

Original Article

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF METHANOLIC ROOT EXTRACT OF
TABERNAEMONTANA ALTERNIFOLIA L

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ABSTRACT

Objective: The infectious diseases caused by bacteria are a major problem and most of them are resistant to the present antibiotics. Also the free radicals act on structural and functional architecture of the cell in turn lead to cytotoxicity and genotoxicity of the cell. In this regard plants would have molecules alternative to antibiotics with higher safety, efficiency and will play a key role in maintaining human health.

Methods: In this study antimicrobial activity of methanolic crude extract of *Tabernaemontana alternifolia* root extract was determined by an agar gel diffusion method against *Bacillus flexus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus aureus* and *E. coli* bacteria, *Aspergillus terreus* and *Scopulariopsis sp. fungi*. Antioxidant potential of root extract was determined by ABTS assay and DPPH method.

Results: The methanolic root extracts of *T. alternifolia* possess significant antibacterial activity and reducing power. The significant inhibition was observed at 250, 500, 750, 1000 µg/ml for *Bacillus flexus*, *Proteus aureus* bacteria. *Staphylococcus aureus* was inhibited at 1000 µg/ml concentration of the plant extracts. Crude extract inhibited DPPH with IC₅₀ value 250 µg/ml and ABTS with IC₅₀ value 600 µg/ml. No antifungal activity was observed.

Conclusion: The overall result can conclude that *T. alternifolia* root possess marked antibacterial activity and anti oxidant activity at lower concentration of plant extract.

Keywords: *Tabernaemontana alternifolia*, Antibacterial, ABTS, DPPH.

INTRODUCTION

Infectious diseases are one of the main reasons for early deaths; antibiotics are used for the treatment which has limitations since microorganisms are developing resistance against antibiotics [1-7]. This resistance is very complex in immunocompromised host [8]. Microorganisms are resistant to the antibiotics which are constantly used for infections. *Staphylococcus aureus* resistant to methicillin and *Pseudomonas aeruginosa* is multidrug resistant [4, 5]. Along with it the antibiotics have adverse side effects to the host like hypersensitivity, immunosuppressant and allergic reactions which has led to immense clinical problems to treat infectious diseases [9]. So novel therapy or antimicrobial compound is inevitable.

Free radicals, which are generated in several biochemical reactions in the body, have been implicated as mediators of many diseases, including cancer, atherosclerosis and heart diseases, inflammation, aging etc [10-16]. Although these free radicals can be scavenged by the *in vivo* produced antioxidant compounds, the endogenous antioxidants are insufficient to completely remove them and maintain a balance [17-19]. Synthetic antioxidants, such as butylated hydroxyl toluene [BHT] and butylated hydroxyl anisole (BHA), which are effective in their role as antioxidants, are commercially available and currently used in industrial processes.

However, since suspected actions as promoters of carcinogenesis and other side effects have been reported, their use in food, cosmetic and pharmaceutical products has been decreasing [20-23]. As a result, dietary antioxidants are required to counteract excess free radicals. Scientific data indicates diet rich in vegetables, fruits, grains are lower risk of oxidative damage. Plant metabolites like Vitamin C, Vitamin E, Phenolic compounds, carotenoids, lycopenes, flavonoids play a major role as reactive oxygen species and there by maintaining normal cell growth and genome of the cell [10]. Therefore plants play a major role in maintaining normal physiological function and reducing the risk of diseases. Several plants have been worked in this aspect and the present study was to determine an efficient antioxidant and antibacterial activity from plant extract.

Tabernaemontana. alternifolia belonging to apocynaceae family (syn. *Ervatamia heyneana*, *Tabernaemontana heyneana* Wall] is included in the oldest script *Amarakosam* or *Namalingkanusasanum* written by Amarasshimhan in between somewhere in 1-6 century AD [24] plant can be used for various medicinal problem such as venereal diseases, gonorrhoea, respiratory problems, nervous disorders, diabetes, chronic bronchitis, rheumatism, cardiotoxic ailments, antioxidant and antiproliferative, and snake bite [1, 10, 24-28]. Previous work on various parts of this plant (leaves and stem bark) has proved potent antioxidant and antibacterial and antifungal activity [1, 10, 25].

MATERIALS AND METHODS

Collection of plant and extraction of crude extract

Tabernaemontana alternifolia L collected from Kollur, Kundapura taluk, Udupi District. The plant was authenticated at National Ayurveda Dietetics Research Institute, Bangalore, Karnataka. The plant roots collected were free of disease and injury, washed several times with distilled water and shade dried. The powdered roots (40g) were extracted using methanol (400 ml) in soxhlet extractor solvent. Extract was concentrated using rotary vacuum evaporator and the residue obtained, was dried, weighed. The photochemical qualitative analysis was performed [29-30].

