Elucidation of Antibacterial and Antioxidant Activities of Sesuvium portulacastrum leaf extracts

Dr. Deepthimahanthi Divya[1*], Dr. Tasneem Jahan[1], Mrs. Nachuri Kavya[1], Dr. Poonam Dev[1], Dr. Govindarao K[2].

[1]Department of Zoology, St. Ann's College for women, Hyderabad, Telangana, India-500028

[2]Department of Zoology and Aquaculture, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India- 522510

[*] E-mail: divyamahanthi21@gmail.com

Abstract:

Mangroves have evolved several physiological and morphological changes over the course of time to withstand the harsh environmental conditions of the coastal regions. They provide a safe niche for a uniquely diverse flora and fauna. The evolutionary adaptation to harsh climate has provided mangroves with novel secondary metabolites and bioactive compounds which have found to be beneficial for human consumption. These bioactive compounds found in the Mangroves can also curb the growth of bacteria. *Sesuvium portulacastrum*, is a Mangrove, commonly called as the "sea purslane" which has been found to contain Antioxidant and Antibacterial properties. In the present study, crude extracts of *S. portulacastrum* have been made from Acetone, Diethyl ether, Petroleum ether and water. Acetone crude extract was found to exhibit phytochemicals such as alkaloids, saponins, flavonoids, proteins and Anthraquinones. Diethyl ether crude extract has shown the presence of Tannins, Saponins, Phenols and Proteins. Aqueous extract of *S. portulacastrum* has shown the presence of most of the phytochemicals such as alkaloids, tannins, saponins, glycosides, steroids, phenols, anthraquinones and reducing sugars, on the other hand, petroleum ether crude extract had shown the presence of only two phytochemicals *viz.*, glycosides and steroids. Based on the phytochemical screening, the Antibacterial test was assayed through agar well diffusion method using the aqueous extract of *S. portulacastrum* against *Bacillus thuringiensis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. It can be observed from the experiment that the maximum Zone of Inhibition was noted against *Klebsiella pneumoniae* (1.2±0.4 mm) and the minimum Zone of Inhibition was observed against *Bacillus thuringiensis* (0.5±0.02 mm). Similarly assays like DPPH and FRAP of the aqueous leaf extract have shown that *S. portulacastrum* has significant Antioxidant properties as well. The IC_{50} values of DPPH were noted to be 289.92 µl/ml and the FRAP assay has shown an increase in the reduction power with a range between 9.33 ± 0.11 µg/ml at 15.62 µg/ml concentration and 44.16 ±0.21 µg/ml at 250 µg/ml concentration.

Key Words: Mangroves, Antibacterial activity, Phytochemicals, *Sesuvium portulacastrum*

Introduction:

The world is inundated with a vast biodiversity, of which one unique ecosystem is the Mangrove ecosystem. The mangroves have evolved evolutionarily in such a way that several physiological and morphological adaptations help them survive in the harsh climes of the coastal regions. Globally, the mangroves are present in 123 countries and territories, with about 40% of the mangrove cover which is found along the coasts of South and Southeast Asia. India contributes to 3% of the total mangrove cover in South Asia. The area of interest for the present study is in Andhra Pradesh state, which currently has 404 sq. km of mangrove cover, of which Krishna District has 137 sq. km. [8, 28].

There is a need to conserve the mangrove forests as they has the evolutionary advantage of having adaptive features which are endowed with novel bioactive compounds and secondary metabolites which help them sustain in harsh conditions. Metabolites like alkaloids, terpenoids, saponins, phenolics, steroids and flavonoids are found in plants and they provide them with the ability to prevent the growth of unrequired bacteria, fungi and other parasites, they also show sensitivity towards heavy metals which are found in the soil etc. The bioactive compounds are a rich source of therapeutic precursors and also serve as industrial raw materials [22].

World is constantly plagued with an array of infectious diseases which is the leading cause behind premature deaths. Most of the notorious infectious diseases are caused by the bacteria. It has become a challenge for the scientists and pharmacists all over the world to design new therapeutic drugs against superbugs like *Candida auris*, *Clostridiodes difficile*, *Neisseia gonorrheae* etc., which have become resistant to most of the synthetic drugs produced in the world. The need of the hour is to explore the antibacterial potential of mangrove medicinal plants and to exploit them for large-scale drug production [23].

