathapuram Dt.



Organic Matter

The organic matter content of soils ranged from 0.65 to 1.28 %. The soil sample collected from the month of December has high organic matter content as compared to other soil samples collected in the year of 2013 (Figure 4).

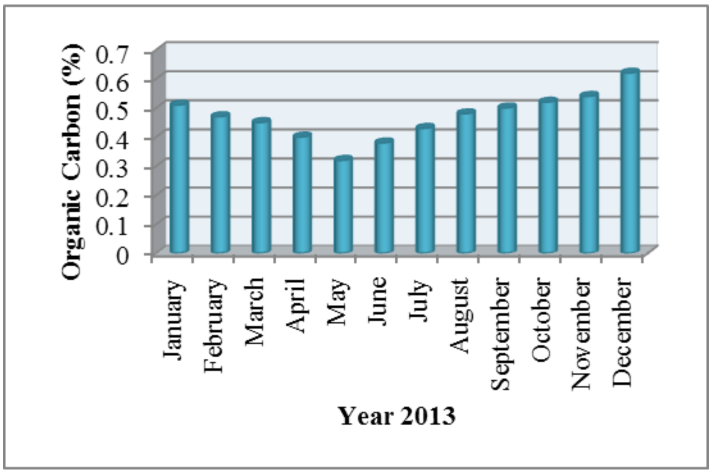
Figure 4. Monthly variation of Organic Matter recorded in soil samples collected from mangrove environment of Karankadu, Ramanathapuram Dt.



Organic Carbon

The organic Carbon content showed variations between the soil samples. The minimum of 0.32% was recorded in the sample collected during the month of May and the maximum of 0.62% was recorded in the sample collected during the month December in the year of 2013 (Figure 5).

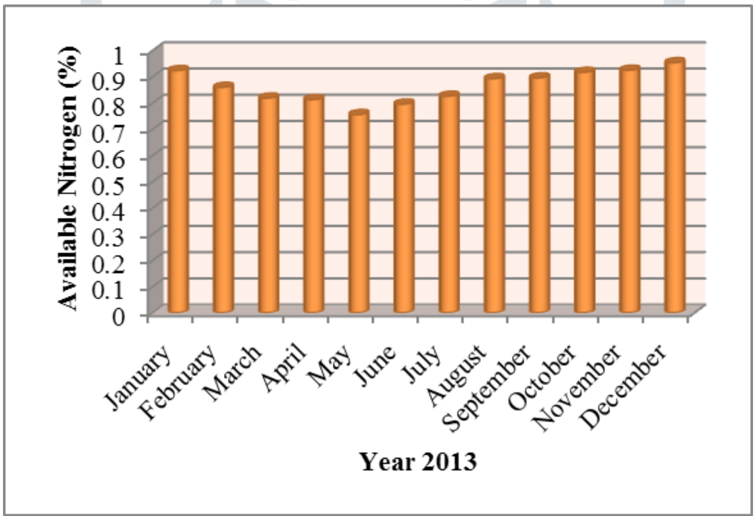
Figure 5. Monthly variation of Organic Carbon recorded in soil samples collected from mangrove environment of Karankadu, Ramanathapuram Dt.



Available Nitrogen

Available Nitrogen content of the soils showed slight variations, ranged between 0.756 and 0.954%. The minimum was recorded in the sample was collected during the month of May and the maximum was recorded during the month of December at mangrove environment in 2013 (Figure 6).

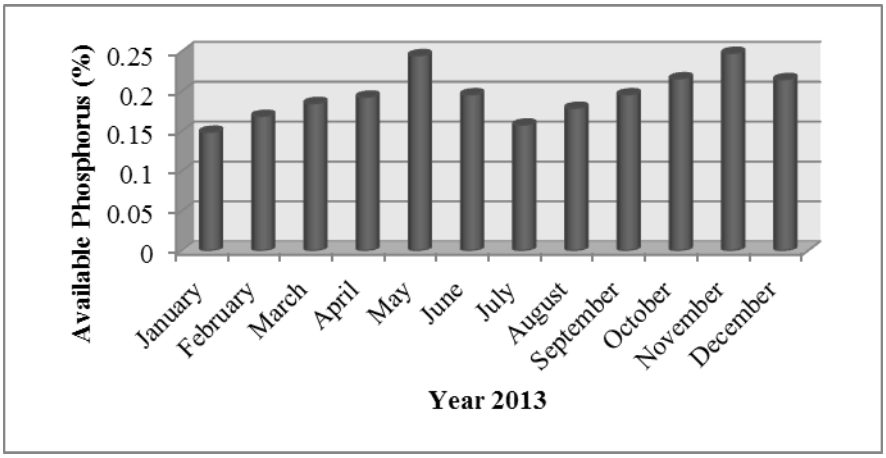
Figure 6. Monthly variation of available Nitrogen recorded in soil samples collected from mangrove environment of Karankadu, Ramanathapuram Dt.



