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EVALUATION OF PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF VARIOUS HALOPHYTES COLLECTED FROM PICHAVARAM REGION

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Abstract–Mangrove halophytes are highly influential in economical utilities such as food, fuel and Medicinals. Antioxidant and Phytochemical activities were highly significant in these samples. The synthesis of metabolites such as phenols, flavonoids and other bioactive molecules were observed and analysed. In the current script the phytochemical screening and antioxidant assays such as DPPH and ABTS were carried out for aqueous, methanol and hexane extracts of mangrove halophytes such as *Ipomoea I. pes-caprae*, *Sesuvium portulacastrum* and *Suaeda maritima.* The preliminary phytochemical screening indicates the presence of different phytoconstituents present in the free samples. For antioxidant activity DPPH radical scavenging assay and ABTS radical scavenging assay were performed.

INTRODUCTION

In the 21st century, Halophytes are eligible sources to restore contaminated and saline lands. They grow even in semiarid areas due to tolerance in salinity (Kafi and Khan, 2008). Research has proven that the Soil polluting heavy metals such as Cu, Zn and Ni failed to impact these plants. They are beneficial alternative to traditional crops and can also be used as feedstocks. The osmotic adjustment in these types of plants was impressive so that they can adopt even in low water level. The rhizobacteria which are salt tolerant were isolated from the rhizosphere of halophytes which enhance the plant growth (Jha *et al.,* 2015, Shukla *et al.,* 2012, Bharti *et al.,* 2013, Ramadoss *et al.,* 2013, Goswami *et al.,* 2014, Sharma *et al.,* 2016, Yaun *et al.,* 2016). Mangrove plants need attention and restoration since they safeguard coasts from erosion and provide habitats for various kinds of aqua species. Phytoremediation, artificial agro-ecosystems to yield food and fuel for the increasing human population were some of the benefits included in mangrove domestication. It can increase the level of food production up to 70% by 2050 (Etesami *et al.,* 2018). The future upholds combat against climate change and pollinator crisis with the lead of mangrove plants. Halophytes are the source of powerful antioxidants, phytochemicals and bioactive metabolites. Among them *Ipomoea I.pes-caprae* is a kind with starchy root which is both diuretic and purgative (Heatwole *et al.,* 2011). They contain saponin and used for relief in bladder disease. It is reported that an unnamed alkaloid has been present in the leaves of *Ipomoea I.pes-caprae* and the leaves have beneficial effect on bed sores. The antidotes for venomous fish have been extracted from leaves of *Sesuvium portulacastrum* and they are antiscourbutic (Chandrasekaran *et al.,* 2011). *Suaeda maritima* is edible and can be consumed raw or cooked. The juice extracted from this plant leaves is used for curing heart, liver and lipid disorders. There are some other halophytes beneficial in phytoremediation and economical usage (Banerjee *et al.,* 2008; Muthazhagan *et al.,* 2014). This manuscript exposes the analysis of phytoconstituents and antioxidants present in mangrove halophytes such as *I.pes-caprae*, *Sesuvium portulacastrum* and *Suaeda maritima* from their

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extracts.

MATERIALS AND METHODS

Collection and Processing of samples

The mangrove plant species were collected from Pichavaram mangrove forest (Lat. 110 20′ N; Long. 79°47′ E), Tamilnadu located in south East coast of India. The halophytes were selected and placed in sealed plastic bags. In the laboratory, plant materials were thoroughly washed using sterile water in order to eliminate surface contaminating microbes and air dried. The plant sample was ground into coarse powder using a domestic grinder and the powder was then passed through a sieve.

Preparation of Plant extracts

The extracts of the plant materials were obtained using the cold maceration method. Fifty grams of powdered plant materials *Ipomoea I.pes-caprae*, *Sesuvium portulacastrum* and *Suaeda maritima* were weighed into separate sterile conical flasks. The samples were extracted using 250 ml aqueous, methanol and hexane separately and left for 48 hours at room temperature. The resultant suspensions were filtered into sterile conical flasks. They were labeled appropriately and used for further studies.

Phytochemical screening of Halophytes

The extracts of samples such as *Ipomoea I.pes-caprae*, *Sesuvium portulacastrum* and *Suaeda maritima* were subjected to phytochemicals analysis. The aqueous, methanol and hexane extracts were analysed for different phytoconstituents such as alkaloids, glycosides, saponins, steroids, terpenoids, flavonoids, phenolic compounds etc.,

Antioxidant assay –DPPH radical scavenging assay

DPPH radical scavenging assay of samples (*Ipomoea I.pes-caprae, Sesuvium portulacastrum* and *Suaeda maritima*) were performed according to modified method described by Perumal *et al*., (2018). In brief, 0.135 mM DPPH was prepared in methanol. Different concentration of extract (5, 10, 20, 40, 80, 160 and 320 µg/ml) was mixed with 2.5 ml of DPPH solution. The reaction mixture was vortexed thoroughly and kept at room temperature for 30 min. The Absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as the reference standard. The ability of plant extract to scavenge DPPH radical and control was calculated from the following formula: respectively.

