

Original Article

STUDIES ON ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF LEAF EXTRACTS OF
SYZYGIUM ALTERNIFOLIUM (WT.) WALP

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ABSTRACT

Objective: To study the antimicrobial and antioxidant properties of *Syzygium alternifolium* (SA) leaf extracts by using *in vitro* methods.

Methods: Hexane (HE), ethyl acetate (EAE), ethanol (EE) and water (WE) extracts of *S. alternifolium* leaf, were screened for antimicrobial and antioxidant activities using *in vitro* methods. Total phenolic and total antioxidant capacity of SA- leaf extracts were quantified as gallic acid equivalents, using the spectrophotometric method.

Results: Among the test extracts ethanol and aqueous extracts exhibited strong antioxidant activity than that of hexane and ethyl acetate. Free radicals, DPPH and H₂O₂ were significantly inhibited by aqueous extract (IC₅₀ 4µg and 10 µg/mL), while nitric oxide was strongly inhibited by ethanol extract (IC₅₀ 30 µg/mL). Ethanol and water extracts contained the higher amount of total phenolic content (29±0.4 and 30±0.8 mg GAE/g dwt) and total antioxidant activity (40±0.1 and 49±0.7 mg AAE mg/g dwt), respectively. The antioxidant activity of ethanol and water extracts exhibited a dose dependent activity and well correlated to its phenol concentration, which is not followed as in hexane and ethyl acetate extract. Further, the leaf extracts were investigated for antimicrobial activity, on five bacterial and one fungal strain using disc diffusion and micro dilution methods. Of the test extracts, hexane extract, showed pronounced antimicrobial spectrum, ethyl acetate and ethanol extracts showed feeble activity, while aqueous extract showed faint inhibition against the test pathogens.

Conclusion: The present observations suggest that *S. alternifolium* leaves may be considered as a new source for antioxidant and antimicrobial constituents for therapeutic use.

Keywords: *Syzygium alternifolium*, Antimicrobial. Antioxidant.

INTRODUCTION

The demand for medicinal plants in health care is about 70-80% [1]. It is believed that the use of plants or plant based materials for medicinal purposes has been associated with fewer side effects. The therapeutic effects of medicinal plants were shown to be associated with their chemical constituents. The natural compounds are reported to have different pharmacological activities i. e., antimicrobial, antiulcer, anticholinesterase, anticancer, spasmolytic, antiviral, antioxidant and analgesic [2].

The genus *Syzygium* (Family: Myrtaceae) comprises about 500 species throughout the globe and most of them are evergreen trees and shrubs. Several species are grown as ornamental/tree crops for glossy foliage and a few produce edible fruits. *Syzygium alternifolium* (Wt.) Walp. (SA) is an endemic aromatic tree, distributed in Assam and Andhra Pradesh states, India [3]. In local language (Telugu) it is known as mogi/ movi. The plant parts used in traditional medicine to cure various diseases viz., tender shoots, fruits and leaves for dysentery, diabetes, seeds for diabetes, ulcers, dysentery, burning sensations in stomach and joint pains [4].

Several *Syzygium* species were reported to possess antibacterial [5;6;7], antifungal [8] anti inflammatory [9] and antioxidant [10] activities. *S. alternifolium* was reported to possess hypoglycemic and antihyperglycemic activity [11] and antimicrobial activity [12;13;14]. The phytochemical studies revealed that only flavonoids and terpenoids were reported from the leaf [15] and the plant material has been unexploited much for detailed studies. Based on review of literature, it was found that, very few reports [12; 13] were noticed on antimicrobial activity of SA-leaf extracts and there are no previous reports observed on free radical scavenging activity of *S. alternifolium* leave as far as the authors concern. Hence, the present study designed to evaluate the antimicrobial and antioxidant activity of SA-leaf extracts, by using spectrophotometric studies.

MATERIALS AND METHODS

Chemicals

All the chemicals and the reagents used in the present study are hexane, ethyl acetate, ethanol, dimethyl sulphoxide, ampicillin, tetracycline, vancomycin, peptone, sodium chloride, beef extract, agar-agar, Folin-Ciocalteu reagent, sodium carbonate, sulphuric acid, sodium phosphate, ammonium molybdate, diphenyl-2-picrylhydrazyl (DPPH), gallic acid, ascorbic acid, hydrogen peroxide, butylated hydroxytoluene (BHT), xylene orange, ammonium ferrous sulphate, sodium nitro prusside (SNP) naphthyl - ethylenediamine (NED) and Griess reagent are analytical grade.