Preparation of inoculum and Antibacterial and Antifungal activity by gel diffusion method

Pure cultures of *Bacillus flexus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus aureus* and *E. coli* were procured from MTCC. The anti fungal activity was performed with *Aspergillus terreus* and *Scopulariopsis spp.*

The lyophilized culture was subcultured for further use. The bacterial culture was inoculated into nutrient broth and allowed to grow until it reaches an optical density of 1.00 later maintained and preserved. About 25 mL of sterile nutrient agar media was poured into sterile petriplate containing 0.1 ml of bacterial culture

[containing 1×10^6 cfu/ml]. The well was bored in agar using 8 mm sized cork borer. Same procedure was followed for fungal culture [containing 1×10^8 cfu/ml] using potato dextrose agar media. 50 μ l of extract with different concentrations [250, 500, 750, 1000 μ g/ml] was added to the wells and antibiotic [1 mg/ml] at the centre well was added. The plates were incubated at 37 °C for 24 hours for bacteria, 28 °C for 48 hrs for fungi and the zone of inhibition was measured [25].

In vitro antioxidant assay

2, 2'-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid]] Radical Cation Scavenging Method: ABTS assay [31]

Solution I: 2 mM solutions of ABTS prepared using distilled water.

Solution II: 17 mM Potassium persulfate solution is prepared using distilled water.

0.3 mL of solution II was added to 50 mL of solution. This reaction mixture was kept in room temperature for the formation of pre-formed radical monocation of 2, 2'-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid] overnight in dark before use.

Different concentrations of plant extract and ascorbic acid [standard] were weighed separately and dissolved in DMSO solution. The test sample contains 1 ml of distilled DMSO and 0.2 mL of various concentrations of plant crude extract or standard, 0.16 mL of ABTS was added. After 20 min absorbance was measured spectrophotometrically at 734 nm.

Blank was maintained with DMSO. Samples, blank, and positive control [ascorbic acid] were assessed in triplicates. IC50 value obtained is defined as the concentration of the sample required to inhibit 50 % ABTS radical mono cation.

2,2-diphenyl-1-picrylhydrazyl[DPPH] radical scavenging assay

The compounds were tested for the scavenging effect on DPPH radical according to the method of Pan *et al.*, [32]. 0.2 mL of each compound solution [50 μ g] in ethanol [95 %] was added to 8 mL of 0.004 % [w/v] stock solution of DPPH in ethanol [95 %].

Scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm until the reaction reached the steady state, using a UV-Visible. All determinations were performed in triplicate. The DPPH radical scavenging activity [S%] was calculated using the following equation:

$$S\% = \frac{[A_{\text{control}} - A_{\text{sample}}]}{A_{\text{control}}} \times 100,$$

Where A_{control} is the absorbance of the blank control [containing all reagents except the extract solution] and A_{sample} is the absorbance of the test sample. Samples, blanks, and positive controls [ascorbic acid] were assessed in triplicate.

Table 1: Antibacterial activity against the *Proteus*

Standard/Extract	Concentration of extract [μ g/ml]	Zone of Clearance [cm]
Antibiotic	1000	1.1
Root extract	250	0.5
Root extract	500	0.7
Root extract	750	1
Root extract	1000	1.2

Table 2: Antibacterial activity against the *Bacillus Flexus*

Standard/Extract	Concentration of extract [μ g/ml]	Zone of Clearance [cm]
Antibiotic	1000	2
Root extract	250	0.6
Root extract	500	0.9
Root extract	750	1.2
Root extract	1000	1.3

Table 3: Antibacterial activity against the *Staphylococcus aureus*

Standard/Extract	Concentration of extract [μ g/ml]	Zone of Clearance [cm]
Antibiotic	1000	1.6
Root extract	250	No clearance
Root extract	500	No clearance
Root extract	750	No clearance
Root extract	1000	0.7



Fig. 1: Inhibition zones observed with extract in *Proteus*

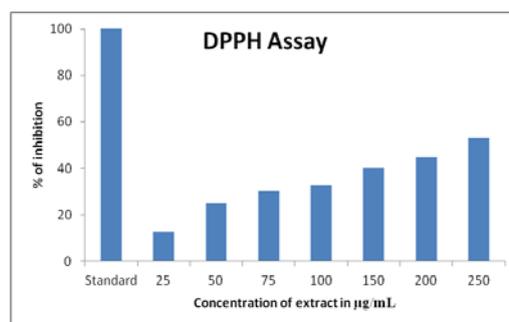


Fig. 2: Schematic representation of % inhibition of DPPH scavenging activity

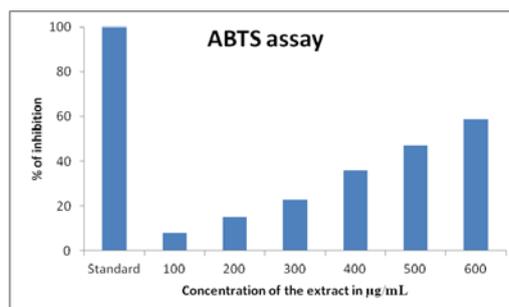


Fig. 3: Schematic representation of % inhibition of ABTS

RESULTS

The phytochemical analysis showed the presence of steroids, glycosides, alkaloids, flavanoid, phenolics and terpenoids, whereas the carbohydrates and proteins are absent.

