ABSTRACT

Objectives: The aim of the present study was to evaluate the bioactive, antioxidant, and antibacterial activity of methanolic extracts of medicinal plants *Erythrina variegata* L. and *Sauropus androgynus* L.

Methods: Total phenolic, flavonoid, tannin content, total antioxidant capacity, 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity, and reducing power assay of the extracts were determined by spectrophotometric methods.

Results: Total phenolic, flavonoid, and tannin content was more in *E. variegata* compared to *S. androgynus*, and the alkaloid, saponin, and carotenoid content was high in *S. androgynus*. The overall antioxidant activity of *S. androgynus* was found to be higher than *E. variegata*. Antibacterial activities of the selected plants were studied against human pathogenic organisms, namely *Escherichia coli* and *Staphylococcus aureus*.

Conclusions: The present study reveals that the selected plants would exert several beneficial effects by virtue of their natural antioxidant activity; further characterization of the phenolic composition is needed and could be harnessed as drug formulation.

Keywords: Anti-bacterial, Anti-oxidant, Bio-active, 1, 1-diphenyl-2-picrylhydrazyl scavenging activity; Medicinal plants, Reducing power assay

INTRODUCTION

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism [1]. The most common reactive oxygen species (ROS) include superoxide (O$_2^-$) anion, hydrogen peroxide, peroxyl radicals, and reactive hydroxyl (OH) radicals. The nitrogen derived free radicals are nitric oxide (NO) and peroxynitrite anion. Majority of the diseases/disorders are mainly linked to oxidative stress produced due to free radicals [2]. ROS have been implicated in over a hundred of disease states which range from arthritis, connective tissue disorders to carcinogenesis, aging, physical injury, infection, and acquired immunodeficiency syndrome [3]. Oxidative process is one of the most important routes for producing free radicals in foods, drugs, and even in living systems [4]. Herbal drugs containing free radical scavengers are known for their therapeutic activity [5]. Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids, flavonoids, and phenolic compounds are more effective [6]. They are known to inhibit lipid peroxidation (by inactivating lipooxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions [7]. Medicinal plants are delivering new drugs and many of the modern medicine indirectly, and these medicinal plants have organized by refined traditional medicine practices that have been used for 1000 of years by people in the world. Since the past few decades, researchers have more attention on medicinal plants, screening for their biological and pharmaceutical properties of the medicinal plants [8,9]. Medicinal plants are considered as clinically effective and safer alternatives to the synthetic antibiotics [10]. Plants produce a very impressive array of antioxidant compounds that include carotenoids, flavonoids, and tannins to prevent the oxidation of the susceptible substrate [11]. Phenolic compounds with antioxidant activity, which are widely distributed in many fruits, vegetables, and tea, are believed to account mainly for the antioxidant capacity of many plants [12-14]. Natural antioxidants have become the target of a great number of research studies in finding the sources of potentially safe, effective, and cheap antioxidants [15]. Hence, the present study was made to analyze the phytochemicals, bioactive compounds, antioxidants, and reducing power assay and also antibacterial properties of medicinal plants, namely *Sauropus androgynus* L. and *Erythrina variegata* L against *Escherichia coli* and *Staphylococcus aureus*.

METHODS

Plant materials used

The medicinal plants such as *E. variegata* L and *S. androgynus* L were collected from in and around area of Mangalore and Belthangady (Western Ghats of Karnataka). The plants were identified and authenticated by Dr. SharathChandra, Assistant Professor, Department of Botany, Canara College, Mangalore, Karnataka, India.

Microbial strains

Bacterial pathogens such as *E. coli* (MTCC 40) and *S. aureus* (MTCC 86) were obtained from the Institute of Microbial Technology, Chandigarh, India. The bacterial cultures were inoculated in nutrient broth for 18 h, and the suspensions were serially diluted to get approximately 10$^{-5}$ dilution.

