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# Assessment of Biochemical, Phytochemical and Antioxidant Activities of Eight Mangrove Plant Leaf Extracts

M. KIRAN KUMAR
Research Scholar
Department of Biotechnology, Andhra University
Visakhapatanam, Andhra Pradesh, India.
S. J. MOUNIKA
Research Scholar
Department of Biotechnology, Andhra University
Visakhapatanam, Andhra Pradesh, India
T. J UDAY RANJAN
Research Scholar
Department of Marine Living Resources, Andhra University
Visakhapatanam, Andhra Pradesh, India
Dr. P. SUDHAKAR RAO
Assistant Professor
Department of Biotechnology, Andhra University
Visakhapatanam, Andhra Pradesh, India
Prof. B. V. SANDEEP
Head & Professor
Department of Biotechnology, Andhra University
Visakhapatanam, Andhra Pradesh, India

**Abstract:**

*The present study is aimed to determine the biochemical, mostly protein concentrations, antioxidant activities and phytochemical constituents in eight mangrove plant leaves existing in Bhavanapadu creek area, specifically Acanthus ilicifolis L. , Aleropus littoralis L., Avicennia marina L., Ceriops tagal L., Rhizophora mucornata L., Sesuvium portulacastrum L., Suaeda maritima L., Zizania aquatica L. Mangrove plants are more susceptible for oxidative stress and damage due to high contents of salt and minerals inside these plants. Therefore, this study was carried out to explore eight mangrove leaf extracts chiefly for antioxidant and*

*phytochemicals by well known methods. Results showed that the tested leaf extracts possess significant antioxidant and phytochemical activities along with fine protein content. The conclusion of this study is that the tested leaf extracts can serve as natural antioxidant agents, setting ahead the possibility of employing them for therapeutic purposes.*

**Key words:** Mangroves, phytochemicals and antioxidants.

## Introduction

Free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), induce oxidative damage to biomolecules, such as deoxyribonucleic acid (DNA), lipids, proteins, and carbohydrates. Oxidative damage of these biomolecules can lead to mutations and contribute to carcinogenesis and the aging process. Antioxidants of plant origin are believed to help protect the cells from free radical damage. Therefore, several studies in recent years showed that polyphenols in plants scavenge active oxygen species and effectively prevent oxidative cell damage. For this reason, considerable attention has already been focused on the isolation, characterization, and utilization of natural antioxidants as potential disease preventing agents. However, little is known about the antioxidant potentials of Indian mangroves. **(Foukia *et al.,* 2014; Krishnamoorthy *et al.,* 2011)**. Mangroves represent a large multiplicity of plant community of divergent families being adapted to saline environment and are best developed on estuarine tropical world particularly in continuous exposure of tidal influence. Mangroves being salt forbearing plants are more vulnerable for oxidative stress and damage owing to high contents of salt and minerals inside these plants. Therefore, self defence against this oxidative stress appeared in the form of high antioxidant and phytochemicals in these plants **(Nirjhar Dasgupta *et al.,***

**2012).** Thus the key objective of this study was to assess four enzymatic antioxidants such as superoxide dismutase, catalase, peroxidase and glutathione reductase and also in quantifying the phytochemicals, specifically total phenols, flavonoids, alkaloids and tannins in eight mangrove plants in Bhavanapadu creek area to be precise *Acanthus ilicifolis L. , Aleropus littoralis L., Avicennia marina L., Ceriops tagal L., Rhizophora mucornata L., Sesuvium portulacastrum L., Suaeda maritima L., Zizania aquatica L.*

## Materials and Methods

### Chemicals

Chemicals and reagents used for biochemical, antioxidant and phytochemical estimations were purchased from Merck. All additional chemicals used were analytical grade. Altogether the experiments were performed at room temperature unless otherwise stated.

