INTRODUCTION

Plants are generally found to be potential source of powerful drugs. Plant derived products are present in 14 of the 15 therapeutic categories of pharmaceutical preparations that are currently recommended by medical practitioners. Despite the advances in chemical science plants still remain the main reservoirs of natural medicine. The pharmacological industries have produced a large number of new antibiotics in the last three decades, but the resistance to these drugs by microorganisms has increased. In addition to this, antibiotics are sometimes associated with adverse effects on the host, for e.g. allergic reactions, immune-suppression etc. Contrary to the synthetic drug, antimicrobials of plant origin are not associated with any side effects. Hence there is a need to establish the pharmacological activities for identifying the various crude drugs for potency.

Free radicals, the unstable species are generated in our body during the normal metabolic processes and during exposure to adverse patho-physiological conditions. Antioxidants are radical scavengers which protect the human body against the free radicals. Phytochemicals such as phenolics, flavonoids, carotenoids etc. are gaining increased attention due to their antioxidant, anticarcinogenic and other health promoting properties. With these viewpoints Syzygium caryophyllatum (L.) Alston was chosen for pharmacological and phytochemical evaluation.

S. caryophyllatum (L) Alston is a low altitude evergreen tree; where the stem bark is traditionally used in the treatment of diabetes mellitus. They possess creamish white flowers borne in corymbose cymes and round, purple, juicy and edible fruits. The species of Syzygium are reported to possess antibacterial, antidiabetic, antifungal, antihyperglycemic and hypoglycemic activities. Essential oil from several species of Syzygium have been isolated, characterized and their bioactivity studied. The flower buds of Scaryophyllatum (L) Alston have been reported to possess antioxidant activity. However, the other plant parts like leaf and bark have not been evaluated for their chemical and biological activity.

MATERIALS AND METHODS

Plant Material

The leaves and bark of Syzygium caryophyllatum were collected around the Mangalore University campus, India during the month of September. The collected plant materials were dried in an incubator maintained at 37 °C and mechanically reduced to a coarse powder and stored at 4 °C until use.

Preparation of the Extract

50 g each of the powdered leaves and bark were soxhleted with methanol at 30 °C for 5 hrs. The aqueous extract was obtained by soaking 50 g each of the powdered materials in water for 96 hrs with stirring at regular intervals. The supernatant was filtered through a three layered muslin cloth. The methanolic and aqueous extracts obtained were evaporated to dryness and stored at 4 °C for further use.

Microbial Isolates

A panel of microorganisms like Staphylococcus aureus (NCIM 2079), Bacillus subtilis (ATCC 6633), Escherichia coli (NCIM 2931), Pseudomonas aeruginosa (NCIM 2200), Klebsiella pneumonia (NCIM 2957) and Proteus vulgaris (NCIM 2813) were used for the microbial sensitivity assay. The microbial isolates were procured from National Chemical Laboratory, Pune, India.

Antibacterial Assay

The antibacterial assay was carried out by following the Agar disc diffusion method. The extracts were dissolved in DMSO (Dimethyl Sulfoxide) to a final concentration of 25 mg/ml. The Muller Hinton Agar plates were inoculated with (overnight 12 hr containing 10−5 CFU/ml) bacterial cell suspension by spread plate method. Sterile filter disks of 6 mm diameter were impregnated with 30 µl extracts and allowed to dry. Streptomycin (10 µg/ml) and DMSO were used as positive and negative controls respectively. The plates were incubated at 37 °C for 24 hr. At the end of the incubation period, the antibacterial activity was evaluated by measuring the inhibition zone.

The MIC (Minimum Inhibitory Concentration) was determined by microdilution method. The lowest concentration i.e. the highest dilution of the active ingredient that inhibited the growth of the bacteria completely was considered as the MIC.