Available Phosphorus

The available Phosphorus content of soils ranged from 0.149 to 0.248%. The soil sample collected from the month of November has high Phosphorus content as compared to other soil samples collected in the year of 2013 (Figure 7).

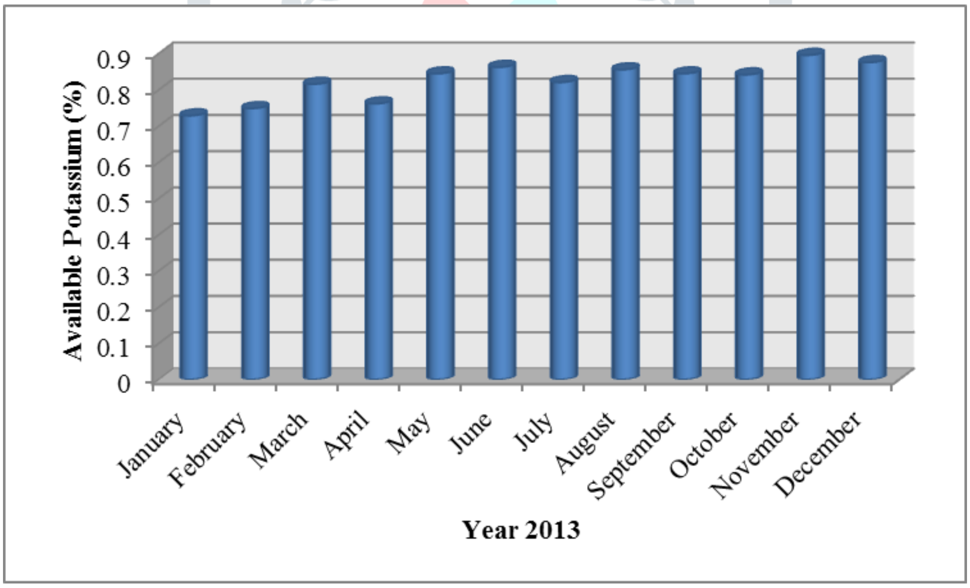
Figure 7. Monthly variation of available Phosphorus recorded in soil samples collected from mangrove environment of Karankadu, Ramanathapuram Dt.



Available Potassium

Available Potassium content showed slight variations between 0.729 to 0.896%. The minimum was recorded collected during the month of January and the maximum was recorded during the month of November in the year of 2014 (Figure 8).

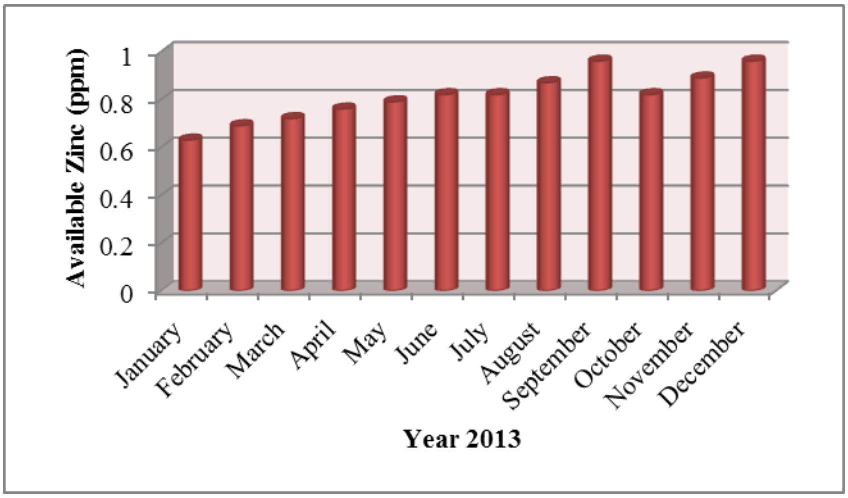
Figure 8. Monthly variation of available Potassium recorded in soil samples collected from mangrove environment of Karankadu, Ramanathapuram Dt.