Plant extract showed significant antibacterial activity against *Proteus aureus* [table 1] *Bacillus flexus* [table 2] and *Staphylococcus aureus* [table 3] but could not inhibit *Pseudomonas aeruginosa* and *E. coli*. The antibiotics showed maximum inhibition at 1 mg/ml concentration. The extract exhibited minimum inhibition at 250 μ g/ml and maximum inhibition at 1000 μ g/ml concentration towards *Bacillus flexus* and *Proteus aureus* [fig. 1]. In *Staphylococcus*

aureus minimum inhibition was observed at maximum concentration only. The extract did not possess antifungal activity against the species used.

The plant extracts possess potent antioxidant activity as in DPPH assay the extract could inhibit 50% of the activity at a concentration of 250 µg/ml (fig. 2) and in ABTS at a concentration of 600 µg/ml (fig. 3).

DISCUSSION

The antibacterial activity conducted on above mentioned microorganisms and antioxidant activity indicates *T. alternifolia* as a potent plant. In the present study, extract inhibited *Staphylococcus aureus* at a concentration of 1000µg/ml which is same as that of antibiotic concentration. Increase in concentration of extract more than antibiotic concentration may be required to get the maximum inhibition of bacteria. The study is similar to the results obtained in the aqueous extracts of *T. alternifolia* stem bark which exhibited antibacterial activity against Gram-positive microorganisms, particularly against clinical isolates of MRSA and vancomycin resistant *S. aureus* [VRSA][1]. The minimum inhibitory concentration [MIC] of extract against the isolates ranged from 600–800 µg/ml. *In vitro* antibacterial effect of *T. alternifolia* leaves shows significant inhibition on *K. pneumonia* and *S. typhii* [25]. *T. alternifolia* stem and leaves exhibited antibacterial activity against Gram negative bacteria, but root extract strongly inhibits Gram positive bacteria than Gram negative bacteria. Roots did not possess antifungal activity against the isolates used where as *T. alternifolia* leaves showed inhibition for fungal species *Rhizopus mucor* and *Trichoderma viridins*. *Tabernaemontana heyneana* Wall. Leaves showed both antifungal and antibacterial activity inhibiting *K. pneumoniae* [26±1.0 mm] and *S. typhii* [9.0±2.0 mm] maximally and minimally and *Rhizopus mucor* [25±0.0 mm] and *Trichoderma viridins* [6.0±1.0 mm] maximally and minimally respectively [25]. Similarly the *T. divaricata* L showed potential inhibitory activity against *Staphylococcus aureus*, *Streptococcus agalactiae*, *E. coli*, *Streptococcus uberis* [26].

Root extract of the plant showed prominent ABTS and DPPH activity and the IC 50 values were as mentioned in the results. Root is very significant than *Tabernaemontana heyneana* wall leaves in which DPPH and ABTS IC 50 values were 507 and 537 µg/ml respectively[10]. Further purification of the compound may show much lower the IC50 values. The purified one of the alkaloid fraction of *Tabernaemontana catharinensis* exhibited DPPH scavenging activity with IC50 value 37.18[11]. The crude root extract of this plant has good antioxidant activity compared to other parts which is already established by previous works.

The DPPH radical scavenging capacity of samples *Tabernaemontana catharinensis* A. DC. Fruits and branches were studied and observed that they were dose-dependent, and the branches obtained better antioxidant capacity [AC] than fruits. For fruits, it was observed that the more polar fractions, EtAc and n-BuOH, had higher scavenging capacity toward DPPH with the IC50 values of 181.82 µg/ml and 188.24 µg/ml respectively. For branches, the n-BuOH fractions exhibited high scavenging activity toward DPPH with the IC50 of 78.19 µg/ml[33]. This indicates that the *Tabernaemontana* family plants have the potent antimicrobial and antioxidant activity.

CONCLUSION

The result from the plant analysis clearly indicates that the roots of this plant possess potent

Antibacterial and antioxidant activity which could be further studied by purification process and could be of commercial use.

CONFLICTS OF INTERESTS

Declared None

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