### Sample collection

The leaves of eight mangrove plants namely *Acanthus ilicifolis L., Aleropus littoralis L., Avicennia marina L., Ceriops tagal L., Rhizophora mucornata L., Sesuvium portulacastrum L., Suaeda maritima L.* and *Zizania aquatica L.,* were collected from Bhavanapadu creek, Tekkali, Andhra Pradesh, India. Healthy leaves were selected and washed thoroughly with running tap water and were evaluated for biochemical, antioxidant and for phytochemical, activities.

### Total protein extraction and estimation

Total leaf proteins were extracted and estimated separately by the poly vinyl pyrrolidine (PVP) precipitation method **(Ferreira *et al.,* 2002)**. Fresh leaf tissues (0.5 g) were homogenized in 50 mM sodium phosphate buffer containing 10% (w/v) insoluble PVP using a pre chilled mortar and pestle

and incubated overnight at $40^0$C. The homogenates were centrifuged at 14000 rpm for 20 min at $4^0$C (Remi Instruments, India). The supernatant was kept under -$20^0$C for protein estimation. The total protein content was estimated using the method of **Lowry *et al.,* (1951)**. To 1 ml of each test sample, 4 ml of freshly prepared alkaline solution (prepared by mixing 50 ml of 2% $Na_2CO_3$ in 0.1 N NaOH and 1 ml of 0.5% $CuSO_4.5H_2O$ in 1% sodium potassium tartrate) was added at room temperature and kept undisturbed for 10 min. Subsequently, to each of these mixture tubes 0.5 ml of Folin-Ciocalteau reagent was added and after half an hour, the OD of each was measured at 750 nm using spectrophotometer against the blank (without protein sample). The total protein content in each sample was calculated by referring the ODs of test sample with the standard curve of BSA. Supernatant was used for the estimation of enzymatic antioxidants.

## Estimation of enzymatic antioxidants

### Assay of superoxide dismutase

The assay of superoxide dismutase was carried out based on the reduction of Nitroblue tetrazolium (NBT) **(Beuchamp and Fedovich, 1976)**. To 0.5 ml of leaf extract, 1ml 125mM of Sodium Carbonate, 0.4 ml of 24µM NBT and 0.2 ml of 0.1mM EDTA were added. The reaction was initiated by adding 0.4 ml of 1mM Hydroxylamine hydrochloride. Zero time absorbance was taken at 560 nm using spectrophotometer, followed by recording the absorbance after 5 min at 25˚c.The control was simultaneously run without leaf extract. Units of SOD were expressed as amount of enzyme required for inhibiting the reduction of NBT by 50%. The specific activity was expressed in terms of Units per mg of protein.

**Assay of Catalase**

Catalase activity was determined by the titrimetric method **(Chance and Maehly, 1995).** To 1ml plant extract, 5 ml of 300 µM phosphate buffer (pH 6.8) containing 100 µM hydrogen peroxide ($H_2O_2$) was added and left at 25°C for 1 min. The reaction was arrested by adding 10 ml of 2% sulphuric acid, and residual $H_2O_2$ was titrated with potassium permanganate (0.01N) till pink colour was obtained. Units of enzyme activity were expressed as ml of 0.1N potassium permanganate equivalents of $H_2O_2$ decomposed per mg protein per min.

**Assay of Peroxidase**

Assay of Peroxidase activity is carried out according to the procedure of **Malik and Singh (1980)**. 3.5 ml of phosphate buffer (pH 6.5) was taken in a clean dry cuvette, 0.2 ml leaf extract and 0.1 ml of freshly prepared o-dianisidine solution was added. The temperature of assay mixture was brought to 28˚C- 30˚C and then placed the cuvette in the spectrophotometer set at 430nm.Then, 0.2 ml of 0.2M $H_2O_2$ was added and mixed. The initial absorbance was read and then, at every 30 sec intervals up to 3 min. A graph was plotted with the increase in absorbance against time. From the linear phase, the change in absorbance per min was read. Water blank was used in the assay. The enzyme activity was expressed in units per mg of protein per min.