Several independent studies conducted on the mangrove plants have proven their efficacy in treating bacterial, fungal and other microbial infections. Amongst them, *Sesuvium portulacastrum* L., has proven to be an excellent antibacterial and antioxidant agent. Traditionally, *S. portulacastrum*, commonly known as the "sea purslane" is consumed by the native people as a salty, leafy vegetable. It is also used to treat various diseases and ailments such as epilepsy, scurvy, conjunctivitis, leprosy, haematuria, liver or kidney disorders etc. The GC-MS analysis of the methanolic extracts of *S. portulacastrum* has shown the presence of chemical constituents such as pyrrole derivatives, butanoic acid, ascorbic acid, octadecanoic acid and hentriacontane which act as antioxidant, antimicrobial, antiulcerogenic and anticancer agents [3]. Alshrari *et al.,* [2] stated that the methanolic and chloroform extracts of the leaves and stem of *S. portulacastrum* were efficacious in inhibiting the growth of *E. coli, P. vulgaris, S. aureus* and *K. pneumoniae*. The hexane concentrate of *S. portulcastrum* was effective against bacterial strains such as *P. aeruginosa,* and *E. coli* in comparison to ethyl acetate and methanolic extracts [14]. The aim of the present study is to explore the antibacterial as well as antioxidant properties of the leaf extracts of *Sesuvium portulcastrum*.

Materials and methods

Collection of Plants and Extraction:

Leaves of *S. portulacastrum* were collected from Gilakaladindi mangroves (16°0' N latitude and 81°10'E longitude) located in Andhra Pradesh, India. The source was identified at Botanical Survey of India (BSI), Hyderabad. Leaves of the plant were cleaned, shade dried and pulverized into fine powder using pestle and mortar. The powdered plant material was extracted by using acetone, diethyl ether, petroleumether and water as solvents by using Soxhlet apparatus. The extracts obtained were filtered using Whatman No. 1 filter paper and they were stored in a refrigerator for use in subsequent experiments.

Phytochemical screening

Screening for the phytochemicals of the aqueous extract of *S. portulacastrum* was carried out to detect the presence of alkaloids, saponins, glycosides, fats, phenols, tannins, flavonoids, terpenoids, steroids, phytosterols, anthraquinones, cardiac glycosides, reducing sugars and proteins using standard methods [12].

***In-vitro* antibacterial activity**

To determine the antibacterial activity of aqueous extract of *S. portulacastrum* against pathogens like *Staphylococcus aureus* (MTCC737), *Pseudomonas aeruginosa*, (MTCC 1688), *Bacillus thuringiensis* (MTCC 1953) and *Klebsiella pneumoniae* (MTCC 3384), agar well diffusion method was used. Mueller-Hinton Agar medium (MHA) was poured carefully into the petriplates and by using an inoculum with a size of 106 colony forming units (c.f.u)/ml of bacteria [24]. These wells were made in MHA plates using a cork- borer of 8mm. Then, 40 µl of extract was loaded into the wells and amikacin was used as a positive control. The prepared petriplates were incubated at 37ºC for 12 h to initiate the growth of the bacteria. After incubation, zone of inhibition diameter was observed and the wells made in the petriplate were measured and tabulated.

***In-vitro* antioxidant activity**

The antioxidant activity of aqueous extract of *S. portulacastrum* was analysed through DPPH radical scavenging assay and FRAP assay.

DPPH radical scavenging activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging capacity of the aqueous extracts of *S. portulacastrum* was determined and compared with a standard ascorbic acid as positive control and methanol are used as standard and blank [19]. DPPH solution at different concentrations was mixed with leaf extract in 1:1 ratio and incubated for 30 mins and the observations were recorded using spectrophotometer at 517 nm.

The DPPH radical scavenging activity was determined by the following equation:

$$\%\ \text{DPPH radical scavenging activity} = \left[\frac{(A0 - A1)}{A0}\right] \times 100$$

Ferric reducing antioxidant power (FRAP) assay:

FRAP assay was conducted in accordance to the method of Benzie and Strain [5]. The reagent for the experiment was prepared in acetate buffer (300mm) by mixing 10mm 2,4,6-tri (2-pyridyl-s-triazine) (TPTZ) solution in 40mM HCl and 20mM $FeCl_3$ solution in the proportion of 10:1:1 (v/v), which isfollowed by incubation for 15 min at 37 °C for furtheruse. The sample extract and standard ascorbic acid at different concentrations 15.62, 31.25, 62.5, 125, 250 and 500µl/ml are added to FRAP reagent. The absorbance was measured at 593 nm and the results were recorded as µg of ascorbic acid equivalents (AAE) per ml.

Results and Discussion

Phyto-chemical screening

Qualitative estimation of *S. portulacastrum* leaf extracts were analysed for the presence of phytochemicals and the results were **tabulated (1).** The analysis revealed that majority of phytochemicals like alkaloids, tannins, saponins, glycosides, steroids, phenols, anthraquinones and reducing sugars are found to be present in aqueous extract and other solvent extracts like acetone, diethyl ether and petroleum ether showed positive results only for Saponins and Proteins and negative for all other phytoconstituents. In comparison, acetone extract has shown the presence of

alkaloids, saponins, flavonoids, proteins and anthraquinones; Diethyl ether exhibited the presence of tannins, saponins, phenols and proteins. Petroleum ether tested negative for almost all the phytochemicals except glycosides and steroids.

