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Original Research Article

Antioxidant and Antibacterial Activities of Sagina Procumbens

Shahbaz Wakeel[1], Zaheer Abbas[2] and Muhammad Usman[*1]

[1]Department of Chemistry, Allama Iqbal Open University, Islamabad Pakistan
[2]Department of Chemistry, Minhaj University Lahore, Pakistan
[*1]Department of Chemistry, Government College University Faisalabad, Pakistan

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Abstract

Sagina Procumbens, a traditional medicinal plant, was examined for its antioxidant activity with the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay, its total flavonoid levels with the Folin-Ciocalteu method, and its total phenolics with the Folin-Ciocalteu method. According to the findings of the study, the ethanolic extract of Sagina Procumbens leaf has a percentage of antioxidant activity (AA%) of 86.61, which was shown to be greater when compared to other extracts of buds and stem. In a similar vein, the methanolic extract of the stem has a high flavonoid concentration, whereas the phenolic level of the ethanolic extract of the bud is significantly higher. Antimicrobial activity exhibited by S. procumbens has a very significant zone of inhibition against gram-positive as well as gram-negative bacterial strains. The findings of this study indicated that plants are excellent sources of natural antioxidants, and that both ethanolic and methanolic extracts of plants contain a sizeable quantity of antioxidant chemicals.

Keywords: *Sagina Procumbens, extraction in different solvents, antioxidant activity, Total phenolic contents, Total flavonoid contents, Antimicrobial activity.*

1. Introduction

Bioactive chemicals have traditionally been derived from plants. The wide availability of chemicals in a natural product like plant extracts opens up endless doors for the discovery of new medicines. Antibacterial drug resistance is a growing problem due to the rapid evolution of germs that cause disease (Al-Dhabi *et al*., 2015, Al-Dhabi *et al*., 2016, Barathikannan*et al*., 2016). That's why scientists from the biological, chemical, and pharmaceutical fields are constantly on the lookout for undiscovered chemicals in the natural world. More and more people are paying attention to medicinal plants because of the wide range of beneficial metabolites found in them (Bonjar and Farrokhi, 2004, Feigin*et al*., 2017, Vetrimurugan*et al*., 2017).

In the past, people relied on plants as a form of alternative medicine. Nowadays, many medications are made from plants, and hence have no or little adverse effects. Plants are a source for several compounds. Chemicals like this are crucial in the production of pharmaceuticals and personal care products. Plant-based chemicals have long been recognized for their use in the pharmaceutical sector. Many plants have yet to be identified or analyzed for their phytochemical constituents (Jardim*et al*., 1998).

*Corresponding author's ORCID ID: 0000-0002-4146-6232
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Roots, stems, flowers, and leaves, all of which are a part of medicinal plants, all have their own unique set of antibacterial qualities (Elango*et al*., 2016, Fowsiya*et al*., 2016, Glorybai*et al*., 2015, Haritha*et al*., 2016). In traditional medicine, it serves many purposes, including as a pest deterrent, blood thinner, fungal and bacterial killer, and anticoagulant (Helan*et al*., 2016, Ilavenil*et al*., 2017, Park *et al*., 2017). This restorative herb has been shown to speed up hair growth and aid in wound healing (Park*et al*., 2017). Many nations have turned to herbal remedies to fill their healthcare voids (Awe and Omojalasola, 2003, Surendra*et al*., 2017, Roopan*et al*., 2019). Activities, such as antibacterial ones, were demonstrated by medicinal plants (Abo *et al*., 1999).

Validating what is known about medicinal plants in a given region requires both understanding of traditional medical systems and the collaborative efforts of traditional healers and modern scientists (Hamill *et al*., 2000). Even though traditional medicine is widely utilized in underdeveloped and emerging nations, very little is known about the biological role and chemical makeup of medicinal plants (Tabuti*et al*., 2003).

