International Journal on Emerging Technologies **12**(2): 155-160(2021)

ISSN No. (Print) : 0975-8364
ISSN No. (Online) : 2249-3255

Antimicrobial, Antioxidant and Antidiabetic Potential of *Suaeda fruticosa* L.

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(Received 27 March 2021, Revised 27 May 2021, Accepted 12 June 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: In traditional medicine, *Suaeda fruticosa* has importance due their usage for therapeutic purposes. They have antibacterial, antioxidant, and anticancer properties reported by few studies. In Pakistan, *Suaeda fruticosa* is commonly found but their biological potential has not been determined. Therefore this study was carried out with the aim to determine the antimicrobial, antioxidant and antidiabetic potential of *Suaeda fruticosa* L. The *S. fruticosa* whole plants were collected in Latamber areas of district Karak in August 2019. The collection process was carried out in flowering season which helped in identification process. Different plants extract were prepared and then antibacterial, antidiabetic and antioxidant potential of different extracts were determined. The antibacterial activity was determined using the well diffusion technique. The antioxidant activity was determined by ferric reducing antioxidant power assay while antidiabetic activity was determined by α-Glucosidase Inhibition Assay. All the fractions of *S. fruticosa* have shown activity against all the bacteria. The study showed that the extracts of *Suedea fruticosa* have concentration dependent antimicrobial activities against *E. coli, S. aureus, P. aureginosa* and *K. Pneumoniae*. In *S. fruticosa*, all extracts showed a significant level of antioxidant activity, ranging from 11.98 mmol to 27.52 mmol Fe(II)/g in dry plant. In the α-glucosidase assay, the methanol extract showed a significant impact, dependent on concentration. Our study concludes that *S. fruticosa* have excellent antibacterial, antidiabetic and antioxidant activities. Further study to determine the toxicity is recommended.

Keywords: Antimicrobial; Antioxidant; Antidiabetic; *Suaeda fruticosa* L

I. INTRODUCTION

Scientists are working on medicinal plants to extract different drugs and medicine for the welfare of humanity. They want to isolate different compounds from plant to cure various diseases and then try to synthesize these compounds in laboratories in bulk. In 1978 World Health Organization (WHO) has stressed on the research and activities related to medicinal plants for the promotion and usage of these plants for health care process scientifically and conventionally. The use of medicinal plant products proved the healing, defensive, remedial competencies in many chronic diseases [1]. There were few medicinal products isolated from the different parts of plant but in present about 35,000 to 70,000 different species in form of plants or their extracts are in usage in human societies. Nowadays, various projects and researches are in progress to extract and isolate the natural products from plants to promote the culture of introducing new method to help societies in a better ways in friendly environment. More than 50% natural products and their derivatives from plants are in use as medicine for clinical objective and locally prescribed for the various chronic diseases [2]. Approximately 90 % of total medicines belong to plants. Most of the plants have been used for the ailment of various diseases in ancient era [3]. The world health organization (WHO) suggested that more than 80 % people are related and depends on traditional medicine extracted/isolated from plants for their primary health care needs. Vast areas of peoples are related to medicinal plants and their products which required their needs in the form of finance. Sufficient revenue is generated in the development of indigenous medicine and the use of medicinal products for the treatment of various diseases [4]. Pakistan is a rich source of medicinal plants and approximately 600 to 700 medicinal plants have been investigated and observed. In mountain areas, peoples are using plants species for various ailment as they have easy access and also know their usage and importance. The hilly areas peoples prefer medicinal plants species over synthetically made products [5]. Pakistan has wide spread mountainous, valleys and lands which contain

various types of herbs, shrubs and trees. These different varieties of plants have medicinal compounds but few of them are known for medicinal activities. Large varieties of plants are not investigated for their medicinal approaches and yet have no access to them. A proper approach and concentration may be made to explore the medicinal properties of those plants which are not yet studied for research purposes [6-8]. *Suaeda fruticosa* belong to Amaranthaceae family, classified under Chenopodiaceae. The general English term for *Suaeda fruticosa* is "Alkali Seepweed" and its name in Urdu is "Laani" [2]. These plants have small leaves and falls in annual herbaceous category and germinating in saline soil.