Plant material and preparation of extracts

Syzygium alternifolium (Wt.) Walp. leaves were collected from Tirumala hills of Eastern Ghats, Andhra Pradesh. The voucher specimens were identified with the help of regional and local floras [16] and the same was deposited at Sri Krishnadevaraya University herbarium (SKU), Anantapur.

The collected leaves were cut into small pieces, shade-dried, powdered and subsequently extracted with 250 mL of hexane (HE), ethyl acetate (EAE) and ethanol (EE) (Merck) using Soxhlet apparatus. The final residue was soaked overnight in 250 mL of sterile double distilled water (WE). The extracts were filtered and concentrated under reduced pressure below 40 °C to dryness. The extracts were stored at 4 °C and used for antimicrobial and antioxidant activities.

Antimicrobial studies

Fifty milligrams of leaf extracts were dissolved in one mL of dimethyl sulphoxide (DMSO) and 10 µL (500 µg/disc) portions were impregnated on sterilized Whatmann No.1 filter paper discs (6 mm

diameter). The solvent was allowed to dryness at room temperature in a laminar air flow bench.

The microbial strains viz., *Bacillus cereus* (BC) MTCC1429, *Staphylococcus aureus* (ST) MTCC737, *Escherichia coli* (EC) MTCC1687, *Pseudomonas aeruginosa* (PA) MTCC1688, *Klebsiella pneumoniae* (KP) MTCC109 and *Candida albicans* (CA) MTCC183, obtained from the Microbial Type Culture Collection Centre, Institute of Microbial Technology (IMTECH), Chandigarh, India, were used in the study.

The antimicrobial activity of the extracts was evaluated by disc diffusion method [17]. Filter paper discs containing 500 µg/disc were placed on surface of the solidified nutrient agar medium, pre seeded with microbial strains (10⁵CFU/ mL). Standard antibiotics, viz., ampicillin, tetracyclin and vancomycin (30 µg/disc) obtained from Hi-media, Mumbai, were used as positive controls. The discs containing petroleum ether, ethyl acetate, ethanol or DMSO served as negative controls. The plates were incubated for 24 h at 37 °C and the diameter of the inhibition zones was recorded. Three independent trials were conducted for each concentration to confirm the activity. The minimum inhibitory concentration (MIC) was determined using a common broth microdilution method in 96-well micro titer plates [18;19]. Two fold dilutions of each extract were carried out, starting from 5 to 0.15 mg/mL. Of the aliquots (10 µL) of different microbial suspensions (10⁵CFU/ mL) were added to each well.

Plates were incubated for 18 h at 37 °C and were examined with Elisa reader (TECAN, Sunrise, China) at 620 nm. The lowest concentration of the extract showing no growth has been considered as its MIC. The solution of DMSO (100 µL/mL) served as the negative control. All the samples were tested in triplicates to confirm the activity.

Estimation of total phenolic content

Total phenolic content (TPC) of the extracts was estimated by using Folin-Ciocalteu reagent and expressed as gallic acid, (a standard natural phenolic compound) equivalents [20]. The leaf extract of 10 µL was incubated with 0.1 mL of commercial Folin-Ciocalteu reagent. After 10 minutes, 300 µL of 20% aqueous sodium carbonate was added and incubated in dark for 2 h and made up to 2 mL with water. The intensity of the colour is directly proportional to the phenolic content of the extracts. The reaction mixture in triplicates was read at 765 nm, against the reagent blank, results were calculated and represented as milligrams of gallic acid equivalents (mg GAE/ g dry weight) of the sample.

Determination of total antioxidant activity

The total antioxidant capacity (TAC) of extracts was evaluated with phosphomolybdenum reagent using ascorbic acid as the standard compound [21]. The results were expressed as milligrams of equivalent of ascorbic acid (mg AAE/g dwt) per gram dry weight.

DPPH radical-scavenging activity

1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of the *S. alternifolium* leaf extracts was measured at 517 nm by spectrophotometer method [22]. A 1 mL solution of 0.004% DPPH solution was added to SA- leaf extracts at different concentration. The solution in the test tubes was shaken well and incubated in the dark for 30 min at room temperature. The disappearance of violet color of methanolic DPPH solution indicates scavenging capacity of the extract considered as the positive reaction.

Hydrogen peroxide scavenging activity

This was performed by the ferrous ion oxidation-xylene orange (FOX) assay [23]. The plant extracts in different concentrations were mixed with 40 mM H₂O₂, incubated in dark for ten minutes, and 0.2 mL of FOX reagent was added. Disappearance of blue color was considered as a positive reaction and the test samples were measured spectrophotometrically at 584 nm and compared with a standard antioxidant (vitamin C) and the values of per cent inhibition were calculated by using the formula mentioned in statistical analysis section.