% DPPH inhibition = [(OD of control - OD of test)/ (OD of control)] ×100

The IC_{50} values of the given sample

Solvent	SP µg/ml	IP µg/ml	SD µg/ml
Water	>320	63.24	>320
Methanol	>320	35.38	>320
Hexane	>320	129.09	>320

The standard drug (Ascorbic acid) was 22.92 µg/mL

Antioxidant assay –ABTS radical scavenging assay

ABTS radical scavenging assay of samples (*Ipomoea I.pes-caprae, Sesuvium portulacastrum*) and were performed according to the modified method of Perumal *et al.*, 2018. The ABTS (7 mM, 25 ml in deionized water) stock solution was prepared with potassium persulfate ($K_2S_2O_8$) (140 mM, 440 µl). Different concentrations of test samples and standard (Ascorbic acid) was mixed with the ABTS working solution (2.0 ml) and the reaction mixture was allowed to stand at room temperature for 20 min; then, the Abs was measured using an ultraviolet-visible spectrophotometer at 734 nm. The radical scavenging activity was given as ABTS radical scavenging effect and calculated by the equation:

ABTS radical scavenging effect (%) = [(A0 - A1)/ A0]×100.

Where, A0 is the control

A1 is the test

The IC_{50} values of the given sample

Solvent	SP µg/ml	IP µg/ml	SD µg/ml
Water	243.52	36.311	23.03
Methanol	135.52	29.516	184.98
Hexane	>320	67.31	>320

The standard drug (Ascorbic acid) was 23.03 µg/ml

RESULTS AND DISCUSSION

Phytochemical screening

The aqueous, methanol and hexane extracts of mangrove species such as *Ipomoea I.pes-caprae*, *Sesuvium portulacastrum* and *Suaeda maritima* were screened for the presence of phytoconstituents shown in table. In the current study, the

Table 1. Phytochemical Analysis -*Ipomoea Pes-Caprae*

Phytochemical Compound	Screening test	Water	Methanol	Hexane
Alakaloids	Wagners reagent test	+	+	-
Reducing sugar	Molischs test	+	++	++
Glycosides	Keller Kellianis test	++	+++	+++
Flavonoids	Alkaline reagent test	+++	+	++
Phenols	Ferric chloride	++	++	+++
Amino acids & protein	Ninhydrin test	++	+++	-
Saponins	Foam test	++	+	+
Steroids	Liebermann-Burchard test	-	++	+++
Tannins	Barymers test	-	+++	++
Terpinoids	Salkowskis test	++	++	+++

Table 2. Phytochemical Analysis- *Sesuvium portulacastrum* L

Phytochemical Compound	Screening test	Water	Methanol	Hexane
Alakaloids	Wagners reagent test	+++	+++	++
Reducing sugar	Molischs test	++	++	+++
Glycosides	Keller Kellianis test	-	++	++
Flavonoids	Alkaline reagent test	++	++	+++
Phenols	Ferric chloride	+++	+++	+++
Amino acids & protein	Ninhydrin test	-	++	-
Saponins	Foam test	++	+++	+++
Steroids	Liebermann-Burchard test	+++	++	++
Tannins	Barymers test	-	-	+++
Terpinoids	Salkowskis test	+++	+++	++

Table 3. Phytochemical Analysis -*Suaeda maritime*

Phytochemical Compound	Screening test	Water	Methanol	Hexane
Alakaloids	Wagners reagent test	+	+++	+
Reducing sugar	Molischs test	+	-	+
Glycosides	Keller Kellianis test	+	-	+
Flavonoids	Alkaline reagent test	+	++	+
Phenols	Ferric chloride	+++	++	++
Amino acids & protein	Ninhydrin test	+	++	-
Saponins	Foam test	+++	+++	+++
Steroids	Liebermann-Burchard test	-	-	-
Tannins	Barymers test	+++	+++	+++
Terpinoids	Salkowskis test	+++	+++	+++

phytochemical analysis and antioxidant assays were performed using aqueous, methanol and hexane extract of *Ipomoea I.pes-caprae*, *Sesuvium portulacastrum* and *Suaeda maritima.* Phytochemical Analysis of *Ipomoea Pes-Caprae* extracts observed the presence of Reducing sugar, Glycosides, Flavanoids, Phenols and Terpenoids in common and the maximum number of compounds were observed in methanolic extract and similar results were studied by Rokad *et al.,* 2018.