Preparation of plant extract

The leaf samples were separated from each plant and were washed with running tap water, surface sterilized in distilled water, blot it dried and powdered. Different solvents, namely aqueous, hexane, ethanol, and methanol were used for the extraction of leaf materials. The extraction was carried out using Soxhlet apparatus. The extracts obtained from solvents were concentrated using rotary vacuum evaporator and then dried. The extract thus obtained was used for various analysis.

Qualitative phytochemical screening

Phytochemical screening was carried out for the leaf extracts as per the standard methods [16,17].
Bioactive compounds

Determination of total phenolic

The total phenolic content was determined using Folin-Ciocalteu (FC) method [18]. 0.1 ml of sample was made up to 0.25 ml with distilled water and mixed with 0.25 ml of FC phenol reagent. After 3 min, 0.5 ml of 20% sodium carbonate solution was added to the mixture and made up to 5 ml by adding distilled water. The resultant mixture was kept in the dark for 30 min, after which its absorbance was read at 760 nm. The results were expressed as µg of Gallic acid equivalents/mg of extract.

Determination of total flavonoids

0.5 ml of the sample was mixed with 1.5 ml methanol, 0.1 ml of 10% AlCl₃, 0.1 ml of 1M potassium acetate and 2.6 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The amount of flavonoid content was expressed as µg of tannic acid equivalents/mg of extract.

Determination of tannin

The tannin content was estimated by the method [20] with slight modifications. 20 µl of the sample was aliquoted into a test tube containing 980 µl of distilled water. To this, 500 µl of 1% K Fe(CN)₆ and 100 µl of 1% ferric chloride (FeCl₃) were added and made up to 3 ml with distilled water. After 10 min, the reaction mixture was measured using a UV spectrophotometer at 720 nm. The tannin content was expressed as µg of tannic acid equivalents/mg of extract.

Determination of alkaloid

To 2.5 g of sample, 200 ml of 10% acetic acid in ethanol was added and covered with aluminum foil. Then, the reaction mixture was allowed to stand for 4 h. The extract was concentrated on a water bath to one-quarter of the original volume after filtration. To this extract, concentrated ammonium hydroxide was added dropwise until the precipitation was complete. Then, the precipitation was collected, washed with dilute ammonium hydroxide and filtered. The residue was the alkaloid, which was dried and weighed [21].

The alkaloid content was determined using the following formula:

\[ \text{Percentage of alkaloid} = \left( \frac{\text{Final weight of the sample} - \text{Initial weight of the extract}}{\text{Initial weight of the extract}} \right) \times 100 \]

Saponin determination

Saponin content was determined with slight modification [22]. 20 g of sample was dispensed in 200 ml of 20% ethanol, and the suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered, and the residue was re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a crucible and was dried in a hot air oven to a constant weight. The saponin content was calculated in percentage.

Determination of β-Carotene

100 mg of each plant extracts were dissolved in 10 ml of acetone:hexane mixture (4:6) and centrifuged at 6000 rpm for 3 min. The O. D of the filtrate was read at 453, 505, and 663 nm. The assay was carried out in triplicates. The results were mean±standard deviation and expressed as µg of carotene/d g of extract [23].

Total antioxidant capacity

The total antioxidant capacity was determined by the method [24]. The methanol extract was added into a series of test tubes containing methanol and mixed with 2 ml of phosphomolybdic acid reagent solution. Then, the tubes were kept in a water bath for 90 min at 95°C. The resultant mixture was cooled to room temperature, and the absorbance was read at 695 nm against blank. The experiment was conducted in triplicates and values were expressed as equivalents of ascorbic acid (mg)/g of the sample.

1. 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay

DPPH free radical scavenging assay was measured by the method [25]. Various concentrations (20–100 µg/ml) of a leaf extract (2 ml) were taken in a number of vials containing 3 ml of 0.1 mM methanolic solution of DPPH. The test tubes were shaken gently and kept for 30 min at room temperature in the dark. An optical density of the sample was measured at 517 nm against blank. Ascorbic acid was used as the standard control. All the tests were performed in triplicates.

Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula:

\[ \text{Percentage inhibition} = \left( \frac{\text{O. D of Control} - \text{O. D of Sample}}{\text{O. D of Control}} \right) \times 100 \]

Reducing power assay

Different concentrations of the extract were prepared in methanol solvent and assayed with 2.5 ml of 0.2 M phosphate buffer followed by 2.5 ml of freshly prepared 1% potassium Ferricyanide (K₃Fe(CN)₆). This mixture was incubated for 20 min at 50°C. To this, 2.5 ml of 10% Trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. 2.5 ml of the clear extract was assayed with 2.5 ml of methanol and 0.5 ml of 0.1% FeCl₃. The absorbance was measured at 700 nm. The experiment was conducted in triplicates, and the reducing power was expressed as (µg) equivalents of ascorbic acid/g of the extract [26].

Antibacterial activity

Agar well diffusion assay

The antimicrobial activity was measured by agar well diffusion assay [27]. The plant extracts were allowed to diffuse out into the medium and to interact in a plate freshly seeded with the test organisms. Petri plates containing 15 ml of nutrient agar medium were seeded with the bacterial strains. Each labeled medium plate was uniformly inoculated with a test organism using a sterile cotton swab. Wells were punched, and 25, 50, 75, and 100 µl of the methanolic plant extracts were added. The plates were then incubated at 37°C for 24 h. Ampilox and Chloramphenicol (0.05% each) were used as positive control and analysis was done in triplicates. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. The diameter of the zone of inhibition was measured in millimeters [28].

Determination of minimum inhibitory concentration (MIC)

MIC is usually considered as the most basic laboratory measurement of the activity of antimicrobial agents against microorganisms. The MIC was determined using a methanolic extract of plants (S. andrographis L. and E. variegata L) which inhibits the visible growth of microorganisms. Different dilutions (10–100%) of the plant extracts were assayed against the test organisms. The tubes were incubated at 37°C for 24 h. Distilled water was used as negative control. After incubation, MIC of each sample was determined by reading the optical density at 600 nm in UV spectrophotometer. The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth [29].

Statistical analysis

The experimental results were expressed as mean±standard error means (SEM) of triplicates. Analysis of data was carried out by applying one-way analysis of variance (Software-Minitab 17). p-value <0.05 (p<0.05) was considered as statistically significant.

RESULTS

Qualitative phytochemical analysis revealed that all the aqueous, ethanolic, and methanolic leaf extracts showed the presence of carbohydrates, proteins, amino acids, glycosides, phenols, tannins, flavonoids, alkaloids, cardiac glycosides, saponins, and terpenoids with...
Table 1: Qualitative analysis of phytochemicals in S. androgyrus L. and E. variegata L.

| Tests          | S. androgyrus L. | E. variegata L. |
|----------------|------------------|-----------------|
|                | Aqueous          | Ethanol         | Methanol |
| Carbohydrate   | +                | +               | +        |
| Protein        | +                | +               | +        |
| Amino acids    | +                | +               | +        |
| Alkaloids      | +                | +               | +        |
| Saponins       | +                | +               | +        |
| Flavonoids     |                  | +               | +        |
| Glycosides     |                  | +               | +        |
| Phenols        |                  | +               | +        |
| Terpenoids     | -                |                  |          |
| Tannins        | +                |                  |          |

*: Present, -: Absent. S. androgyrus: Sauropus androgyrus, E. variegata: Erythrina variegata, SEM: Standard error of the mean.

Table 2: Quantitative analysis of phytochemicals in S. androgyrus L. and E. variegata L.

| Phytoconstituents | S. androgyrus L. | S. androgyrus L. | E. variegata L. |
|-------------------|------------------|------------------|-----------------|
|                   | Aqueous          | Ethanol          | Methanol |
| Total phenolic (mg GAE/g) | 276.86±0.49 | 310.96±0.49 | 214.86±0.27 |
| Tannin content (mg TAE/g) | 28.51±0.17 | 31.44±0.8 | 20.05 mg |
| Alkaloid content (mg/g) | 6.60±0.002 | 5.70±0.124 | 6.609±0.002 |
| Saponin content (mg/g) | 20.067±0.028 | 15.08±0.038 | 20.067±0.028 |
| β-carotene content (mg/g) | 304.0±0.005 | 213.7±0.003 | 304.0±0.005 |

Values are expressed as mean±SEM, n=3, P<0.05 considered as significant.