Common names for Sagina Procumbens include "procumbent pearlwort," "birdeye pearlwort," and "matted pearlwort," all of which refer to members of the same pink family of blooming plants. It's a perennial plant that grows in mats or clumps of

smooth, green leaves, often confusingly resembling a mossy area. The linear leaves can grow to be about 2 cm in length. A single flower forms the inflorescence, and it has four or five sepals and four or five tiny white petals (or none at all). The word "sagina" is derived from the Latin word for "fodder," and the genus Sagina was originally named after the spurrey plant, which is now classified as its own genus, Spergula. What we mean when we say "procumbens" is that we are lying flat on the ground or inching forwards (Botanical Society of Britain and Ireland 2015). This plant can be found in many different parts of the world, but it is very common in Asia. This plant has not been validated for its antibacterial potential, despite the fact that numerous medicinal herbs have been studied for this and other biological functions (Balandrin*et al*., 1985). Sagina Procumbens was thought to have several chemicals with biological activity. The study set out to determine whether or not Sagina Procumbens has any useful biological properties.

2. Material and Method

Collection of Plant Sample

The specimen was taken from the MianIshaq Nurseries, and identified there. Once the stem, leaves, and buds were extracted, they were air dried at room temperature in the shade, then milled into a powder for further extraction using various solvents.

Extraction into Different solvents for Biological Activities

To make a powder, we washed, dried in the shade, and then pulverized the plant pieces we had collected. Methanol, ethanol, acetone, and n-Hexane were the solvents of choice for extracting the plant material. The plant powder was mixed with the appropriate solvent at a 1:10 (w/v) ratio in a clean flask. The flasks were incubated at 37 degrees Celsius and 10,000 rpm for four days. Then, it is filtered through No. 1 Whatman's filter paper, condensed using a rotary evaporator, and chilled to 4 degrees Celsius so that additional analysis can be performed (Den *et al*., 2020).

Total Phenolic Content

The total phenolic content was determined by using the Folin- Ciocalteu reagent and the same methodology published by Ainsworth and Gillespie (2007), with some minor adjustments. The sample extract (100 L) was mixed with the diluted FolinCiocalteu reagent (200 L) (10%) and vigorously vortexed. The absorbance at 765 nm was then measured after each sample was treated with Na2CO3 (800 L of 700 mM) and left to stand at room temperature for 2 hours. When converting to GAE from mg/g, gallic acid was employed as the benchmark.

Total Flavonoids Content

The method for measuring total flavonoid content was adapted from that published by Anjum*et al*., (2013). After taking a 1mL sample extract in a test tube, 3mL of 5% NaNO2 was added, followed by 0.6mL of AlCl3 (10%) after 5 minutes and 2mL of NaOH (1M). The 510 nm absorbance reading was recorded. Catechin is the benchmark (in milligrams per gram of dry substance).

DPPH Free Radical Activity

A sample (50 L) was diluted in 5 mL of DPPH methanol solution (0.004%). After incubation for 30 minutes at room temperature, the absorbance was measured at 517 nm.

I % = (A blank –A sample /A blank) *100

In which Ablank is the absorbance of DPPH solution and Asample being the absorbance of sample (Bozin*et al*., 2006).

Antimicrobial Activities

Extracts from plants were tested for their ability to kill off certain strains of bacteria, including Gram-negative E. coli and Gram-positive Bacillus subtilis (CLSI, 2007).

Anti-Bacterial Assay by Disc Diffusion Method

The nutrient agar solution (28.08 g/L) was autoclaved at 121 degrees Celsius for 15 minutes. When the media reached room temperature after being sterilized, various bacterial species were introduced. Positive control was ampicillin, and negative control was distilled water. Solution was poured onto sterile discs prepared from Whatsmann filter paper No. 1, which were then placed on solidified media poured into petri dishes. Millimeter-scale readings from a zone reader confirmed the presence of a distinct zone of inhibition around the antibacterial extract (Banerjee *et al*., 2014).

Statistical Evaluation

Minitab version 10 was used for the two-way analysis of variance and the least significant difference between the solvents.

3. Results and Discussion

Total Phenolic Contents

Phenols are important compounds that are widely distributed across plant kingdom. There may be a connection between the total phenolic contents and total antioxidant activity of the plant extracts, and this connection might be shown by qualitative and quantitative research into phenolic content in plants (Demain*et al*. 2009). Polyphenolic compounds have recently been shown to have an important role in warding off degenerative diseases like cardiovascular disease, cancer, and neurodegenerative disorders. Diets high in polyphenols have been shown to have

beneficial effects on health, and the processes behind this are becoming clearer thanks to the antioxidant properties of these compounds.

