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# Evaluation of Antioxidant Polyphenols from Selected Mangrove Plants of India

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Mangroves and halophytes are a unique group of vascular plants that occur in saline coastal habitats. Some mangroves and halophytes are used for a wide range of conditions including bacterial, fungal and viral diseases. These specialized plants are known to tolerate extreme environmental conditions. However, little is known on the antioxidant potentials of India's mangroves and halophytes. We have evaluated 5 halophytic plants (*Arthrocnemum indicum, Suaeda monoica, S. maritima, Sesuvium portulacastrum* and *Ipomoea pes-caprae*) and 8 mangrove plants (*Avicennia officinalis, Bruguiera cylindrica, Ceriops decandra, Rhizophora apiculata, R. mucronata, Aegiceras corniculatum, Excoecaria agallocha* and *Acanthus ilicifolius*) collected from Tamil Nadu (India) to determine total polyphenol content and antioxidant activity (by 2,2-diphenyl-1,1-picrylhydrazyl DPPH free radical scavenging assay against α-tocopherol). The total polyphenol content ranged from 23.5 to 384.2 mg/g dry weight and the highest free radical scavenging activity was found in *E. agallocha* (30.3 µg/mL). Moreover, higher DPPH radical scavenging activity was also found in species such as *B. cylindrica* (42.9 µg/mL), *C. decandra* (51.9 µg/mL), R. apiculata (64.9 µg/mL), *A. corniculatum* (74.3 µg/mL), *R. mucronata* (79.7 µg/mL) and *I. pes-caprae* (83.7 µg/mL), respectively. Between mangroves and halophytes, the polyphenols was significantly higher among 8 mangrove species than that of 5 salt marsh halophytes. Our research indicates that India's mangrove plants have the potential in scavenging free radicals and can be a vital source of antioxidant phytochemicals.

**Key Words: Mangroves, Halophytes, Total polyphenol, Radical-scavenging properties, India.**

## INTRODUCTION

India is one among the 25 hotspots of the richest and highly endangered eco-regions of the world[1,2]. India's diverse plant species are mainly found in various types of forests such as tropical, sub-tropical, temperate, sub-alpine, alpine, dry open, open, evergreen, deciduous, littoral and mangrove that occupy 20 % of the total geographical area[3]. Mangrove forest is

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considered to be one of the highly endangered ecosystems and uninterrupted man-made disturbances ranging from deforestation to pollution threatens their survival throughout Asia[4,5]. Mangrove plants require specific conditions to grow, thus restricting their geographic range. Plants that live in mangrove ecosystem are adopted to encounter high salinity, tidal extremes and heavy winds. Mangroves occur in 121 countries covering 15 million ha worldwide. Asia harbors the largest mangroves in the world and India alone contributes for 3 % of the global mangrove habitat[6].

Mangrove ecosystem is a natural resource for tannin and the timbers produced are of great value. For example, the heavy hard wood of *Rhizophora* used for making boats is resistant to termites and marine organisms[7]. Mangrove plants are used in folklore medicine to treat various diseases for centuries[8,9] (Table-1)[9-32]. Some mangrove plants have been screened for their antiviral, antibacterial, antiulcer and antiinflammatory activities[11,23,33-36] (Table 1)[9-32].

Mangrove plants like *B. cylindrica, R. apiculata, R. lamarkii* and *R. mucronata* are rich in polyphenols[37,38]. Some mangrove plants have been investigated for the production of tea[39]. The ashes of *A. indicum* have been prescribed to treat snakebites[9]. Traditional healers in Africa use *S. portulacastrum* to treat kidney problems, fever and scurvy[12,13]. India's traditional healers used *A. officinalis* to treat smallpox infections in the past[9] (Table-1)[9-32]. The leaves of *A. ilicifolius* have been used to treat snakebite[9]. The bark and wood of *E. agallocha* used for flatulence in Thailand[19]. In Sri Lanka, the burning wood of *E. agallocha* has been used in the treatment of leprosy, while roots of this plant powdered with ginger has been used as an embrocation for swelling hands and feet[18]. The polysaccharide extracted from *R. mucronata* apparently showed *in vitro* anti-HIV activity[23].

The antioxidant compounds in a typical diet are mostly derived from plant sources and polyphenolic components of higher plants act as antioxidant or other mechanisms contributing to anti-carcinogenic action[40]. The antioxidants from natural sources are given preference than synthetic sources and the DPPH method has been widely applied for estimating antioxidant activities recently[41]. Research also indicates that the DPPH test is particularly suitable for the evaluation of antioxidant activity of crude extracts[42]. Besides, evaluation of the antioxidant polyphenols from less known ethno-medicinal plants from Asia are urgently needed[43].