While in *Suaeda maritima* the presence of Alkaloids, Flavonoids, Phenols, Saponins, Tannins and Terpenoids in common and the highest number of phyto compounds were seen in both aqueous and hexane solvent. In antioxidant assays such as DPPH and ABTS lowest IC50 value was noticed in hexane extract compared to both aqueous and methanol extract in all three halophytes. When compared with other solvents hexane showed better result as like the previous study of Bulti nayak *et al.,* (2018). The maximum IC50 is found in methanol compared to aqueous and hexane extract. In *Ipomoea I.pes-caprae*, the maximum IC50 value is 90.12 µg/mL for DPPH and 77.34 µg/ml for ABTS. In *Sesuvium portulacastrum*, the maximum IC50 value is 23.88 µg/ml for DPPH and 72.14 µg/ml for ABTS. Whereas in

Suaeda maritima, the maximum IC50 value is 17.14 µg/ml for DPPH and 76.01 µg/mL for ABTS in methanol and showed similar results compared to that of Roy *et al.*, 2018.

Antioxidant assay radical scavenging assay report

Table 1. Anti-oxidant assay (DPPH) of *Ipomoea I.pes-caprae* sample using different Solvent

Con. (µL)	Ascorbic Acid	Water	Methanol	Hexane
5	15.02865	9.519612	13.79462	4.660633
10	23.93125	12.34024	15.02865	5.837104
20	55.7955	14.89643	38.56324	16.28959
40	70.38343	41.20758	59.9383	26.1991
80	79.41825	62.49449	70.20714	39.54751
160	90.39224	68.97312	78.27237	51.71946
320	94.18246	79.37417	90.12781	71.90045

Table 2. Anti-oxidant assay (DPPH) of *Sesuvium portulacastrum* sample using different Solvent

Con. (µl)	Ascorbic Acid	Water	Methanol	Hexane
5	15.02865	7.007492	6.919348	0.497738
10	23.93125	8.373733	8.285588	1.58371
20	55.7955	9.343323	9.563684	3.076923
40	70.38343	10.88585	10.66549	4.570136
80	79.41825	11.94359	12.20802	5.972851
160	90.39224	13.44204	15.95416	7.19457
320	94.18246	14.58792	23.88717	8.190045

Table 3. Anti-oxidant assay (DPPH) of *Suaeda maritima* sample using different Solvent

Con. (µl)	Ascorbic Acid	Water	Methanol	Hexane
5	15.02865	6.919348	5.729396	0.135747
10	23.93125	8.50595	6.96342	1.764706
20	55.7955	9.563684	8.417805	3.076923
40	70.38343	10.92993	9.607757	4.38914
80	79.41825	12.29617	10.75364	5.565611
160	90.39224	13.53019	12.20802	7.013575
320	94.18246	16.08638	17.14412	8.00905

Antioxidant assay radical scavenging assay report

Table 4. Anti-oxidant assay (ABTS) of *Ipomoea I.pes-caprae*sample using different Solvent

Con. (µl)	Ascorbic Acid	Water	Methanol	Hexane
5	11.73689	20.67928	20.679278	5.256623
10	36.24248	29.66466	30.782459	15.02483
20	51.76268	46.38865	46.302666	31.25
40	67.1969	50.5589	51.805675	42.34272
80	76.44024	65.73517	72.226999	56.08444
160	88.04815	72.18401	77.343078	60.76159
320	92.08942	76.18229	85.081685	77.02815

Table 5. Anti-oxidant assay (ABTS) of *Sesuvium portulacastrum* sample using different Solvent

Con. (µl)	Ascorbic Acid	Water	Methanol	Hexane
5	11.73689	10.23216	9.200344	1.490066
10	36.24248	11.82287	10.40413	3.890728
20	51.76268	12.94067	11.6509	5.256623
40	67.1969	18.18573	19.4325	8.774834
80	76.44024	23.25881	36.28547	13.8245
160	88.04815	38.69304	56.74979	15.02483
320	92.08942	72.14101	71.88306	18.66722

Table 6. Anti-oxidant assay (ABTS) of *Suaeda maritime* sample using different Solvent

Con. (µl)	Ascorbic Acid	Water	Methanol	Hexane
5	11.73689	10.66208	7.824592	7.698675
10	36.24248	11.77988	9.286328	9.933775
20	51.76268	14.35942	14.23044	15.06623
40	67.1969	23.25881	16.89596	22.51656
80	76.44024	33.61995	34.90972	30.91887
160	88.04815	46.6466	54.29923	37.33444
320	92.08942	67.06793	76.01032	44.70199

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