Nitric oxide scavenging activity

Different concentrations of SA leaf extracts were pre-incubated with 6 mM sodium nitro prusside (SNP) for 5 min. Aqueous solution of SNP at physiological pH spontaneously generates nitric oxide [24] which interacts with oxygen to produce nitric ions that can be estimated using Griess reagent. The absorbance of the chromophore formed during the diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine was read at 546 nm.

Statistical analysis

The protective effect of different extracts against different radicals was calculated as follows:

% inhibition = (control-sample/control) x 100. Ascorbic acid and gallic acids were used as a reference compounds. All the results were expressed as mean ± standard deviation of mean (S. D.).

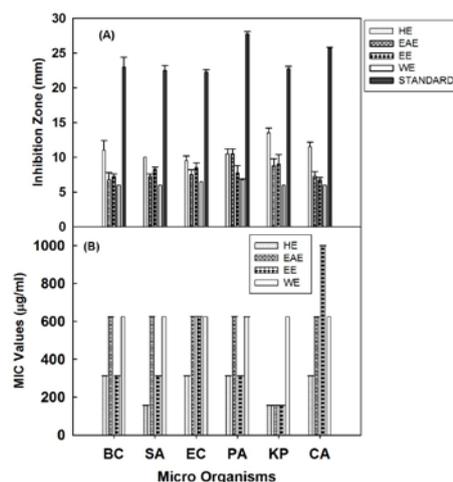


Fig. 1: It shows antimicrobial activity of SA- leaf extracts by disc diffusion method (A) and minimum inhibitory concentration (MIC)s of each extract against test pathogen (B). HE: hexane, EAE: ethyl acetate, EE: ethanol, WE: water extract. Standards used Ampicillin for gram positive bacteria, tetracycline for gram negative bacteria and vancomycin for *Candida* species

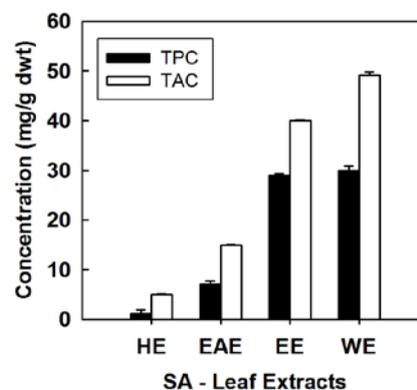


Fig. 2: It shows total phenolic content (TPC) and total antioxidant capacity (TAC) of SA- leaf extracts. HE: hexane, EAE: ethyl acetate, EE: ethanol, WE: water extract

RESULTS

Antimicrobial studies

The antimicrobial activity of the extracts was measured by observing bacterial free zones formed around the discs. The standard antibiotics,

penicillin, tetracycline and vancomycin used as positive controls, whereas DMSO serves as negative control. The *in vitro* antimicrobial activities of *S. alternifolium* leaf extracts were summarized in fig. 1A. The HE extract exhibited the broad spectrum of inhibition against the test pathogens in the following order of KP>CA>BC>PA>ST>EC. The ethyl acetate extract showed maximum inhibition on KP, followed by ST, EC and PA. The EE and WE extract exhibited faint inhibition against test pathogens (fig. 1A). The MIC values of hexane extract of SA revealed that 156 µg/mL against KP and ST, 312 µg/mL for BC, EC PA and CA. With the exception of KP (MIC 156 µg/mL), ethyl acetate extract exhibited MIC values at 625 µg/mL (fig. 1B). Ethanol extract expressed MIC values 156 to 625 µg/mL for bacterial species, while *Candida* seems to be the least sensitive (1000 µg/mL) organism.

Total phenolic content

The total amount of phenolic content in HE, EAE, EE and WE extracts of SA leaves were determined by Folin-Ciocalteu reagent method and expressed as gallic acid equivalents per gram dry weight (dwt). The total phenolic content in the test extracts of SA leaves was in the range 1.13±0.81 mg GAE/g in HE, 7.06±1.7 mg GAE/g in EAE, 29.04 ±0.4 mg GAE/g in EE and 30.0±0.8 mg GAE/g dry weight (dwt) in WE. The maximum phenolic compounds were extracted in water extract (fig. 2).