**Assay of Glutathione reductase**

Glutathione Reductase Activity (GR) was measured according to **Carlberg and Mannervick (1985)**, following the decrease in absorbance at 340 nm induced by oxidised glutathione in the presence of NADPH in phosphate buffer, pH 7.8. Absorbance changes were read between 1 and 10 min. Glutathione reductase activity was expressed as µg of glutathione utilized per mg of protein.

**Methanolic extract preparation**

Methanolic extract of fresh leaves was prepared according to modified method of **Farrukh *et al.,* (2006)**. 10 grams of leaf material were soaked separately in (twice i.e., 2×100ml) of methanol and for 8-10 days at room temperature in dark conditions, with stirring at every 18 h using a sterile rod. The final extracts were filtered using a Whatman No 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator (Buchi rotavapour-114) and stored at 4°C for further use. Each extract was resuspended in the respective solvent that is methanol to yield a 40 mg/ml stock solution, this methanolic extract was used for the estimation of phytochemicals.

**Estimation of total phenolics**

The amount of total phenolics in extracts was determined according to the Folin- ciocalteu procedure **(Javanmardi *et al.,* 2003)**. Samples (200 µl) were introduced into test tubes. One milliliter of Folin ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured. The total phenolic content was expressed as mg of gallic acid equivalent per ml of extract as calculated from standard gallic acid graph.

**Estimation of total flavonoids**

Total flavonoid content of the extract was determined according to a modified colorimetric method **(Bao *et al.,* 2005)**. Leaf extract (1.0 ml) was mixed with 1ml of distilled water and 75 µl of a 5% $NaNO_2$ solution. After 5 min, 75 µl of 10% $AlCl_3.H_2O$ solution was added. After 5 min, 0.5 ml of 1M Sodium hydroxide was added. The solution was mixed well and kept for 15 min. The increase in absorbance was measured at 510 nm using a UV-Visible spectrophotometer. The total flavonoid content was calculated using standard quercetin calibration

curve. The results were expressed as milligrams of quercetin equivalents (QE) per ml of extract.

**Estimation of total tannins**

The total tannins were determined using the method of **Folin-ciocalteau (1927)**, briefly, 0.1 ml of leaf extract, 6.5ml of water and 0.5 ml of Folin- ciocalteau reagent and 1.5 ml of 20% sodium carbonate at overnight standard solution were added and incubated at 1 hour the absorbance of sample was measured in spectrophotometer at 725 nm and the results were expressed as mg of tannic acid equivalents per ml of extract.

**Estimation of total Alkaloids**

Total Alkaloid content was estimated by the method of **Sreevidya and Mehrotra (2003)**. A standard solution was prepared by dissolving 5 mg of boldine and leaf extract separately in 5 ml of warm distilled water each. Five ml of boldine solution/sample extract was adjusted to pH 2-2.5 (with 0.01 M HCl), and 2 ml of DR (Dragendorff's reagent) was added to form an orange precipitate that was centrifuged at 5000 rpm for 15 min. Afterwards, DR was added to the supernatant to check for complete precipitation. A 2 ml amount of 1% sodium sulfide was added to the residue to form a brownish black precipitate which was centrifuged at 5000 rpm for 15 min. Complete precipitation was checked by further adding 1% sodium sulfide. The resulting residue was dissolved in 2 ml of nitric acid with warming and sonication and then made up to 10 ml with distilled water. A 5 ml amount of 3% thiourea was added to 1 ml of the resulting solution to form a yellow bismuth complex, of which the absorbance was measured at 435 nm. All the assays were performed in triplicate. The amount of bismuth present in the boldine solution/extract was achieved from the calibration curve of bismuth nitrate. The results were expressed as boldine, considering that is a monobasic alkaloid,

and therefore the complex formed with bismuth follows a 1:1 stoichiometry.

### Statistical analysis

The results of *in vitro* studies were given as Mean±Standard Deviation (SD) obtained from three independent experiments, and analyzed with Student's t-test for paired data and a 'p' value less than 0.05 was considered as significant difference in the analysis.