The *Suaeda fruticosa* has distribution in many parts of the world. It is generally found in the Atlantic coastal areas of Portugal and southern Spain, in the Arabian Peninsula, Afghanistan, Pakistan, India, Iran, the Horn of Africa, Canary islands, south east of England, Bangladesh and France. The soils where these are found can be salty marshes which are sandy in nature, alluvial land flooded regions of coasts soil with enormous amount of clay, dry places, flats of salts and the bases of dry mountains. They have associations with grass Odyssea paucinervis and Tamarix usneoides [9]. Shrubs and plants like *Capparis decidua*, *Salvadora oleoides*, *Salvadora persica*, and including *Tamarix dioica* grow in habitats of salinity [10]. *Suaeda fruticosa* seeds and leaves are utilised as a phytoremediation technique and have been recognised as safe for human food or fodder [11, 12]. A newly discovered polysaccharide from *S. fruticosa* exhibited antioxidant, anti-filamentary, antinociceptive, hypoglycaemic, and antihyperlipidaemic activities in in vitro and ex vivo tests [13, 14]. When related to other halophytes in the same family, such as Salsola kali [15], *Suaeda fruticosa* shoots and leaves are rich in phenols, flavonoids, tannins, alkaloids, saponins, proanthocyanins, and carotenes, indicating a remarkable pharmacological range. The seeds and young shoots of *Suaeda fruticosa* have been used for food. Arabs use it as a fodder for their camels. This plant is the source of a huge quantity of sodium, so this has potential usage in soap making and washing soda and also in the industries of the glass making [16, 17]. Their seeds are the source of extraction of edible oil; the plant is also the source of fatty acids which are unsaturated. In structure, this plant is obligate halophyte. *Suaeda fruticosa* is utilized for fertility of soil and as a treatment of salinity. Experts suggest the cultivation of *Suaeda fruticosa* on large scale for the remedy of metals which are toxic and contaminate soil [18, 19].

In traditional medicine, *Suaeda fruticosa* has importance due their usage for therapeutic purposes. The edible *S. fruticosa* exhibits hypoglycemic and hypolipidaemic properties in this scenario [20]. Leaf extracts of *suaeda fruticosa* are utilized in the cure of opthalmia [21] and antibacterial, antioxidant, and anticancer properties have also been documented.[22]. Very limited data is available about the biological potential of *Suaeda fruticosa* in the literature.

In Pakistan, *Suaeda fruticosa* is commonly found but their biological potential has not been determined. Therefore this study was carried out to determine the biological activities of *Suaeda fruticosa* L. This study will help the researcher for further study to determine the toxicity and then utilization of this plant for treatment purposes.

III. MATERIAL AND METHODS

A. Plant Collection

The *S. fruticosa* whole plants were collected in Latamber areas of district Karak in August 2019. The collection process was carried out in flowering season which helped in identification process. It was identified at the Department of Botany, University of Science and Technology, Bannu. The plant specimens were deposited in Botany Department for future reference.

B. Preparation of Plant Extract and Fractionation

Collected sample material (1Kg) of *S. fruticosa* (Whole) were protected from direct sun light in order to avoid any decomposition in its constituents and were dried in the shade for more than seven days at room temperature and were then grounded to a fine powder by using clean grinder. The powder was weighed on digital balance. The net weight of powder was 500 g. The powdered plant material of *S. fruticosa* was dissolved at room temperature in 80% aqueous methanol. To get a crude extract, the methanolic extract was evaporated using a rotary evaporator at decreased pressure. Care was taken to keep temperature at normal level in order to avoid any decomposition. The initial crude was defatted with hexane and then resulted extract was suspended in water and was extracted successively with dichloromethane and ethyl acetate by using separating funnel. The solvent-solvent extraction was based on polar dissolve polar and like dissolve like substances.