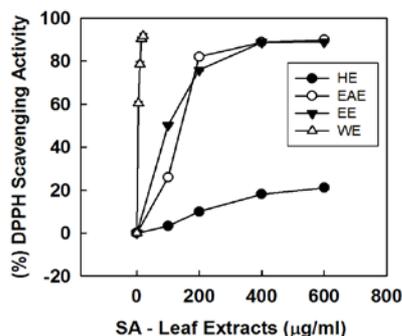


Fig. 3: It shows DPPH scavenging capacity of SA- leaf extracts. HE: hexane, EAE: ethyl acetate, EE: ethanol, WE: water extract

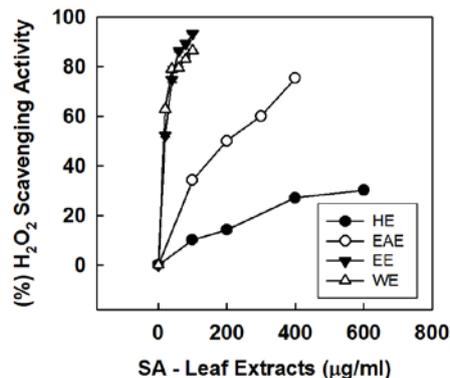


Fig. 4: It shows hydrogen peroxide scavenging activity of SA- leaf extracts. HE: hexane, EAE: ethyl acetate, EE: ethanol, WE: water extract

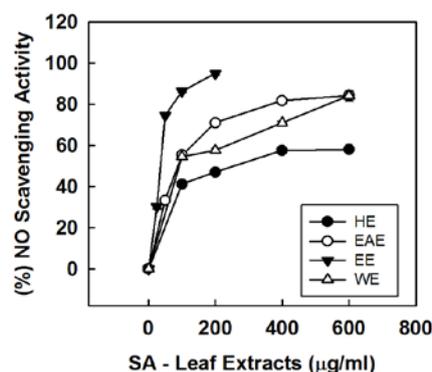


Fig. 5: It shows nitric oxide scavenging capacity of SA- leaf extracts. HE: hexane, EAE: ethyl acetate, EE: ethanol, WE: water extract

Table 1: It shows 50 % inhibition (IC₅₀ values) of the *S. alternifolium* leaf extracts and standard compounds against different free radicals (µg/ml)

Type of extract/Standard compound	DPPH	H ₂ O ₂	NO
Hexane	800±0.6	800±0.18	300±0.32
Ethyl acetate	100±0.12	200±0.24	100±0.42
Ethanol	60±0.2	20±0.51	30±0.87
Water	4±0.12	10±0.51	90±0.19
*Standard	2±0.2 ^G	45±0.45 ^A	10±0.6 ^G

*Standards: A: ascorbic acid; G: gallic acid

Total antioxidant capacity

The results on total antioxidant activity of SA leaf extracts was expressed as ascorbic acid equivalent (AAE) are presented in fig. 2. Among the test extracts, water and ethanol extracts showed maximum antioxidant activity, while HE extracts showed significantly less activity.

DPPH scavenging activity

The effect of SA leaf HE, EAE, EE and WE extracts and gallic acid on DPPH reducing capacity revealed that both extracts and gallic acid showed dose dependent DPPH reducing capacity (fig. 3). The highest DPPH radical scavenging activity was shown by the water extract (IC₅₀ – 4 µg/mL) and the second highest was found in ethanol (IC₅₀ – 60 µg/mL), while the lowest was observed in hexane extract (IC₅₀ – 800 µg/mL). IC₅₀ value represents the concentration of the test extract requires for the inhibition of test radical 50% (Table 1).

Hydrogen peroxide scavenging assay

The results of hydrogen peroxide scavenging activity of SA leaf extracts and ascorbic acid showed that the entire test extracts

expressed concentration dependent inhibition (fig. 4). Among the test extracts WE strongly inhibited hydrogen peroxide radical. The concentration of SA leaf extracts needed for 50% inhibition (IC₅₀) was found to be 10 to 200 µg/ml (table1).

Nitric oxide scavenging activity

In the present study, the results indicate that the HE, EAE, EE and WE extracts of SA leaves and gallic acid exhibited strong nitric oxide radical scavenging activity by decreasing the nitrite concentration in the assay medium. Fig. 5 showed that the extracts showed the percentage of inhibition in a concentration dependent manner. The concentration of SA leaf extracts needed for 50% inhibition (IC₅₀) was found to be 30 – 300 µg/mL (Table 1).

DISCUSSION

The present study has designed to evaluate the antimicrobial and antioxidant activity of HE, EAE, EE and WE extracts of SA leaf extracts by *in vitro* methods. Previous reports on antimicrobial activity of SA – leaf chloroform, ethyl acetate, ethanol, methanol and water extracts significantly inhibited Gram positive bacteria [12;13].