## Results and Discussion

### Biochemical components

Among all eight mangrove samples, leaves of *Avicennia marina* have high amount of protein content with 880±0.02 µg/ml and *Sesuvium portulacstrum* contain low protein content with 450±0.03 µg/ml, where as *Zizania aquatica* and *Suaeda maritima* encompass moderate amounts of proteins by 680±0.04 and 400±0.02 µg/ml respectively. Comparable sort of study was carried out by **Chekuboyina Ravi Kiran *et al.,* (2012)** among germinating and non germinating *Ceiba pentandra* seed extracts. The outcomes acquired were presented in table.1.

### Enzymatic antioxidants

The control of steady-state ROS levels by SOD is an important protective mechanism against cellular oxidative damage, since $O_2^{\cdot-}$ acts as a precursor of more cytotoxic or highly relative ROS **(Mittler *et al.,* 2004)**. SOD has been established to work in collaboration with POD and CAT which act in tandem to remove $O_2^{\cdot-}$ and $H_2O_2$ respectively **(Blokhina *et al.,* 2003)**. Highest activity of superoxide dismutase was observed in extract of *Avicennia marina L.* with 2.89±0.01 Units per mg of protein. Lowest activity observed in *Zizania aquatic*a *L.* by 1.60±0.04 Units per mg of protein. Superior SOD activity can be triggered by increased production of ROS or it might be a

protective measure adopted by mangrove leaves against oxidative damage. In the present study, CAT action was also examined. CAT activity could be an indication of the cellular evaluated ROS, since the amount of CAT present in aerobic cells is directly proportional to the oxidative state of the cells **(Apel *et al.,* 2004)**. Among all the eight mangrove plants highest activity of catalase was observed in *Avicennia marina* with 2.20±0.05 $H_2O_2$ decomposed per mg protein per min, lowest activity observed in *Acanthus ilicifolis L.* through 1.45±0.01 $H_2O_2$ decomposed per mg protein per min. Moderate activities were observed in *Rhizophora mucornata L.*, and in *sesuvium portulacstrum L.* with 1.46±0.02 and 1.85±0.05 $H_2O_2$ decomposed per mg protein per min correspondingly.

In plants, Glutathione reductase and POD were considered to be associated with a number of essential metabolic processes, such as cell elongation, lignification, phenolic oxidation, pathogen defence and defence against stress **(Lewis *et al.,* 1993; Passardi *et al.,* 2005)**. Peroxidases are substantially more efficient on a molar basis than other enzymes. Glutathione reductase acts as a radical scavenger, membrane stabilizer and precursor of heavy metal binding peptides. Highest activity of glutathione reductase was showed by *Sesuvium portulacstrum L.* with 0.42±0.05 µg of glutathione utilized per mg of protein, and lowest activity by *Ceriops tagal L.* with 0.10±0.05 µg of glutathione utilized per mg of protein. Modest activities were experiential in *Avicennia marina L.* and in *Rhizophora mucornata L.* through 0.3±0.04 and 0.31±0.01 µg of glutathione utilized per mg of protein. Alongside with that highest and lowest peroxidase activities were observed in *Avicennia marina L.*, and *Ceriops tagal L.* with 8.83±0.03 and 0.20±0.01 units per mg of protein per min respectively. Outcomes were laid on sight in table.2.