Little is known on the antioxidant potentials of India's mangroves and halophytes. We have evaluated 5 halophytes (*Arthrocnemum indicum, Suaeda monoica, S. maritima, Sesuvium portulacastrum* and *Ipomoea pes-caprae*) and 8 mangroves (*Avicennia officinalis, Bruguiera cylindrica, Ceriops decandra, Rhizophora apiculata, R. mucronata, Aegiceras*

TABLE-1
TRADITIONAL MEDICINAL USES AND BIOLOGICAL ACTIVITIES OF 13 SPECIES OF HALOPHYTIC AND MANGROVE MEDICINAL PLANTS FROM INDIA

| Name of the species | Traditional medicinal uses | Biological activities |
|---|---|---|
| *A. indicum* | Ashes-Snakebite, scorpion sting, alexipharmic in ayurveda[10] | – |
| *S. monoica* | Ointment for wounds[10] | Antiviral[11] |
| *S. maritima* | Cures hepatitis[12] | Antiviral[11] |
| *S. portulacastrum* | Kidney diseases[13] fever and scurvy[14] | Antiviral[11] |
| *A. officinalis* | Smallpox[10], joint pain, urinary disorders, bronchial asthma, stomach disorders[15] | – |
| *A. ilicifolius* | Snakebite, skin diseases, smallpox and ulcer[15] | Antiviral[11]; leishmanicidal[16]; antioxidant[17]; antitumor and anticarcinogenic[18] |
| *A. corniculatum* | – | Antiviral[11] |
| *E. agallocha* | Swelling hands and feet[19]; flatulence[20]; epilepsy, antiinflammation[15] | Antiviral[11]; antitumor[21]; antioxidant[22]; *in vitro* propagation[23] |
| *B. cylindrica* | – | Antiviral[11] |
| *C. decandra* | Cures hepatitis[12] | Antiviral[11] |
| *R. apiculata* | Diarrhoea, nausea, vomiting and amoebiasis[15] | Antiviral[11,24]; larvicidal[25,26] |
| *R. mucronata* | Bark-powerful astringent useful in diabetics, hemorrhage and angina[10] | Antiviral, anti-HIV[11,27] |
| *I. pes-caprae* | Diarrhoea, pains, vomiting, inflammation of legs, piles[10] | Antinociceptive[28]; antispasmodic[29]; antiinflammatory[30]; inhibition of platelet aggregation[31]; jellyfish poison[32]; insulogenic and hypoglycemic[33] |

*corniculatum, Excoecaria agallocha* and *Acanthus ilicifolius*) collected from Tamil Nadu, India and determined the total polyphenol content and their antioxidant activity by 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) free radical scavenging assay against α-tocopherol.

## EXPERIMENTAL

**Description of study area and collection of samples:** Medicinal plants were collected from Pichavaram mangrove forest (area 1400 ha) located between Vellar and Coleroon estuaries (latitude 11° 22′ N to 11°30′ and longitude 79°45′ E to 79°52′) in Cuddalore (India). The mangrove forest area comprises about 51 islands with their sizes ranging from 10 $m^2$ to 2 $km^2$. About 40 % of the area is covered by water ways, 50 % by forest and the rest by mud flats and sandy/salty soils. There are numerous creeks and canals traversing the forest with a depth ranging from 0.5 to 1.5 m and discharging freshwater into the mangrove ecosystem. The climate is sub-humid (< 30 °C) with an average rainfall of 1310 mm. The rainfall occurs mostly during the northeast monsoon season (October-December) annually[44].

**Collection of plant samples:** Mangrove plant samples were collected between 06:00 to 09:00 h during May 2006 by sailing in a rowboat through the mangrove swamp with minimum or no disturbance to the floral and faunal communities. Leaves from 5 halophytes and 8 mangroves were collected, stored in separate polythene bags and transported to the laboratory for processing the same day (Table-2).

**Analysis of total polyphenols in dry plant materials:** The collected plant samples were identified and authenticated by the Botany Department (Annamalai University) and a voucher specimen for each species was preserved at the university herbarium. Vitamin C (ascorbic acid), luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma Chemicals (St. Louis, MO, USA). All other chemicals were of analytical reagent grade. Polyphenols in dry plant materials were determined spectro-photometrically using the Folin-Ciocalteu reagent based on a colorimetric oxidation/reduction reaction. To 0.2 mL of diluted aqueous acetone sample, 1 mL of Folin-Ciocalteu reagent (Merck, diluted 10 times with water) was added. After that, 0.8 mL of 7.5 % $Na_2CO_3$ was added and mixed thoroughly. After 0.5 h of standing, the absorbance was measured at 765 nm. The amount of total polyphenols was calculated as a chlorogenic acid equivalent from the calibration curve of chlorogenic acid standard solutions. All measurements were done in triplicate.