Numerous therapeutic plants have high concentrations of polyphenolic substances like phenolic acids, tannins, and flavonoids (Dong*et al*., 2009). Phenolic acids are extensively distributed secondary aromatic plant metabolites. These phenolic acids can be distinguished from one another by their hydroxycinnamic and hydroxybenzoic structures, both of which are naturally occurring. The antioxidant and health benefits of foods have been linked to their phenolic acid content, as well as their color and flavor (Maga*et al*., 2008).

Figure 1 shows that the total phenolic content of the ethanolic extract of Sagina Procumbens leaf is much higher than that of the other extracts tested (65.181.04). Leaf extract by acetone showed similarly low phenolic levels. The polarity of the solvent we utilized for extraction explained the variation in phenolic content. When compared to other solvents, ethanol was discovered to be the most polar. To put it another way, the phenolic content of Sagina Procumbens leaves is highest in ethanolic extracts and lowest in acetone extracts (31.621.07 mg/g). The total phenolic content of the methanolic extract of Sagina Procumbens stem is also quite high (45.680.71). The phenolic content of leaf extract prepared with acetone was likewise found to be quite low.

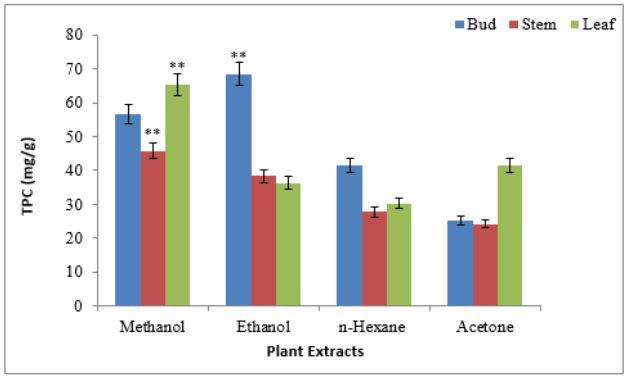

Figure 1. Total phenolic contents of Saginaprocoumbens

When compared to other extracts, the ethanolic extract of Sagina Procumbens bud has a very high total phenolic content (68.380.99). The phenolic content of bud extract prepared with acetone was also found to be low (25.131.05). The ethanolic extract of Sagina Procumbensas buds had a higher total phenolic content than the stems and leaves. Because this plant species has not been studied, it is obvious that bud extract of ethanol contains more phenolic compounds than stem and leaves.

Total Flavonoid Contents

Flavonoids have a variety of pharmacological and biological functions, including metal chelation, scavenging of reactive oxygen species and chain-breaking antioxidants, reduction of agents, quenching of singlet oxygen, and other related actions (Naseer*et al*., 2014). Several illnesses of the neurological system and inflammation are treated with antioxidants (Babu*et al*., 2011).

Water-soluble complexes like flavonoids and lipid-soluble complexes like phenolic acids are two types of phytochemicals. Total flavonoid content was estimated using a method that has been utilized in numerous previous studies (Ghimeray*et al*., 2009).

Figure 2 shows that the total flavonoid content of Sagina Procumbens stem extracts prepared using methanol and n- hexane are, respectively, 78.530.84 and 31.020.73.

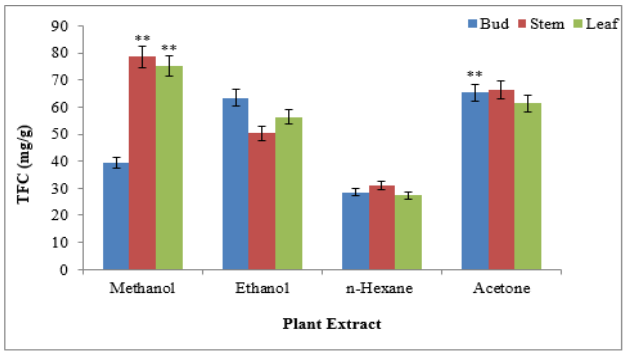

Figure 2. Total Flavonoid Contents of Sagina Procoumbens

Flavonoid concentration was found to be much higher (66.351.10) in acetone extract of leaf than in phenolic content, perhaps due to the polarity of the solvents used. To a similar extent, the flavonoid content of Sagina Procumbens leaves extracted with methanol is higher than that of leaves extracted with n-hexane (27.440.96 mg/g). Sagina Procumbens bud acetone extract had the highest total flavonoid concentration (65.270.84 mg/gof) when compared to other extracts. N-hexane bud extract, on the other hand, is not very rich in flavonoids. Flavonoids are the polyphenolic substances that have shown promise for improving human health; they have been shown to have antiviral, anti-inflammatory, anticancer, antihaemolytic, and anti-oxidative actions (Buhler and Miranda, 2000).