## Phytochemicals

Phytochemicals are invaluable sources of raw material for both traditional and orthodox medicine. Methanolic extracts of leaves containing bioactive agents such as alkaloids, tannins and phenolic thus, readily present themselves as a good source of raw material in modern and traditional medicine. Phenolics are able to scavenge reactive oxygen species due to their electron donating properties. In many *in vitro* studies, phenolic compounds demonstrated higher antioxidant activity than antioxidant vitamins and carotenoids. These antioxidants can act against various degenerative diseases such as Alzheimer, Parkinson and cancer. Phenolic compounds and their antioxidant nature are important in disease prevention in plants and animals. The grades of total phenolic content were expressed as mg of gallic acid equivalents per ml of extract. From the preliminary phytochemical studies of tested leaf extracts total phenol content of the eight mangroves was ranged from 0.72±0.012 to 0.51±0.017. Superior amount of phenolic content was present in the *Rhizophora mucornata L.* with 0.72±0.012, while *Zizania aquatica L.* had low phenolic content with 0.51±0.017. Moderate amount of phenolics were observed in *Avicennia marina L.* and *Ceriops tagal L* by 0.66±0.09 and 0.61±0.012 respectively.

Flavonoids are a major group of secondary compounds that act as primary antioxidants or free radical scavengers and antimicrobial or free radical scavengers **(Polterait, 1997)**. The total flavonoid content of the tested extracts ranged from 0.61±0.019 to 0.31±0.017 mg quercetin equivalents per ml. Peak total of flavonoids observed in *Zizania aquatica L.* with 0.61±0.019 mg quercetin equivalents per ml. whereas lowest value perceived in *Suaeda maritima L.* with 0.31±0.017 mg quercetin equivalents per ml and moderate value of flavonoids observed in *Rhizophora mucornata L.* with 0.56±0.037 mg quercetin equivalents per ml. Along with the above phytochemicals sensible amounts of alkaloids and tannis were

also observed in all eight tested mangrove plants. Alkaloids and their synthetic derivatives are being used as basic therapeutic agents for their analgesic, antispasmodic and bactericidal effects and also alkaloids and flavonoids inhibit certain mammalian enzymatic activities such as those of phosphodiesterase, prolonging the action of cyclic-AMP. Alkaloids also affect glucagons and thyroid stimulating hormones **(Okaka *et al.,* 1992)**. The end results of alkaloids were expressed in mg boldine equivalents per ml of extract. Tannins have shown potential antiviral **(Lü L, Liu SW *et al.,* 2004)**, antibacterial **(Akiyama *et al.,* 2001)** and antiparasitic effects **(Kolodziej *et al.,* 2005)**. It is believed that tannins isolated from the stem bark of *Myracrodruon urundeuva* may have neuroprotective functions capable of reversing 6-hydroxydopamine-induced toxicity. The plant has shown promise as a potential therapeutic agent, which may be beneficial in patients with neurological disease **(Nobre-Junior *et al.,* 2007)**. Souza *et al.* discovered that the tannins isolated from the stem bark also have anti-inflammatory and antiulcer activity in rodents, showing a strong antioxidant property with possible therapeutic applications **(Souza *et al.,* 2006)**. Foods rich in tannins can be used in the treatment of HFE hereditary hemochromatosis, a hereditary disease characterized by excessive absorption of dietary iron, resulting in a pathological increase in total body iron stores. Tannins can also be effective in protecting the kidneys. The end results of tannins were uttered in mg tannic acid equivalents in ml. All the above results were in accordance with the previous reports **(Ravi Kiran *et al.,* 2012)**. Preceding results were put on view in table.3.

**Table: 1 Total protein content of leaves of eight mangroves**

| S.No | Plant name | Leaves (µg/ml) |
|---|---|---|
| 1 | *Acanthus ilicifolis L.* | 690 ± 0.03 |
| 2 | *Aleropus littoralis L.* | 450 ± 0.04 |
| 3 | *Avicennia marina L.* | 880 ± 0.02 |

| | | |
|---|---|---|
| 4 | *Ceriops tagal L.* | 530 ± 0.04 |
| 5 | *Rhizophora mucornata L.* | 800 ± 0.04 |
| 6 | *Sesuvium portulacastrum L.* | 430 ± 0.05 |
| 7 | *Suaeda maritima L.* | 660 ± 0.04 |
| 8 | *Zizania aquatica L.* | 680 ± 0.05 |