Phytochemical screening

The resultant extracts were further subjected to phytochemical analysis for the presence of alkaloids (Hagers, Wagners, Mayers test), flavonoids (Shinodas test), steroids (Salkowski test), glycosides (Molishs test, Benedicts test), phenol (Fecl3 test), tannins (Lead acetate test) and saponins (Foam test).
Total Phenolic content determination

Total Phenolic content was determined following the Folin-Ciocalteau (FC) method21. The leaf and bark extracts were dissolved in DMSO to a final concentration of 10 mg/ml.10 µl of aliquot sample was mixed with 2 ml of 2 % Na2C03 and allowed to stand for 2 minutes at room temperature. To this was added 100 µl of 50 % FC reagent and was incubated at room temperature for 30 minutes. The absorbance was measured using a spectrophotometer at 725 nm. The total phenolic content was calculated with Gallic acid standard and expressed in terms of Gallic acid equivalent (mg/g of extracted compound).

Antioxidant Assay

2, 2'-diphenyl-1-picryl hydrazyl (DPPH) Radical Scavenging Assay:

The effect of extracts on DPPH radical was measured based on the scavenging activity of the stable DPPH free radical22. A solution of DPPH (0.135 mM) in methanol was prepared and 1 ml of this solution was mixed with1 ml of varying concentrations of the extracts. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 minutes. The absorbance of the mixture was measured at 517 nm using Ascorbic acid as reference standard. The ability to scavenge DPPH radical was calculated as:

% DPPH radical scavenging activity = [Absorbance of control - Absorbance of sample] / [Absorbance of control]* 100

2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ABTS radical scavenging assay

The antioxidant activity was measured by ABTS radicals scavenging assay23. The stock solution which was allowed to stand in dark for 16 hrs at room temperature contained equal volumes of 7 mM ABTS salt and 2.4 mM Potassium per sulfate. The resultant ABTS solution was diluted with methanol until an absorbance of about 0.70 ± 0.01 at 734 nm reached. Varying concentrations of the plant extracts (1 ml) reacted with 1 ml of ABTS solution and the absorbance was taken at 734 nm between 3-7 minutes using a spectrophotometer. The ABTS scavenging capacity of the extracts was compared with that of ascorbic acid and the % inhibition was calculated as:

ABTS radical scavenging activity %= Absorbance of control- Absorbance of sample/Absorbance of control* 100

RESULTS AND DISCUSSION

Percentage Yield: The yield of leaf using water and methanol were 1.8 % and 25.4 % respectively. The yield of bark using water and methanol were 13.6 % and 23.3 % respectively.

Antibacterial Assay

The activity potentials of the antimicrobial assay employed was assessed by the presence or absence of inhibition zone and zone diameter. Antibacterial activity and MIC of the crude extracts against the bacterial species is represented in Table (1) and Table (2) respectively. Results obtained from disc diffusion method indicated that both extracts possessed moderate activity against gram positive bacteria tested whereas no activity was observed against gram negative bacteria. This is to be expected because the outer membrane of gram negative bacteria is known to present barrier to penetration of numerous antibiotic molecules and the periplasmic space contains enzymes which are capable of breaking down foreign molecules introduced from outside24,25. Thus antibacterial activity observed in the study may at least partly be due to the phytochemical compounds detected26. The positive control Streptomycin exhibited larger zones. This may be related to the purity of the antibiotic used27.

Table 1: Antibacterial activity of the extracts of S. caryophyllatum

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Zone of inhibition (mm)</th>
<th>Streptomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LWE</td>
<td>LME</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>6.06 ±0.09</td>
<td>6.2 ±0.21</td>
</tr>
<tr>
<td>S. aureus</td>
<td>4.16 ±0.16</td>
<td>5.1 ±0.014</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Results are the mean ± SD values of three replicates; LWE=Leaf Water Extract, LME=Leaf Methanol Extract, BWE=Bark Water Extract, BME=Bark Methanol Extract.

Table 2: Minimum Inhibitory Concentration

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC of extracts (mg/ml)</th>
<th>Streptomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LWE</td>
<td>LME</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>S. aureus</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>-</td>
<td>-</td>
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<tr>
<td>K. pneumonia</td>
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<td>-</td>
</tr>
</tbody>
</table>

Results are the mean values of three replicates; LWE=Leaf Water Extract, LME=Leaf Methanol Extract, BWE=Bark Water Extract, BME=Bark Methanol Extract.