DPPH Radical Scavenging Assay

We used the compound's ability to scavenge DPPH free radicals to evaluate its antioxidant activity in a series of synthesized compounds. As a free radical, 1, 1-diphenyl-2-picryl-hydrazyl is violet in color but turns yellow upon reduction. It is soluble in methanol. Antioxidant activity is proportional to an antioxidant's ability to quench the DPPH radical. The percentage of the DPPH radical that is inhibited provides a great explanation of antioxidant efficacy. The presence of antioxidant compounds is confirmed by the radical scavenging activity of ethyl acetate extracts and their ability to donate electrons or hydrogen atoms.

When a 2, 2-diphenyl-1-1-hydrazyl reagent accepts an electron, the molecule becomes diamagnetic and also functions as a free radical. The DPPH assay was proven to be a quick and inexpensive way to determine an antioxidant's effectiveness. The DPPH technique was described by Souri et al.(2008). The byproducts of photosynthesis and photorespiration are reactive oxygen species. When added to samples, DPPH takes a few seconds to lose its purple hue, and after 10 minutes in incubation, its once-vivid hue fades to a pastel yellow.

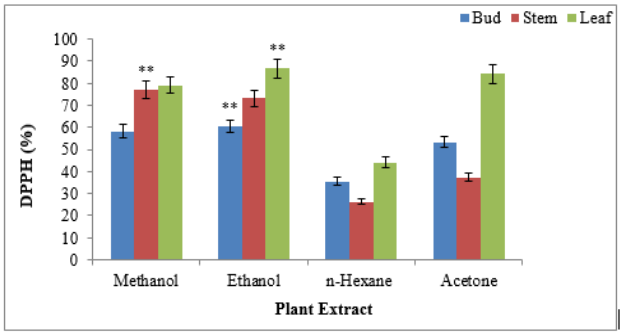

Figure 3. DPPH activity of Sagina Procumbens

Figure 3 shows that the methanolic extract of Sagina procoumbens's stem has the highest DPPH activity (77.121.02%), while the n- hexane extract has the lowest (26.30.67%). Due to the polarity of the solvents, it was also observed that the ethanol extract of the leaf exhibited a considerably high (86.611.07) percentage of DPPH activity. The percentage of DPPH activity in n-hexane extract is lowest (44.211.20). The DPPH activity of ethanolic extracts of Saginaprocoumbens buds is comparable to that of n-hexane extracts, whereas the DPPH activity of n-hexane extracts is the lowest (35.460.94%). In addition, the DPPH concentration of ethanolic extract of Sagina procoumbens leaf was found to be substantially higher than that of bud and stem extracts obtained using different solvents.

Antimicrobial Activity of Sagina Procoumbens

Antimicrobial activity was tested in Sagina Procumbens extracts. Some extracts had a wide range of activity, as evidenced by a distinct zone of inhibition,

Table 1. Comparative analysis of antibacterial activity of different extracts of stem leaf and bud of Saginaprocoumbens against gram positive (B.subtilis) and gram negative (E.coli) bacterial strainwhile others were ineffective against the strain at hand.