Each value represents the mean±SD of three replicates
P < 0.05 was considered as significant difference

**Table: 2 Enzymatic activities of antioxidants in eight mangroves**

| S.No | Plant name | SOD (Units per mg of protein) | CAT ($H_2O_2$ decomposed per mg protein per min) | Peroxidase (Units per mg of protein per min) | GR (µg of glutathione utilized per mg of protein) |
|---|---|---|---|---|---|
| 1 | *Acanthus ilicifolis L.* | 2.01±0.01 | 1.45±0.001 | 2.70±0.03 | 0.29±0.03 |
| 2 | *Aleropus littoralis L.* | 2.17±0.05 | 2.10±0.06 | 2.28±0.05 | 0.27±0.01 |
| 3 | *Avicennia marina L.* | 2.89±0.01 | 2.20±0.005 | 8.83±0.03 | 0.3±0.04 |
| 4 | *Ceriops tagal L.* | 1.75±0.01 | 2.10±0.001 | 1.82±0.01 | 0.10±0.05 |
| 5 | *Rhizophora mucornata L.* | 2.01±0.01 | 1.45±0.002 | 4.47±0.05 | 0.31±0.01 |
| 6 | *Sesuvium portulacastrum L.* | 1.85±0.05 | 1.85±0.003 | 4.31±0.05 | 0.42±0.05 |
| 7 | *Suaeda maritima L.* | 2.07±0.04 | 2.07±0.001 | 3.20±0.01 | 0.39±0.01 |
| 8 | *Zizania aquatica L.* | 1.60±0.04 | 2.12±0.004 | 3.29±0.01 | 0.23±0.05 |

Each value represents the mean±SD of three replicates
P < 0.05 was considered as significant difference

**Table: 3** Phytochemicals in eight mangroves

| S.No | Plant name | Phenols (mg of gallic acid equivalent per ml) | Flavonoids (mg quercetin equivalents per ml) | Tannins (mg tannic acid equivalents in ml) | Alkaloids (mg boldine equivalents per ml) |
|---|---|---|---|---|---|
| 1 | *Acanthus ilicifolis L.* | 0.55±0.014 | 0.52±0.036 | 0.12±0.036 | 0.15±0.023 |
| 2 | *Aleropus littoralis L.* | 0.71±0.015 | 0.54±0.029 | 0.14±0.025 | 0.17±0.014 |
| 3 | *Avicennia marina L.* | 0. 66±0.009 | 0.55±0.001 | 0.15±0.023 | 0.16±0.017 |
| 4 | *Ceriops tagal L.* | 0.61±0.012 | 0.57±0.030 | 0.19±0.018 | 0.10±0.018 |
| 5 | *Rhizophora mucornata L.* | 0.72±0.012 | 0.56±0.037 | 0.19±0.023 | 0.15±0.019 |
| 6 | *Sesuvium portulacastrum L.* | 0.57±0.010 | 0.45±0.008 | 0.17±0.019 | 0.16±0.019 |
| 7 | *Suaeda maritima L.* | 0.63±0.015 | 0.31±0.017 | 0.19±0.025 | 0.14±0.017 |
| 8 | *Zizania aquatica L.* | 0.51 | 0.61±0.019 | 0.14±0.023 | 0.13±0.017 |

| | | | | | |
|---|---|---|---|---|---|
| | | ±0.017 | | | |

Each value represents the mean±SD of three replicates

$P < 0.05$ was considered as significant difference

## Conclusion

Finally, it can be concluded that the leave extracts of eight mangrove plant possess significant antioxidant activity. The potential of these activities may be due to the presence of phyto-constituents reported in the phytochemical tests and justify its uses as a habitual folk medicine. A comparison among the results guides us to the finale that, constituents of this extracts may provide as a source of drugs useful in the chemotherapy of some inflammatory and infectious diseases and also as an antioxidant agent. However, a more extensive study should necessary to establish the exact mechanism(s) of action and its active compound(s) of this extract.

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