**DPPH-free radical scavenging activity:** The free radical-scavenging activity of polyphenols on the DPPH radical was assessed using the method described by Fenglin *et al.*[45] with some modifications[46]. A stock solution (1 mg/mL) of each extract was prepared and diluted with methanol to various concentrations. An aliquot of 50 µL of each dilution was transferred into a 96-well micro-plate (NUNC, Roskilde, Denmark). A working solution of DPPH (250 µM) in methanol was freshly prepared and then an

TABLE-2
HABITAT AND GROWTH OF 13 SPECIES OF HALOPHYTIC AND MANGROVE MEDICINAL PLANTS FROM TAMIL NADU, INDIA

| Mangrove/ halophyte | Habitat | Name of the species | Family | Local name | Growth form |
|---|---|---|---|---|---|
| Salt marsh halophytes | Backwater | *Arthrocnemum indicum* | Chenopodiaceae | Pavalapoondu | Prostrate under shrub |
| | Backwater | *Suaeda monoica* | Chenopodiaceae | Karu umari | Erect/ascending herb |
| | Backwater | *Suaeda maritima* | Chenopodiaceae | Umari | Erect herb |
| | Backwater | *Sesuvium portulacastrum* | Aizoaceae | Kadal vazhukai keerai | Prostrate herb |
| | Low saline inland | *Ipomoea pes-caprae* | Convolvulaceae | Kuthiraikulambu | Creeper |
| Mangrove | Marshy | *Avicennia officinalis* | Avicenniaceae | Alai athi | Small tree |
| | Backwater | *Acanthus ilicifolius* | Acanthaceae | Kalimulli | Gregarious shrub |
| | Marshy | *Aegiceras corniculatum* | Myrsinaceae | Narikandal | Large shrub/small tree |
| | Backwater | *Excoecaria agallocha* | Euphorbiaceae | Thillai | Small tree |
| | Marshy | *Bruguiera cylindrica* | Rhizophoraceae | Pannukkuchi | Medium buttressed tree |
| | Marshy | *Ceriops decandra* | Rhizophoraceae | Sirukandal | Shrub or small tree |
| | Marshy | *Rhizophora apiculata* | Rhizophoraceae | Surapunnai | Tree |
| | Marshy | *Rhizophora mucronata* | Rhizophoraceae | Surapunnai | Large/small tree |

aliquot of 150 µL was added to each well. After incubation for 0.5 h, the quenching at an absorbance of 490 nm was measured on an ELISA reader (Thermo Labsystems, Cheshire, UK). Each dilution was performed at least in triplicate. Free radical-scavenging activities of test samples and the positive control (vitamin C) were expressed in terms of $IC_{50}$ values, which is the concentration of a sample required to decrease the absorbance at 490 nm by 50 % compared to the control response.

## RESULTS AND DISCUSSION

The results on the estimation of total polyphenols and their free radical scavenging activity for the halophytic and mangrove plants are given in Table-3. The total polyphenol contents of these plants ranged from 23.5 to 384.2 mg/g (dry weight). The highest polyphenol content of 384.2 mg/g (dry weight) was recorded in *C. decandra*. Besides, higher polyphenolic contents were also recorded in *R. apiculata* (302 mg/g dry weight), *E. agallocha* (279.3 mg/g dry weight), *B. cylindrica* (238.8 mg/g dry weight) and in *I. pes-caprae* (209 mg/g dry weight). Local people in India consume cooked leaves of *S. portulacastrum* as a food item and we found a total polyphenol content of 55.1 mg/g dry weight in these leaves (Table-2). Interestingly, we found plants belonged to the same genus to have a wide range of polyphenolic contents. For example, *S. maritima* and *S. monoica* had 23.5 and 57.1 mg/g (dry weight) of total polyphenols when compared to *R. mucronata* and *R. apiculata* that had 157.4 and 302 mg/g (dry weight; Table-3).