Sample extracts	Zone of inhibition (mm)					
	E.coli			*B.subtilis*		
	Stem	**Leaf**	**Bud**	**Stem**	**Leaf**	**Bud**
Methanol	16.00 ± 0.77^{A}	14.00 ± 0.61^{B}	12.00 ± 0.54^{C}	14.00 ± 00.61^{B}	16.00 ± 0.67^{A}	16.00 ± 0.67^{A}
Ethanol	14.00 ± 0.61^{B}	12.00 ± 0.54^{C}	12.00 ± 0.54^{C}	16.00 ± 0.77^{A}	12.00 ± 0.54^{C}	16.00 ± 0.67^{A}
***n*-Hexane**	12.00 ± 0.54^{C}	12.00 ± 0.54^{C}	14.00 ± 0.61^{B}	14.00 ± 0.61^{B}	12.00 ± 0.54^{C}	12.00 ± 0.54^{C}
Acetone	16.00 ± 0.77^{A}	14.00 ± 0.61^{B}	14.00 ± 0.61^{B}	14.00 ± 0.61^{B}	14.00 ± 0.61^{B}	12.00 ± 0.54^{C}
Ampicillin (positive control)	22.00 ± 0.99^{A}	20.00 ± 0.99^{A}	22.00 ± 0.99^{A}	24.00 ± 1.23^{A}	24.00 ± 1.23^{A}	24.00 ± 1.23^{A}

If the results are negative, it means that the plant either did not contain any active compound, or if it did, the concentration was so low that the compound was ineffective.

In comparison to the stem extract, the leaf and bud methanolic extracts of Sagina Procumbens showed significantly higher antibacterial activity (16.000.67) against Gram positive bacteria (B. subtilis). Sagina Procumbens leaf and bud extract are less effective against Gram-negative bacteria (E. coli) than the plant's stem extract (16.000.77). The results were measured against those obtained when using ampicillin (an antibiotic). Stem and bud extracts of Sagina Procumbens have excellent antibacterial activity (16.000.77) against B.subtilis(Gram positive bacteria), whereas stem extracts had good antibacterial activity (14.000.61) against gram negative bacteria (E.coli).

Table 1 displays data indicating that, back Against B.subtilis (Gram positive bacteria), hexane extract of Sagina Procumbens stem has strong (14.000.61) antibacterial activity, while bud extract has high antibacterial activity (14.000.61) against gram negative bacteria (E.coli). Sagina Procumbens stem, leaf, and bud acetone extracts have antibacterial action against B.subtilis(Gram positive bacteria) (14.000.61), whereas stemextract has considerable antibacterial activity against gram negative bacteria (E.coli) (16.000.77). It is evident from the foregoing that the zone of inhibition of antibacterial activity against B.subtilisas is significantly higher for the methanolic and ethanolic extract of the stem of Sagina Procumbens than it is for E.coli. Standard antibiotic treatment was used as a benchmark for all of these findings (ampicilin).

Damage to the bacterial cell wall, membrane, enzyme inhibition or metabolic inactivation, and bacterial nucleic acids molecules are the primary mechanisms by which chemicals of any origin exert their antibacterial effect (Alsughayeret al., 2011). Chemically interfering with the bacterial cell wall or membrane, or entering the cell and chemically altering the proteins or nucleic acids, are all effects of antibacterial chemicals' various pharmacologically active functional groups, which have electron withdrawing or electron giving capacities. Cell growth arrest or death is the cumulative effect of such changes (Kumaret al., 2010).

Some of these chemicals are structural analogues of metabolic intermediates and function as enzyme inhibitors or modifiers of enzyme bases involved in essential catalytic reactions for bacterial cell survival (Cossuet al., 2012). Some sulfonamides, such as sulfanilamide, are structurally similar to tetrahydrofolate, a bacterial precursor to folic acid synthesis; however, because of structural differences, these sulfonamides are unable to enter the active site of the tetrahydrofolate synthase enzyme, thereby halting the growth of the bacteria. Distinct chemicals with possible antibacterial effects have had several different mechanisms reported (Kucukguzel, et al., 2002). Sagina Procumbens has been discovered to have strong potential against pathogens, despite the fact that it has not been the subject of extensive prior investigation.

Conclusion

Plants with medicinal properties have been utilized for ages to heal human illness because of the substances they contain. Flavonoids are a naturally occurring chemical with a wide range of pharmacological effects, including those of an anti-inflammatory, anti-oxidant, and diuretic. Researchers are now able to explore the photochemical and antimicrobial activity of many herbs and medicinal plants thanks to antibiotic resistance available from these sources. Before this experiment, Sagina Procumbens wasn't given much of a second thought, but now that we know it has microbiological resistance against infections and active potential of antioxidant chemicals and is getting a lot more attention.

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