Available Zinc

Available Zinc content ranged from 0.63 to 0.96 ppm, the minimum was recorded during month of January and the maximum was observed during the months of September and December in the year of 2013 (Figure 9).

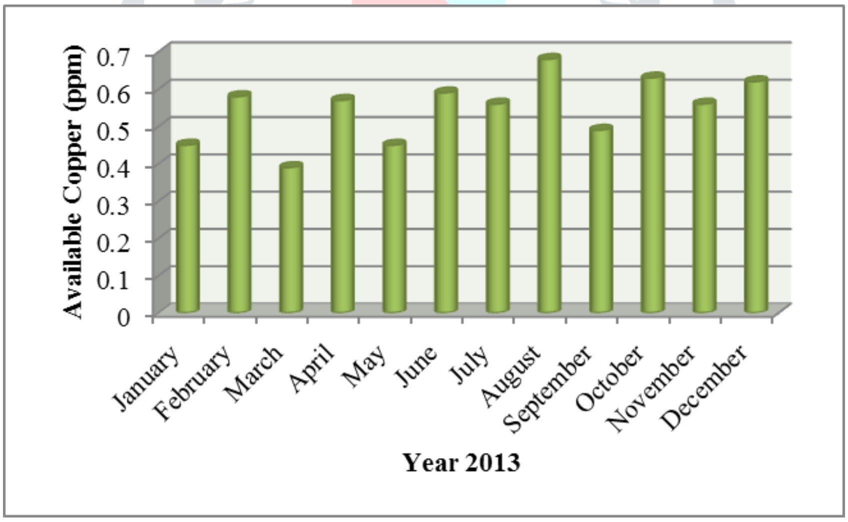
Figure 9. Monthly variation of available Zinc recorded in soil samples collected from mangrove environment of Karankadu, Ramanathapuram Dt.



Available Copper

The available Copper content ranged from 0.39 to 0.68 ppm, the minimum was recorded during month of March and the maximum was observed during the month of August in the year of 2013 (Figure 10).

Figure 10. Monthly variation of available Copper recorded in soil samples collected from mangrove environment of Karankadu, Ramanathapuram Dt.



***3.3. In vitro* antioxidant activities of selected mangrove fungi**

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) free radical scavenging activity

In vitro antioxidant activity of ethyl acetate extract of *D. nanum*, *P. cyaneum* and *T. polysporum* was studied by DPPH free radical scavenging assay, the tested extracts produced considerable antioxidant activity (Figure 11 & 12). The maximum inhibition of DPPH free radical was exhibited *P. cyaneum* (69.54±1.01%) followed by *T. polysporum* (54.48 ±1.05%) and *D. nanum* showed lowest inhibition (48.21±1.01%) at 120 µg/mL concentration. The IC_{50} values of *D. nanum, P. cyaneum* and *T. polysporum* was found to be 124.45, 78.07 and 99.13 µg/mL concentrations respectively. The activity was concentration dependent. The inhibition activity of the test extracts is illustrated in table 2. The inhibition was compared with standard antioxidant L-ascorbic acid.

Table 2. DPPH free radical scavenging activity of ethyl acetate extract of *D. nanum, P. cyaneum* and *T. polysporum*

S. No	Concentration (µg/mL)	Inhibition of DPPH˙ (%)			
		D. nanum	*P. cyaneum*	*T. polysporum*	L-ascorbic acid (Standard)
1	20	16.42±0.81	27.94±1.02	28.16±0.95	29.14±0.12
2	40	25.87±0.94	32.47±0.94	31.45±0.89	34.57±1.06
3	60	27.94±1	44.47±1.16	39.1±1.3	48.65±1.05
4	80	31.75±1.01	51.23±0.99	43.51±1.01	57.24±0.99
5	100	45.78±1.05	62.75±1.01	50.25±1.09	70.78±0.95
6	120	48.21±1.01	69.54±1.01	54.48±1.05	78.46±0.85

Results expressed as Mean ± Standard Deviation (n-3)

Figure 11. DPPH free radical scavenging activity of ethyl acetate extract of *D. nanum, P. cyaneum* and *T. polysporum*