S. portulacastrum has a rich source of phytochemicals like the 22,23-Dihydrostigmasterol, 3,4,5-trihydroxy-(Gallic acid), Benzoic acid, (2R, 3R) -(-)-Epicatechin and also Capsaicin in the ethanolic leaf and stem extracts which were responsible for their antimicrobial nature which was reported by Amad Al-Azzawi *et al.*[1]. Chintalapani *et al*. [9] reported the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, proteins, saponins, sterols, tannins, terpenoids, quinones, diterpenes in ethanol, methanol, acetone and diethyl ether extracts of *S. portulacastrum.* The phytochemicals such as the saponins, tannins, alkaloids, terpenoids and steroids were observed in the methanolic leaf extracts of *Suaeda maritima* [15]. Three mangrove species viz., *Avicennia schaueriana, Rhizophora mangle* and *Laguncularia racemosa* leaf extracts have shown the presence of phytochemicals like alkaloids, saponins, tannins, steroids, coumarins, and triterpenoids. These phytochemicals were analysed to be incredibly helpful in controlling the parasitosis of the intermediate host; *Biomphalaria glabrata* in the lifecycle of the parasite *Schistosoma mansoni*, which is the causative organism of Schistosomiasis disease. The hydroalcoholic extracts of these mangroves were proven to be effective molluscicides as they acted negatively on the biological activities of the host snails [20]. The phytochemical screening of medicinal plants like *Solanum virgianum* and *Physalis angulate* were revealed the presence of phytochemical compounds like phenols, flavonoids, saponins and glycosides [26]. Usually the phytochemicals are of high medicinal importance as they possess anti-bacterial, anti-fungal, astringent, analgesic, anti-viral, molluscicidal, immune boosting, anti-tumor, anti-malraial, ant-parasitic, anti-inflammatory, anti-oxidant properties [13, 10]. The present study revealed the presence of highest number of phytochemicals in the aqueous extract of *S. portulacastrum* indicating the efficacy of aqueous solvent over other solvents for phytochemical extraction. Extracting compounds in the present study as compared to all other extracts, with acetone, diethyl ether and petroleum ether shown very poor extractability of phytochemicals.

***In vitro* antibacterial activity**

Based on the results of phytochemical screening, the aqueous leaf extract of *S. portulacastrum* was selected for the assessment of *in vitro* antibacterial activity as it extracted a greater number of phytochemicals. The aqueous leaf extract of *S. portulacastrum* was selected for antibacterial activity as it possesses a high number of phytochemicals as their positive results exhibited in phytochemical screening. The assay was carried out *in vitro* by following the protocol for agar well diffusion method against *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*. As shown in **Table (2),** the highest zones of inhibition were observed against the *Klebsiella pneumoniae* followed by *Staphylococcus aureus*, then in *Pseudomonas aeruginosa* and the lowest zone of inhibition is seen in the

Bacillus thuringiensis. All the tested strains exhibited the zones of inhibition ranging from 0.5 to 1.2 mm. Mangrove species are most significant in exhibiting antibacterial activity, earlier studies on methanolic leaf extract of a marine mangrove plant *Avicennia marina* have shown the highest antimicrobial activity against *Pseudomonas aeruginosa* [29]. Kolli *et al*. [16] reported the antibacterial activity of methanol, ethanol and hexane extracts of *S. portulacastrum* against *staphylococcus aureus, Streptococcus pyogenes, Escherechia coli* and *Pseudomonas aeruginosa*. *Suaeda nudiflora* crude extracts made with different solvents like hexane, methanol, chloroform and ethyl acetate have shown a higher degree of inhibition zones against a selected group of bacterial strains such as four Gram +ve bacteria *viz., Bacillus subtilis, S. aureus, S. haemolycticus* and *Enterococcus faecalis* and five Gram -ve bacteria such as *Citrobacter sp., Pseudomonas sp., E. coli, Klebsiella pneumoniae* and *Stenotrophomonas maltophila*. Similarly, Divya *et al.* [11] reported that the acetone and diethyl ether extracts of *A. marina* has highest inhibitory activity against *K. pneumoniae*, *S. aureus* and *P. aeruginosa.*