Phytochemical screening

The plant materials were subjected to phytochemical screenings. The results are shown in Table (3). All the extracts showed the presence of saponins, phenols, steroids, tannins and glycosides; whereas alkaloids and flavonoids were present in trace amounts. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. For instance, Saponins are amphipathic glycosides highly toxic to fish and have been used as fish toxins. It is a mild detergent used as antioxidant, anticancer, anti-inflammatory etc. It is also used to allow antibody access in intracellular proteins.

The phenolics also known as polyphenols are derived from aromatic amino acids. Tannins are phenol derivatives. Both tannins and phenols appear to be involved in plant / herbivore interactions. The plant steroids are known to possess insecticidal and antimicrobial properties. They are used in nutrition, herbal medicine and cosmetics28.
Table 3: Preliminary phytochemical screening

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Methanol extracts</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Bark</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- = Absent, + = Present

**Determination of Total phenolic content**

Fig 1 summarizes the content of total phenolics of different extracts. Phenolic compounds are the secondary plant metabolites which contribute to the overall antioxidant property of plants. Many of the phenolics have been shown to contain high levels of antioxidant activities. Moreover, the polyphenolic compounds have been found to be effective in much health-related properties such as antioxidants and anticancer activities. Phenolic compounds undergo a complex redox reaction with Phosphotungstic and Phosphomolybdic acids present in FC reagent. The FC assay is a simple method and is based on the oxidation of phenolics by molybdatungstate in FC reagent to yield a colored product with λmax 725 nm.

The methanolic and aqueous extract of bark contained highest amount of phenolic compounds (324.8 ± 5.23 and 310.5 ± 3.53 respectively) compared to the methanolic and aqueous extract of the leaf (290.6 ± 0.8 and 214.3 ± 0.9 respectively). Our results are in agreement with the other authors who have reported similar results of phenolic content in bark and leaf extracts.

**Scavenging activity on (DPPH) radical**

The measurement of the scavenging activity of DPPH radical allows one to determine exclusively the intrinsic ability of the substance to donate hydrogen atom electrons to this reactive species in a homogenous system. The DPPH radical contains an odd electron which is responsible for the absorbance at 517 nm and also for the visible deep color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. Higher reduction of DPPH is related to the high scavenging activity performed by particular sample. The result revealed that the methanol fraction of the bark exhibited the highest radical scavenging activity with 90.87 % followed by water fraction of bark 72.73 %. Among the leaf extracts the methanol fraction was 87.94 % compared to the aqueous fraction 72.90 %. In overall comparison between bark and leaf it can be confirmed that the bark possesses higher free radical scavenging activity at the concentration of 10 µg/ml. However, in the result the increase in the free radical scavenging capacity of the bark extracts can be explained by the presence of higher phenolic contents.

**ABTS Radical Scavenging Assay**

The ABTS test measures the relative antioxidant ability of the phenol containing sample to scavenge the radical - cation ABTS+ produced by the oxidation of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid). In the absence of phenolics, ABTS+ is rather stable, but it reacts energetically with a H-atom donor, such as phenolics, being converted into a non-colored form of ABTS. The result revealed that the methanol fraction of the bark exhibited the highest radical scavenging activity with 84.02 % followed by water fraction of bark 77.35 %. Among the leaf extracts the methanol fraction was high 61.62 % compared to the aqueous fraction 40.07 %. In overall comparison between bark and leaf it can be confirmed that the bark possesses higher free radical scavenging activity at the concentration of 5 µg/ml.

The antioxidant activities of the extracts are in accordance with their amount of phenolics. The bark methanol extracts contained high phenolic content compared to other extracts, which was responsible for its high antioxidant activity. Several reports have shown a close relationship between total phenolic content and high antioxidant activity.
Fig. 2: DPPH free radical scavenging activity
ASA=Ascorbic acid, BME=Bark Methanol Extract, Bark Water Extract, LME=Leaf Methanol Extract, Leaf Water Extract

Fig. 3: ABTS radical scavenging activity
ASA=Ascorbic acid, BME=Bark Methanol Extract, Bark Water Extract, LME=Leaf Methanol Extract, Leaf Water Extract

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REFERENCES