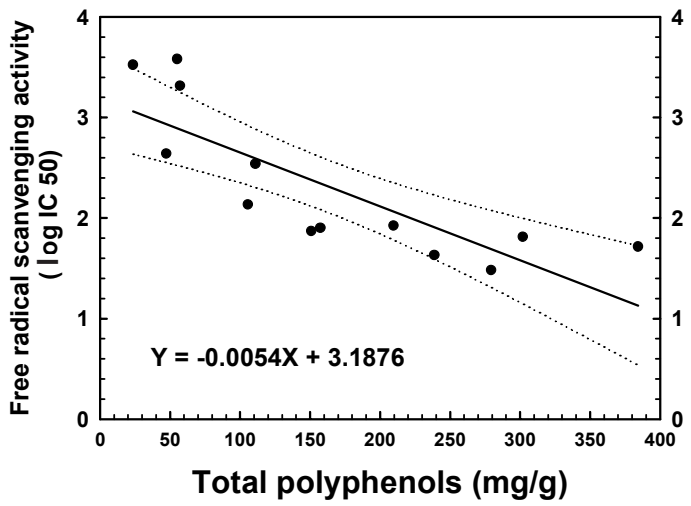


Fig. 1. Correlations of polyphenolic contents and free radical scavenging activity (regression (solid line) with 95% confidence intervals (dotted line) among selected mangrove plants of India

TABLE-3
TOTAL POLYPHENOLS AND FREE RADICAL SCAVENGING ACTIVITY OF 13 SPECIES OF HALOPHYTES AND MANGROVES FROM INDIA

| Name of species | Total polyphenols[a] | Free radical scavenging activity (μg/mL)[b] |
|---|---|---|
| *Arthrocnemum indicum* | 47.3 ± 5.0 | > 250 (437.8 ± 30.9) |
| *Suaeda monoica* | 57.1 ± 6.4 | > 250 (2073.1 ± 871.4) |
| *Suaeda maritima* | 23.5 ± 4.2 | > 250 (3343.0 ± 902.8) |
| *Sesuvium portulacastrum* | 55.1 ± 4.4 | > 250 (3813.7 ± 2101.4) |
| *Avicennia officinalis* | 111.0 ± 15.4 | > 250 (344.4 ± 5.2) |
| *Acanthus ilicifolius* | 105.6 ± 1.0 | 136.3 ± 6.3 |
| *Aegiceras corniculatum* | 150.8 ± 17.2 | 74.3 ± 1.0 |
| *Excoecaria agallocha* | 279.3 ± 26.0 | 30.3 ± 0.3 |
| *Bruguiera cylindrica* | 238.8 ± 28.6 | 42.9 ± 0.1 |
| *Ceriops decandra* | 384.2 ± 26.9 | 51.9 ± 1.8 |
| *Rhizophora apiculata* | 302.0 ± 39.0 | 64.9 ± 0.7 |
| *Rhizophora mucronata* | 157.4 ± 22.9 | 79.7 ± 1.3 |
| *Ipomoea pes-caprae* | 209.7 ± 24.8 | 83.7 ± 2.8 |

[a]The total polyphenols were expressed as mg chlorogenic acid /g dry plant material.
[b]The antioxidant activity was evaluated as the concentration of the test sample required to decrease the absorbance at 490 nm by 50 % in comparison to the control response. The data in parentheses were obtained from concentration- % DPPH remaining profile through extrapolation.
Data are presented as the mean ± standard deviation (n = 3).

We also found significant negative correlation between polyphenols and DPPH-free radical scavenging activity among the 13 species of medicinal plants (Spearman Correlation coefficient = -0.90, $p < 0.001$; Fig. 1). The polyphenolic content was significantly higher in 8 mangrove plants compared to 4 halophytic plants (Wilcoxon Rank Test, $p < 0.01$). However, the DPPH-free radical scavenging activity was significantly lower in 8 mangrove species compared to 4 salt marsh halophytes (Wilcoxon Rank Test, $p < 0.01$), which indicates that mangrove plants have higher potential in scavenging the free radicals.

The values of DPPH free radical scavenging activity are given as $IC_{50}$- the lower the $IC_{50}$ value is the greater the free radical scavenging activity. The $IC_{50}$ values of all the plants in this study ranged from 30.3 to 3813 μg/mL and the α-tocopherol (vitamin E) produced the $IC_{50}$ value of 22.5 μg/mL. Vitamin E, which is a critical component of antioxidant system and can be used as potential therapeutic agent to reduce clinical diseases

associated with increased free radical activity[47]. The highest free radical scavenging activity was recorded in *E. agallocha i.e.* 30.3 µg/mL, which is slightly lower than α-tocopherol. The other higher free radical scavenging activities were recorded in *B. cylindrica* (42.9 µg/mL), *C. decandra* (51.9 µg/mL), *R. apiculata* (64.9 µg/mL), *A. corniculatum* (74.3 µg/mL) and *R. mucronata* (79.7 µg/mL) (Table-3). Any molecule that can donate an electron or hydrogen to DPPH can react with the DPPH and thereby bleach the DPPH absorption.