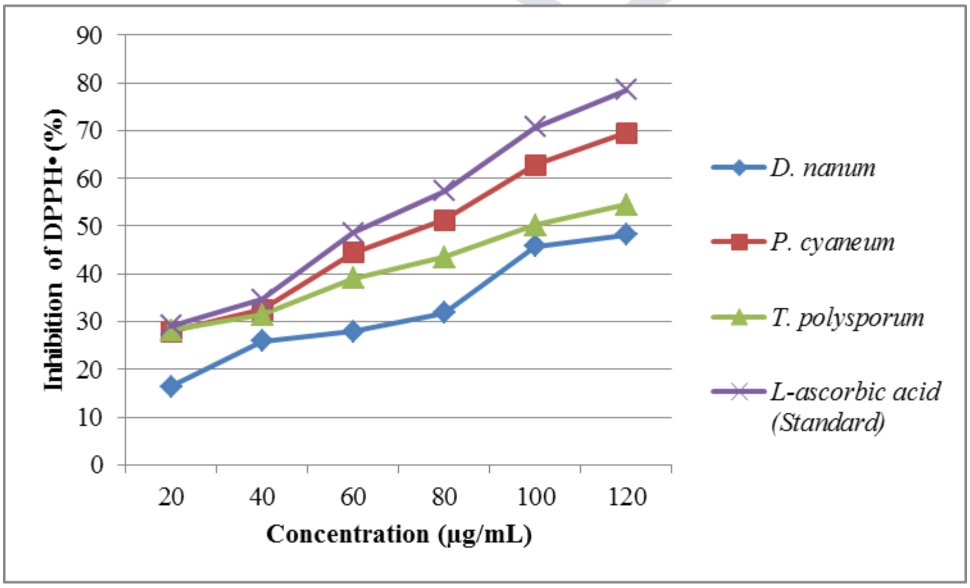
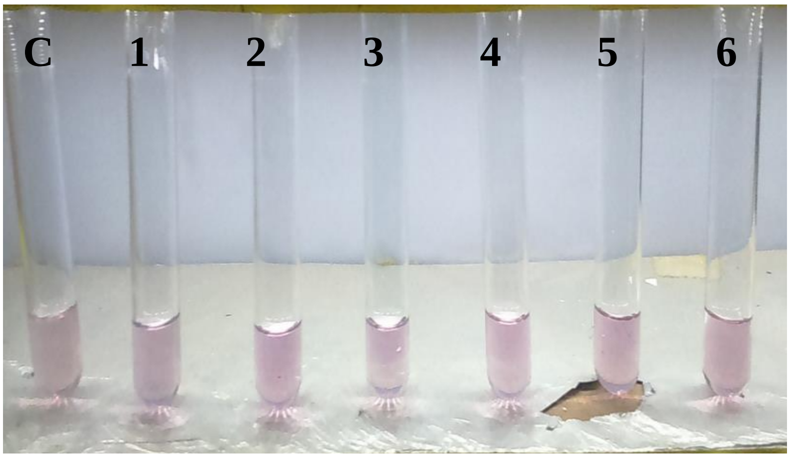
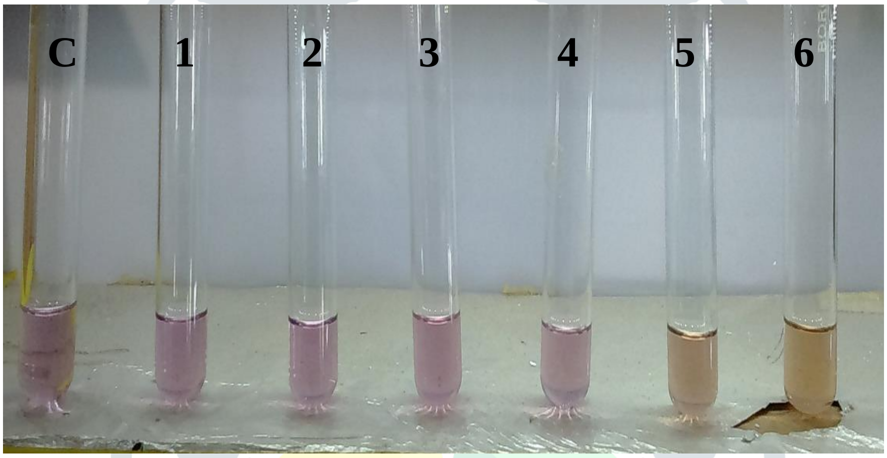