Fig. 1. Separating funnel for fractionation of *S. fructicosa.*

Thin Layer Chromatography (TLC) was utilized to guess the nature of constituents in the extracts and to compare the fractions. A TLC tank and capillary tubes were used for this purpose. The TLC behaviors of these fractions were checked initially by visualizing under UV light followed by Ceric sulfate ($CeSO_4$) as spraying reagent followed by heating. A clear difference in RF ratio of fractions at TLC plates indicated good fractionation.

Fig. 2. Fractions of *S. fructicosa.*

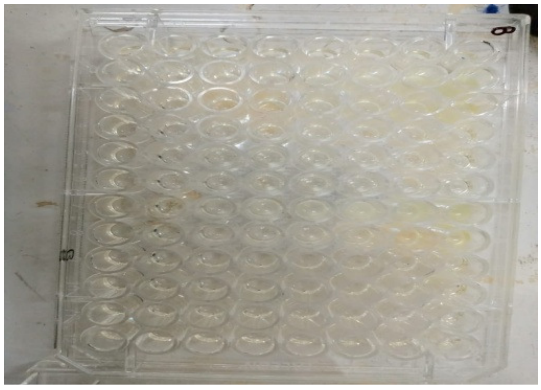

Fig. 3. Microtiter plates used in antibacterial assay

C. Antibacterial Bioassay

For carrying the test of the different antibacterial action of different methanol extracts which were freshly prepared, the micro titer plates were used [3]. A dilute culture amounting to 0.1 ml was dropped on each plate. The plates were solidified for duration of half an hour at temperature of 37℃. The extract of the sample which dissolve in DMSO having different concentrations were prepared in wells having 2 mm diameter. Four bacterial strains namely *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa and Klebsiella pneumonia* were used to determine the antibacterial activity. Ciprofloxacin was used as standard control. The plates were incubated at temperature of 37℃ for 24 hours. The minimum inhibitory concentration (MIC) was calculated.

D. Antioxidant Bioassay

FRAP (Ferric reducing antioxidant power assay) which is method of HAT (Hydrogen atom transfer) was used. FRAP reactant contains 50 mL of acetate buffer and 5 mL of a (10 mmol/L) 2, 4 ,6- tripyridyl- s- triazine (TPTZ) solution in 40 mmol/L HCL plus 5 mL of FeCl3 (20 mmol/L). It was fresh preparation having warming temperature of 37ºC. A 100 µL amount of aliquots were put into 3 mL FRAP reactant. At the same time the reaction mixture's absorption was measured to be 593 nm in Spectro-photometric manner after incubating for the duration of ten minutes at the temperature of 37ºC. Five different concentrations (125, 250, 500, 750, 1000 µmol/L) of $FeSO_4.7H_2O$ were used. The amounts were shown as antioxidant concentration possessing a reduction capacity of ferric which was equal to that of 1 mmol/L $FeSO_4$. For every *S. fruticosa* sample, the antioxidant potential was measured five times. The tubes size for this process was 10 ml each. The concentration of each sample was the same i.e.100ml. The standard was FRAP. For the sake of measuring the inhibition, the freshly prepared solutions having alike concentration were moved by applying dual beam UV/Vis spectrophotometer. The obtained values expressed a corresponding relation in the antioxidant potential at 0.755 nm [23].

E. α-Glucosidase Inhibition Assay

In a reaction mixture (100 µL volume), the mixture comprised of 1.2 µg/mL α-glucosidase, a 25 µL each 250mM buffer of phosphate which has pH 6.8, and drugs of experimentation which was 2.5 mM pNPG. The plates were incubated for 10 minute at 37 ℃ temperature, By using the plate reader of Multiskan, the measurement of absorption of para-Nitrophenol take place during hydrolysis reaction which was catalyzed by enzyme [3].

III. RESULT

A. Antibacterial Activity

The antibacterial data was investigated against four bacterial strains namely *E. coli*, *S. aureus, P. aureginosa* and *Klebsilla Pneumoniae* using ciprofloxacin as a standard drug. The different extracts showed moderate activity against all the tested bacteria as compared to that of the standard drug. By using the agar well diffusion method antibacterial activity was assessed. Antibacterial activity was noted by measuring the MIC values.