The present study results on antimicrobial activity of SA leaf extracts showed that, HE exhibited significant inhibition against test pathogens than other extracts. Gram negative bacterium, KP was strongly inhibited by hexane, ethyl acetate and ethanol extracts, while EC found to be least sensitive organism (fig. 1A & B). The potential antimicrobial activity of the SA leaf extracts might be due to the presence of secondary metabolites like flavonoids, volatile oils and alkaloids. Detailed phytochemical analysis of SA leaf extracts revealed that, presence of flavonoids (eucalyptin and tephrowsin) and terpenoids [15]. Eucalyptin was reported to possess antimicrobial activity [25], while friedelin reported for its antileishmanial activity [26]. The broad spectrum of antimicrobial activity observed in the present study appeared to be due to the individual or synergetic effect of the above mentioned chemical constituents.

The results on total phenolic content and total antioxidant capacity of SA leaf extracts were depicted in fig. 2. It revealed that EE and WE extracts had the higher amount of phenolic content and total antioxidant capacity. There are very few reports on the antioxidant capacity of *Syzygium* species measured by the phosphomolybdenum method. The present observations suggest that the strong antioxidant activity of ethanol and water extracts of *S. alternifolium* leaves might be attributed to the presence of high concentration of phytochemicals such as phenolic compounds [27]. The phenolic compounds present in different parts of a plant contain hydroxyls that are responsible for the radical scavenging redox properties [28]. In the present investigation *S. alternifolium* showed high amount of phenolic content than the previous reports of *S. aqueum* [29] and *S. cumini* [30].

DPPH is widely used free radical to evaluate the antioxidant capacity of natural products from plant and microbial sources. The results on DPPH scavenging activity demonstrates a significant decrease in the concentration of DPPH radical due to the presence of antioxidant compounds in SA leaf extracts (fig. 3). The antioxidant molecule donates hydrogen to scavenge the radical. Out of the four extracts tested water extract strongly inhibited DPPH radical at the lowest concentration (IC₅₀ value 4 µg/mL). All the extracts of SA leaf showed concentration dependent antiradical activity against DPPH radical. A higher DPPH radical scavenging activity is associated with a lower IC₅₀ value. The strong DPPH scavenging activity of WE, may be due to the presence of high amount of phenolic content. The present observations are similar to that of *S. cumini* fruit extract against DPPH radical [31].

Hydroxyl radicals are the major active oxygen species that can cause lipid oxidation and enormous biological damage. H₂O₂ itself it is not very reactive, but it can sometimes be toxic to the cell because it may give rise to hydroxyl radical in the cells. Thus, removal of H₂O₂ is very important for protection of food systems [32]. EE showed maximum inhibition as 93% inhibition to hydrogen peroxide. But WE exhibited strong inhibition by expressing lowest IC₅₀ values than other extracts (IC₅₀ value 10 µg/mL). It clearly showed that, plant extract scavenged H₂O₂ more effectively than ascorbic acid (IC₅₀ value 45 µg/mL).

Nitric oxide (NO) exhibits numerous physiological properties and it is also implicated in several pathological states. This is due to the fact that, the nitric oxide reacts with other radicals, such as superoxide, leads to the formation of more hazardous radicals such as peroxy nitrite anion and hydroxyl radical [33]. The results showed that EE expressed maximum inhibition (94%) and lowest IC₅₀ values to inhibit NO (IC₅₀ value 30 µg/mL).

CONCLUSION

The present study demonstrated that, *S. alternifolium* leaf extracts have significant free radical scavenging and antimicrobial activity. Free radicals, DPPH and H₂O₂ were strongly inhibited by WE extract (IC₅₀ 4 µg and 10 µg/mL), while nitric oxide was strongly inhibited by ethanol extracts (IC₅₀ 30 µg/mL). Accordingly, aqueous and ethanol extracts showed highest total phenolic content (30 and 29 mg GAE/g dwt). The observed antioxidant property of aqueous and ethanol extracts may be due to the presence of higher amount of total phenolic content. Furthermore, hexane, ethyl acetate and

ethanol extracts showed pronounced antimicrobial spectrum. Hexane extract exhibited higher antimicrobial activity as compared to ethyl acetate and ethanol extracts. Gram negative bacterium, *K. pneumoniae* was greatly inhibited by hexane, ethyl acetate and ethanol extracts. Further toxicological/phytochemical studies are required to establish molecular basis to understand the mechanism of action in *in vivo* models. The data suggest that aqueous and ethanol extracts of leaves are potential sources of natural antioxidants, while hexane extract is a source for natural antimicrobial compounds.

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CONFLICT OF INTERESTS

Declared None

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