Despite the fact that more research has been focused to derive drugs from medicinal plants to cure wide range of diseases, the antioxidant activities of many traditional medicinal plants have not been systematically studied due to of lack of popularity. The compounds that possess antioxidant activity can inhibit mutation and cancer because they scavenge free radicals or induce antioxidant enzymes[48]. Many plant chemicals have been reported to have antioxidant, antiallergic, antiviral, antiinflammatory, antiproliferative and anticarcinogenic properties. Some of the Indian medicinal plants have already been proved to have potent antioxidant activity[49]. Interest on plant derived food additives has grown enormously in recent years and herbs and spices usually used to flavor dishes are an excellent source of phenolic compounds that have been reported to show good antioxidant activity[40,50]. Plant extracts might substitute synthetic food antioxidants that may influence human health when consumed chronically[51]. Plant derived food additives, especially polyphenolic compounds have been ascribed health promoting properties in terms of preventing cardiovascular diseases[52].

Polyphenols are especially important antioxidants because of their high redox potentials that allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers[53]. The analysis of total polyphenols in present study showed that halophytic plants contain lesser amount of polyphenolic contents than the mangrove plants. The halophytes survive by minimizing the entry of salt into the plant and by minimizing the concentration of salt in the cytoplasm and species such as *S. monoica*, *S. maritima, S. portulacastrum* and *A. indicum* are herbs or under shrubs. In general, polyphenols are believed to provide a chemical defence against predators and ultraviolet radiation[54]. This becomes true when comparing the polyphenol content of these plants with another halophyte (*I. pes-caprae*) that contained the highest total polyphenol content (209.7 ± 24.8 mg/g dry wt.) among the halophytic specimens examined in this study. The former four halophytes are succulent halophytes adopted with fleshy leaves adapted to grow in salt marsh or backwater habitat whereas the later thrives in sand dunes. The leaf morphology of the sand dune plant is entirely different from the succulent halophytes and the leaf blade receives more radiation.

These morphological and physiological characteristics might have evolved to increase their polyphenolic contents.

The *C. decandra* having highest total polyphenol (302 ± 39.0 mg/g dry wt.) was recommended for the production of tea as it contained significant theaflavin content, which is used as a major parameter in the quality determination of commercial tea. This plant had no toxic effect in mice[39]. Total polyphenol content of *C. decandra* makes a clear evidence for the production of tea from the leaves and the plant can also be included in herbal mixtures as it has many medicinal properties.

DPPH has been widely used to evaluate the free radical scavenging activity of natural antioxidants[55]. It is a paramagnetic compound with an odd electron shows strong absorption band in methanol. The scavenging activity of antioxidants by hydrogen donation decreases the absorbance as the colour changes from purple to yellow. Though several mangroves and halophytes are extensively used in traditional medicine, only some of them were tested for biological activities and a very few were studied for antioxidant activity[56]. In this study, *E. agallocha* had the lowest $IC_{50}$ value, which in turn showed the highest scavenging activity when compared to all other plants tested. The anti-tumor activity of this plant had already been reported[20]. The other major phytochemicals reported from the plant include diterpenoids[57-59] and triterpenoids[60,61]. The present study found all four plants belonged to the family Rhizophoraceae showed potential antioxidant activity, which further supports previous reports that indicated antioxidant enzymes and anti-HIV activity[11,23,62].

The phenolic compounds are known to have direct antioxidant property due to the presence of hydroxyl groups, which function as hydrogen donor[63,64]. There have been many reports for the increase in antioxidant activity with increased total polyphenol content[50,65,66]. In present study, there was no increase in antioxidant activity observed with the increase in total polyphenol. A study conducted by Heo *et al.*[67] with enzymatic extracts of some brown seaweeds, some extracts did not possess antioxidant activity, although they contained similar phenolic content like other extracts studied. Similar findings were reported from some tropical plants consumed in South East Asia[68]. The reason for this may be due to differential response of these polyphenols to Folin-Ciocalteu reagent depending on the number of phenolic groups they possess[69].

In conclusion, the present study confirms the potential free radical scavenging activities of India's mangrove and halophytic plants such as *E. agallocha, B. cylindrica, C. decandra, R. apiculata, A. corniculatum* and *R. mucronata*. These plants can further be investigated for their biological activity against inflammation, cardiovascular diseases, ulcers and cancers influenced by the production of free radicals.

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