All the fractions of *S. fruticosa* have shown activity against all the bacteria. *S. fruticosa* n-hexane and dichloromethane extracts have moderate antibacterial activity against the multidrug resistant strains of *E. coli*, *Staphylococcus, P. aureginosa* and *Klebsilla Pneumoniae* having MIC values of 3.25 mg/ml. The study showed that the extracts *Suedea fruticosa* has concentration dependent antimicrobial activities against *E. coli, S. aureus, P. aureginosa* and *K. Pneumoniae*. (Fig. 4, Fig. 5).

B. Antioxidant Estimation

In *S. fruticosa*, all extracts showed a significant level of antioxidant activity, ranging from 11.98 mmol to 27.52 mmol Fe(II)/g in dry plant (Fig. 6).

C. α- Glucosidase (Antidiabetic)

The anti-α-glucosidase activity of *S. fruticosa* methanol extract was determined using a well-established in vitro assay protocol and was observed as potent [24]. The methanol extract exhibited a strong activity dependent upon concentration. These results indicate that the methanol extract was more potent against -glucosidase as compared to other extracts (Table 1).

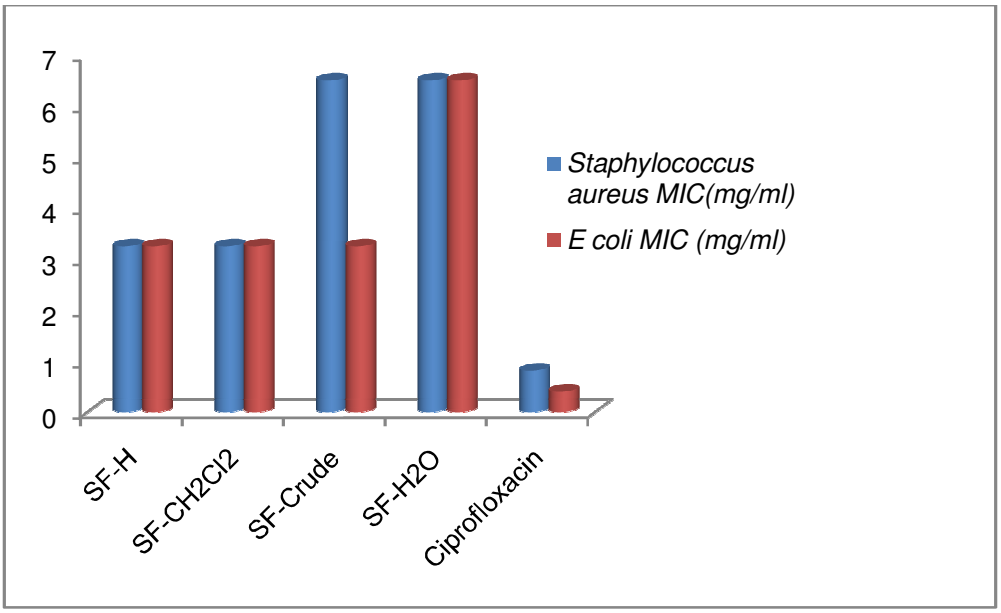

Fig. 4. Antibacterial activities shown by different extracts of *S. fruticosa.*

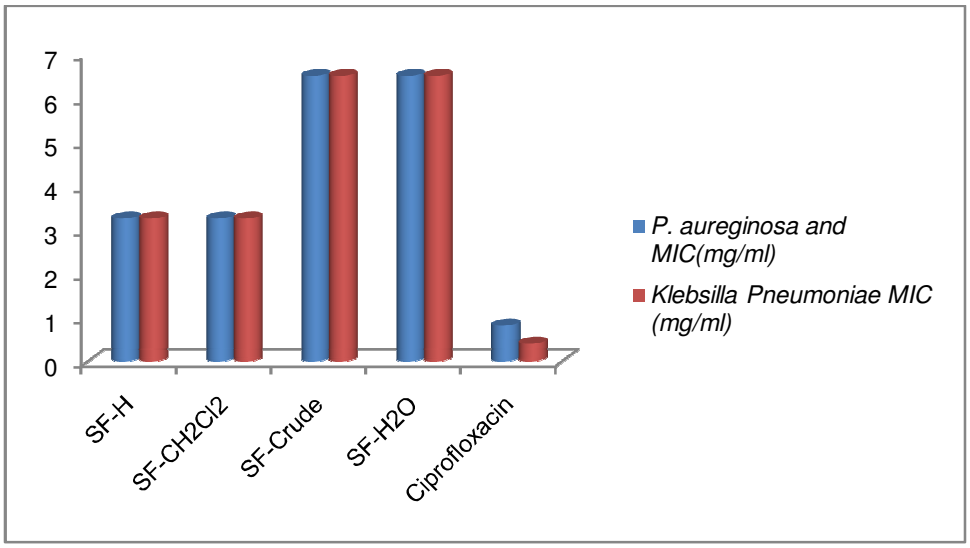

Fig. 5. Antibacterial activities shown by different extracts of *S. fruticosa.*

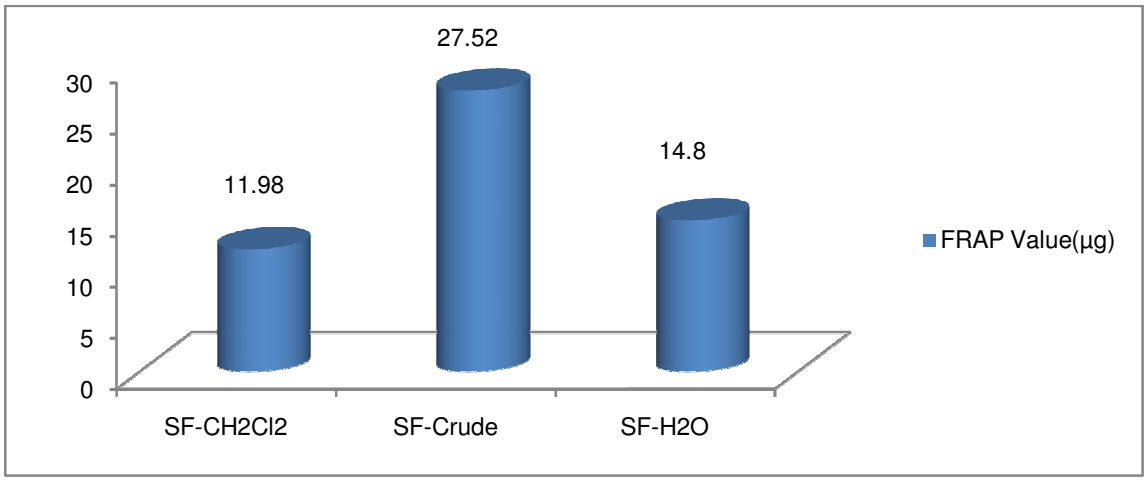

Fig. 6. Ferric reducing antioxidant power of different *Suedea fruticosa* extracts.

Table 1: *Suedea fruticosa* methanol extracts Anti-α-glucosidase activity.

Sample	% inhibition
SF Extraction (0.037mg/ml)	34.8%
Acarbose[1]	65%

[1] 0.019µM

IV. DISCUSSION

With chronic illnesses being the leading cause of morbidity and death globally, there is a growing demand to concentrate on herbal medicinal therapeutic plants from a scientific point of view. The majority of chronic conditions (cardiovascular disease, diabetic problems, and other disorders) are the result of a combination of etiological causes displaying the accompanying symptoms [25]. But, using numerous medicines to prevent and cure these important chronic diseases may result in negative consequences and adverse effects [26]. As a result, covering several targets at the same time with several active principles in a balanced and individualized manner is ideal. Herbal medications are chemically complex mixtures including numerous major and minor elements, typically capable of addressing numerous possible targets when treating a complicated chronic condition. According to evidence suggesting, multiomic approaches such as metabolomics, genomics, transcriptomics, proteomics, epigenomics and others will be extremely useful in reviewing existing treatments to gain new insights and will also provide prospects for the establishment of innovative types of medications.