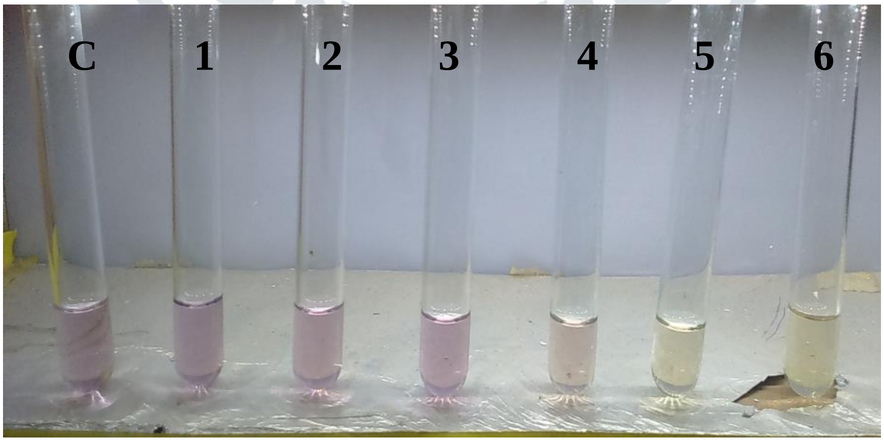
Figure 12. DPPH free radical scavenging activity of ethyl acetate extract of *D. nanum, P. cyaneum* and *T. polysporum*



D. nanum



P. cyaneum



T. polysporum

C – Control, 1 – 20 µg/mL, 2 – 40 µg/mL, 3 – 60 µg/mL,
4 – 80 µg/mL, 5 – 100 µg/mL, 6- 120 µg/mL

4.Discussion:

4.1.Physico- chemical characteristics of mangrove soil

The plants in mangrove may grow in various types of soil; thus, their structure, species composition and vegetation may vary significantly at the local, regional and global scales (Vilarrubia, 2000; Sherman *et al*., 2003). These (Mangrove) soils are of marine alluvium, transported as sediment and deposited by rivers and the sea. The soils are made up of silt, sand and clay in various compositions. The darker-coloured clayey topsoils are less well aerated (Hossain and Nuruddin, 2016).

Study of soil physicochemical parameters is essential to fungal diversity of mangrove environments. The pH, salinity, electrical conductivity, organic matter, available nitrogen, major elements and minor elements of soil such as phosphorus, potassium, Zn, Fe, Cu, Mn, Ca, Mg and Na are affecting the fungal population and diversity. Mangrove forests soils are complex systems resulting from various complicated interactions amongst biotic and abiotic factors, which may change within undersized distances. Soil features such as iron, salinity, sulfide concentrations, nutrients, soil redox potential, organic matter and physiographical position are the vital factors in defining mangrove species composition and structure (Sherman *et al*., 1998; Marchand *et al*., 2004; Otero *et al*., 2009). Research consequences exposed noteworthy differences in the composition of mangrove soils at various depths, total organic carbon content, clay mineralogy and carbon stock (Ferreira *et al*., 2010).

In the present research work, available Nitrogen content of the soils showed slight variations, ranged between 0.756 and 0.954%. A considerable variation was observed in the nutrient values of Phosphorus, Potassium, Zinc, Calcium, Magnesium and Sodium. Rambok *et al*. (2010) reported that the Sibuti mangrove has maximum (25.27%) phosphorus, Sarawak, Malaysia while Sukardjo (1994) reported that the 26.34 ppm of phosphorus in Apar nature reserve mangrove, Indonesia. The lowest available nutrients values recorded in Bangladesh mangrove forest by Khan *et al*. (1993) and highest nutrients values are reported in Indonesia mangroves by Sukardjo (1994).

4.2. Relationship between soil mycoflora and physico- chemical characteristics of soil

Determining the mechanisms regulating the relationships between environmental factors and benthic organisms has been an active research area in estuarine ecology. Microorganisms in mangrove environments are likely to be greatly affected by nutrient availability. Most nutrients in such environments are derived from litter decomposition and from mangrove secretions (Alongi *et al*., 1993).