***In vitro* antioxidant activity**

The DPPH radical scavenging activity and FRAP assays were used to test the antioxidant activity of the aqueous crude leaf extracts of *S. portulacastrum*. As shown in **Table (3),** percentage of DPPH radical scavenging activity at various concentrations ranging from 31.25 to 500 µg/ml was determined along with 50% inhibitory concentration of extract. DPPH is a synthetic free radical mostly used in the experiments to find the radical scavenging activity of antioxidant compounds in the tested extract. The results in the present study revealed significant free radical scavenging activity on DPPH with IC50 value of 289.92 µg/ml. In previous studies, it was reported that the ethanol leaf extract of *Rhizophora mucronata* has shown antioxidant activity and the free radical scavenging activity of 127.5 µg/mL [25]. A study conducted by Sofia and Teresa [27] determined the DPPH activity in leaf, stem and root samples of *E. agallocha*. The leaf extracts have shown maximum IC_{50} at 141.56µg/ml in the methanolic extract of the leaves and the extracts from the stem showed minimum IC_{50} at 931.3µg/ml. The methanol leaf extract of *Avicennia marina* having phenolic compound namely gallic acid was reported for high antioxidant activity using DPPH assay. Similar studies reported on antioxidant activity in *Acanthus ilicifolius* petroleum ether extract exhibited highest activity of 116.75µg/mL [6].

Antioxidants plays an important role in maintaining the oxidative resistance .According to the results, as shown in **Table (4)**, the reducing potential of aqueous leaf extract of *S. portulacastrun* was expressed in ascorbic acid equivalent. The reducing power gradually increased with increase in concentration just as exhibited in DPPH radical scavenging assay with an AAE value of 44.16µg/ml. From the results, the tested leaf extract exhibited moderate reducing activity. The reducing power of leaf extracts could be estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe(II) [7]. Similar studies of Kuthi and Basar [18] reported in the methanol extracts of *Pellacalyx axillaris* provides significant reducing power with equivalent values at

varying IC50 concentrations (1.0mm, 2.69 and 2.97 ug ml).

Conclusion:

With the advent of modern medicine, it has become possible to find a cure for improbable diseases. However, synthetic antimicrobial or antibacterial drugs are now falling short of curing any microbial or bacterial infections owing the growing immunity against these drugs. It has hence, become the need of the hour to use natural medicines or extract effective bioactive compounds from plant species to combat the increase in drug resistance seen in bacteria. It was proven, on numerous occasions that, Mangroves have an abundance of bioactive compounds which are rich in antibacterial and antioxidant properties. *Sesuvium portulacastrum*, which is one such mangrove, also has shown the presence of bioactive compounds such as alkaloids, saponins, anthraquinones, tannins etc., which might prove beneficial. Significant reduction in bacterial growth of *Klebsiella pneumoniae* and other such bacteria will make this mangrove irreplaceable as a bactericidal drug. The DPPH and FRAP assays have also proven that *S. portulaastrum*, has significant antioxidant properties. Therefore, further studies and experimentation on this little explored mangrove might result in the discovery of a novel drug which can cure bacterial and other life-threatening diseases.

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Table 1: Phytochemical screening of *S. portulacastrum* leaf extracts

Name of test	**Acetone extract**	**Di-Ethyl ether extract**	**Petroleum Ether extract**	**Aqueous extract**
Alkaloids	+	-	-	+
Tannins	-	+	-	+
Saponins	+	+	-	+
Flavonoids	+	-	-	-
Glycosides	-	-	+	+
Terpenoids	-	-	-	-
Steroids	-	-	+	+
Phenols	-	+	-	+
Proteins	+	+	-	-
Anthraquinones	+	-	-	+

Reducing Sugars	-	-	-	+

'+' indicates presence and '-'indicates absence

Table 2: The antibacterial activity of the aqueous leaf extract of *S. portulacastrum*

Test organism	Zone of inhibition (mm)
Bacillus thuringenesis	0.5±0.02
Staphylococcus aureus	0.8±0.6
Klebsiella pneumonia	1.2±0.4
Pseudomonas aeruginosa	0.7±0.08

Results were represented as mean ± standard deviation; n=3.

Table 3: Percentage of DPPH radical scavenging activity of *S. portulacstrum aqueous* leaf extract.

Concentration (µg/ml)	Test extract
31.25	4.08 ±0.1
62.5	19.0 ±0.6
125	38.1 ±0.9
250	48.2 ±0.5
500	78.4±0.5
IC50	**289.92µl/ml**

Results are represented as mean± standard deviation; n=3

Table 4: Ferric reducing antioxidant power ability of *S. portulacstrum aqueous* leaf extract

Concentration (µg/ml)	Aqueous extract
15.62	9.33 ±0.11
31.25	20.33 ±0.14
62.5	27.16 ±1.16
125	34.66 ±0.18
250	44.16 ±0.21

Results are represented as mean± standard deviation; n=3