DISCUSSION

The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties. Steroids and triterpenoids show the analgesic properties [30]. Alkaloid contents were studied in 12 leafy vegetables which are capable of reducing headaches associated with hypertension [31]. Many authors have described the potential antioxidant properties of polyphenols. These compounds act as antioxidants by donation of hydrogen atom, as an acceptor of free radicals, by interrupting chain oxidation reactions or by chelating metals [32,33]. Tannins comprise both condensed non-hydrolysable tannins, known as proanthocyanidins, and esters of gallic acid and ellagic acid defined as hydrolysable tannins [34,35]. The decrease in absorbance of β-carotene in the presence of different extracts due to the oxidation of β-carotene and linoelic acid [36]. Antioxidants have already been found in plant materials and supplements. Due to their natural origin, the antioxidants obtained from plants are of greater benefit in comparison to synthetic ones. The use of natural antioxidants from plants does not induce side effects, while synthetic antioxidants were found to have genotoxic effect [37-40]. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [41,42]. Phosphomolybdenum method is based on the reduction Mo (VI) to Mo (V) by the sample anlyte and subsequent formation of green phosphor/ Mo (V) complex at acidic pH. Antioxidant activity may be probably due to phenolic compounds present in the extract. Free radicals are involved in many disorders such as neurodegenerative diseases, cancer, and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. Stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts [43]. It is known that only flavonoids with certain structure and particularly OH position in the molecule can act as proton donating and show radical scavenging activity [44,45]. DPPH solution exhibits purple color generally fades when antioxidant molecules quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably through a free-radical attack on the DPPH molecule) and convert them into a colorless-/bleached product (i.e., 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm band [46]. The reducing
Table 3: Antioxidant activity of S. androgyrus and E. variegata leaf extracts

| Medicinal plants | Total antioxidant activity (µg/mg) | Reducing power assay (µg/mg) | DPPH radical scavenging activity (%) |
|------------------|-----------------------------------|------------------------------|--------------------------------------|
| S. androgyrus    | 250.55±0.49                       | 132.91±0.03                  | 45.38                                |
| E. variegata     | 82.95±0.56                        | 150.07±0.04                  | 4.08                                 |

| Plant            | Zone of Inhibition (mm) | MIC Zone of Inhibition (mm) | MIC |
|------------------|-------------------------|-----------------------------|-----|
| S. androgyrus    | 19.48±0.02              | 10%                         | 17.68±0.15 | 20% |
| E. variegata     | 17.49±0.01              | 10%                         | 16.2±0.2   | 10% |
| Control Ampilox  | 24.2±0.2                | -NA-                        | 22.13±0.11 | -NA- |
| Chloramphenicol  | 22.±0.001               | -NA-                        | 21.7±0.11  | -NA- |

Data expressed as mean±SEM, P<0.05 considered as significant, -NA: Not applicable. Sandanogus: Sauropus androgyrus, E.variegata: Erythrina variegata, DPPH: 1,1-diphenyl-2-picrylhydrazyl, SEM: Standard error of the mean.

Table 4: Antibacterial activity of S. androgyrus and E. variegata plant extracts

| Plant            | S. aureus Zone of Inhibition (mm) | MIC | E. coli Zone of Inhibition (mm) | MIC |
|------------------|----------------------------------|-----|--------------------------------|-----|
| S. androgyrus    | 10%                              | 17.68±0.15 | 20% |
| E. variegata     | 10%                              | 16.2±0.2   | 10% |
| Control Ampilox  | -NA-                             | 22.13±0.11 | -NA- |
| Chloramphenicol  | -NA-                             | 21.7±0.11  | -NA- |

CONFLICTS OF INTEREST
The authors declare that they have no conflicts of interest.

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