Previous research demonstrated that the microbial biomass in mangrove environments is related to the contents of soil organic matter, total nitrogen, and available nitrogen (Zhang, 2002; Cuellar-Gempeler and Munguia, 2013; Thatoi *et al*., 2013a).

In the present study salinity (r =0.860; P< 0.05) and Organic matter (r =0.921; P < 0.01) showed positive correlation in the mangrove soils of Karankadu. In the contrary Sahu *et al*. (2013) reported organic carbon content were significantly correlated with microbial dynamics, whereas salinity had negative relation in mangrove forest of the Devi estuary, Odisha, India. Moreover, Das *et al*. (2012) accounted organic carbon was the most significant factor that regulated the total microbial population in mangrove soil of Sundarban, India.

4.3. *In vitro* antioxidant activities

Free radicals are any chemical species capable of independent existence with one or more unpaired electrons in their outermost shell, which seek out and capture electrons from other substances to achieve neutrality. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, resulting in a chain reaction. If two radicals meet they can combine their unpaired electrons, thus forming a covalent bond. Reactive oxygen species (ROS) means a group of metabolites derived from molecular oxygen (O2). The antioxidant capacity of compounds has been related to the prevention of several diseases including cancer, coronary heart diseases, inflammatory disorders, neurological degeneration, and aging (Valko *et al*., 2007; Cavar *et al*., 2012). Marine-derived fungi are known to be a source of antioxidative natural products (Abdel-Lateff *et al*., 2002; Abdel-Lateff *et al*., 2003).

DPPH reactivity is one popular method of screening for free radical-scavenging ability in compounds, and has been used extensively for antioxidants in fruits and vegetables. This method was first described by Blois (1958) and was later modified slightly by numerous researchers. DPPH is a stable free radical that reacts with compounds that can donate a hydrogen atom. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourises the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. Antioxidant activity is then measured by the decrease in absorption at 517 nm. A huge reduction in the absorbance of the reaction mixture specifies noteworthy free radical scavenging activity of the compound under test (Krishnaiah *et al*., 2011). This method is considered, from a methodological point of view, one of the easiest, most accurate and productive for evaluation of antioxidant activity in fruit juices, plant extracts and pure substances like flavonoids and terpenoids (Alves *et al*., 2010).

In the present study, the maximum inhibition of DPPH free radical was exhibited *P. cyaneum* followed by *T. polysporum* and *D. nanum* showed lowest inhibition at 120 µg/mL concentration. The antioxidant activity of ethyl acetate extract of *P. cyaneum* was found to be comparable to the standard L-ascorbic acid. Similar work was done by Hulikere *et al.* (2016a) who marine fungus *P. citrinum* extract showed a significant antioxidant activity. In another study by Hong *et al*. (2015) who demonstrated *Arthrinium saccharicola* KUC21220 exhibited the highest DPPH radical scavenging activity. And also, *P. cyaneum* ethyl acetate extract exhibited 69.54 % DPPH radical scavenging activity with IC_{50} value of 78.07 µg/mL. On the contrary, Hamed *et al*. (2015) demonstrated chloroform extracts of marine isolate *Circinella muscae* showed strongest antioxidant activity than ethyl acetate extracts. A another study by Li *et al*. (2014) reported ethyl acetate extracts of the marine derived fungus *Aspergillus wentii* EN-48 showed potent antioxidant activity with IC_{50} values 99.4 µg/mL.

5. Conclusion:

Based on the previous and present investigations, it is concluded that physico- chemical characteristics of mangrove soils are widely varied in different mangrove forests of the world. Fungal diversity and distribution were correlated with physico – chemical properties of soil. Salinity (r =0.860; P< 0.05) and organic matter (r =0.921; P < 0.01) showed positive correlation in the year of 2013.The commonly isolated soil mycoflora namely *D. nanum*, *P. cyaneum* and *T. polysporum* were subjected to *in vitro* antioxidant assays and bioactive compound analysis. *In vitro* antioxidant activity of ethyl acetate extract of *D. nanum, P. cyaneum* and *T. polysporum* was studied by DPPH free radical scavenging assay, the tested extracts produced considerable antioxidant activity. The maximum inhibition of DPPH free radical was exhibited *P. cyaneum* followed by *T. polysporum* and *D. nanum* showed lowest inhibition at 120 µg/mL concentration.

Conflict of interest

There is no conflict of interest declared.

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