In our study, the different extracts showed moderate activity against all the tested bacteria as compared to that of the standard drug. All the fractions of *S. fruticosa* have shown activity against all the bacteria. *S. fruticosa* n-hexane and dichloromethane extracts have moderate antibacterial activity against the multidrug resistant strains of *E. coli*, *Staphylococcus, P. aureginosa* and *Klebsilla Pneumoniae bacterial strains* having MIC values of 3.25 mg/ml. The study showed that the extracts of *Suedea fruticosa* have concentration dependent antimicrobial activities against *E. coli, S. aureus, P. aureginosa* and *K. Pneumoniae*. These findings are in line with the findings of previous study who reported that n-hexane extract have moderate activity against many bacterial pathogen [27]. A previous study done by Rashid *et al.* determines the antimicrobial potential of *Suedea fruticosa* and reported that they have potent antimicrobial activity against various bacteria [21].

In *S. fruticosa*, all extracts showed a significant level of antioxidant activity, ranging from 11.98 mmol to 27.52 mmol Fe(II)/g in dry plant. This might be owing to the extract's inclusion of powerful antioxidant phenolic components. These findings back up the ethno-pharmacological usage of *S. fruticosa* in ROS-related diseases. The antioxidant activity of shoot extracts in four solvents (hexane, dichloromethane, methanol, and water) was evaluated in vitro using the oxygen radical absorbance capacity (ORAC) assay, with methanol extract showing the highest antioxidant activity, with an ORAC value of 2.94 0.17 lmol TE/mg, followed by dichloromethane (1.59 0.28 lmol TE/mg). This action was less significant when compared to solvent polarity water and hexane extracts [28]. The methanolic extract of *S. fruticosa* had the highest activity when compared to a medicinal plant (*Juglans regia)* and conventional antioxidants like Trolox (2.17 0.22 and 2.52 0.35 lmol TE/mg, respectively) [29]. Ex vivo, the antioxidant activity of these extracts was determined using a cellular-based test [30]. An earlier study done by Saleh et al reported that *S. fruticosa* hexane extract can be considered as potent anti-cancer agent [31]. The anti-α-glucosidase activity of *S. fruticosa* methanol extract was determined using a well-established in vitro assay protocol and was observed as potent [24]. The methanol extract exhibited a strong activity dependent upon concentration. These results indicate that the methanol extract was more potent against –alpha glucosidase as compared to other extracts. A previous study performs an in vivo study on rats to determine the anti-diabetic activity of *S. fruticosa* and reported that it has high potential as anti-diabetic potential [32]. Another study determines the antidiabetic potential of *S. fruticosa* and reported that methanol extract shows potent antidiabetic activity [18]

V. CONCLUSION

Our study concludes that different fractions of *S. fruticosa* showed moderate activities against the different strain of bacteria. The minimum inhibition concentration (MIC) values of the extracts were less than the MIC value of the standard, ciprofloxacin. The antioxidant data showed that *S. fruticosa* extracts are potent as its antioxidant values are comparable with the standard. The antidiabetic data of the extracts indicated that *S. fruticosa* is an excellent antidiabetic agent. Our study only determine the biological potential of this plant and further study is needed to determine the toxic effects of this plant on human and animal.

VI. FUTURE SCOPE

This study will help the researchers to consider this plant as useful medicinal plant and further work on it to determine the toxic effects of this medicinal plant.

Conflict of interest. The authors declare that they have no conflict of interest.

Acknowledgements. Authors are thankful to Dr Saleem Jan, Assistant Professor, Department of Chemistry, University of Science and Technology Bannu, Bannu-28100, Khyber Pakhtunkhwa, Pakistan for their kind supervision.

Author contributions. All authors contributed equally.

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How to cite this article: Ahmad, I., Gul, H., Noureen, A., Ujjan, J.A., Manzoor, S., Achakzai, W.M., Saddozai, S. and Jan, S. (2021). Antimicrobial, Antioxidant and Antidiabetic Potential of *Suaeda fruticosa* L. *International Journal on Emerging Technologies, 12*(2): 155–160.