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EXPLORING THE THERAPEUTIC PROPERTIES OF *BOUGAINVILLEA GLABRA*: A STUDY OF ITS ANTIMICROBIAL AND ANTIOXIDANT EFFECTS IN METHANOL EXTRACT

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Abstract

The search for phytochemicals with antimicrobial and antioxidant properties has gained significant attention due to their therapeutic application. To fully realize their medicinal applications, it is necessary to screen and identify these compounds from a variety of plant species. The present study aimed to investigate the chemical profile of the methanolic extract of *B. glabra* flowers by performing various qualitative chemical tests. The analysis revealed the presence of alkaloids, flavonoids, phenolic compounds, and tannins in the extract. In vitro antimicrobial assays confirmed the antimicrobial activity of the flower extract against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas putida, Escherichia coli,* and *Candida albicans*. Additionally, the antioxidant properties of the extract were evaluated using three different assay methods, including the quantification of phenolics, flavanoids, and betalains. The results showed high antioxidant activity in the extract, as indicated by its reducing power in the Ferric Reducing Antioxidant Power (FRAP) assay (134.71 ± 0.97 TE/100 g of extract). The presence of natural antioxidants in *B. glabra* flowers can scavenge excess free radicals and prevent oxidative damage, slowing the onset of premature aging symptoms and degenerative chronic diseases. As a result, *B. glabra* flowers have the potential to serve as a natural source of antioxidants for food and nutraceutical product development.

Keywords: *Bougainvillea glabra,* Paper flower, Antimicrobial, Antioxidant, Free radical

Introduction

Plants are a vital source of therapeutic agents, possessing a wide range of biological properties like antioxidant, antibacterial and antifungal activities. Natural antioxidants are wide-range, safe and effective in regulating oxidative stress caused by free radicals [1]. Allopathic medicine primarily employs synthetic or semi-synthetic antibiotics to treat microbial diseases, however, more microbes are resistant to antibiotics resulting in treatment failures. This has led to increased use of plant extracts and their derivatives as a natural alternative [2]. Previous studies have demonstrated the therapeutic potential of several botanicals against chronic diseases such as cancer, diabetes, inflammation, stroke and ageing [3]. These findings suggest that plants continue to be valuable source for the discovery of novel drugs and therapeutic compounds.

Insufficient amounts of antioxidants in human cells, synthetic antioxidants are used to combat oxidative stress, but cause toxicity, carcinogenicity and mutagenic effects [4,5]. Therefore, a natural alternative source of antioxidant is needed, which can be provided as a supplement. Plant-based antioxidants such as vitamins, phenolics, flavonoids, tannins and carotenes have been found to play an essential role in preventing oxidation by reacting with free radicals and protecting cells from damage without causing any side effects [4, 6]. Prior research has shown the positive effects of taking high dietary natural antioxidants on chronic heart diseases and cancer. As a result, a substantial increase in demand for natural antioxidants in pharmaceuticals, nutraceuticals and food additives.

In recent years, growing trends towards organic foods as consumers become increasingly aware of food safety concerns. This has led to a ban on certain pesticides in agriculture and a renewed interest in plant pesticides due to their efficacy, biodegradability and lower toxicity [7]. *Bougainvillea glabra* plant belonging to the Nyctaginaceae family, is one such species that has not been fully exploited. It is a notorious ornamental plant is native to tropical, subtropical and temperate regions. Extracts of *B. glabra* have been used by traditional healers for centuries to treat various ailments such as anti-diabetic, hepatoprotective, insecticidal, anti-inflammatory, anti-diarrheal, anti-ulcer and anti-microbial agents [8,9,10,11].

In the estimated 500,000 higher plants, 6-15% of plant's biological activity and phytochemicals were evaluated [12]. Here, we investigate the antimicrobial activity of *B. glabra* flower extract against a panel of standardized bacteria. Additionally, the antioxidant properties of the methanol crude extract of the plant will be examined.

Methods

Plant and preparation of crude extract

B. glabra flowers were collected from Tirupati, Andhra Pradesh, India and washed the flowers with water, air dry for a week. The dried flowers were pulverized using a mixer grinder to create a fine powder. The 10 g powder was macerated with 100 ml of methanol for 48 hours at room temperature. The solution was filtered through Whatman No. 1 grade filter paper and the filtrate was concentrated by a rotary evaporator at 40 °C. The dried crude extract was weighed and stored at 4 °C for further use.

Phytochemical analysis

The methanolic extracts of the plant were evaluated for the presence of alkaloids, flavonoids, reducing sugars, saponins, phenolic compounds, tannins, proteins and amino acids using standard methods [13].

Antimicrobial susceptibility assays

The antimicrobial activity of *B. glabra* was evaluated against microorganisms including *Staphylococcus aureus, Bacillus subtilis, Pseudomonas putida, Escherichia coli, Candida albicans* by disc diffusion method [14]. Muller-Hinton agar media were prepared and poured into sterilized petri dishes under aseptic conditions. Whatman filter paper discs were impregnated with a 1 mg/ml solution of the *B. glabra* extract and placed on the agar plates. The plates were incubated

at 37 °C for 12-48 hrs. After incubation, the zones of inhibition were measured in millimetres around the filter paper discs, indicating the inhibition of microbial growth.

Antioxidant assays

1, 2-Diphenyl-2-picrylhydrazl (DPPH) scavenging activity

The antioxidant activity of *B. glabra* was evaluated using DPPH radical scavenging assay. The assay was performed as previously described by Ebrahimzadeh method [15]. Different concentrations (0.025-0.5 µg/mL) of the extract were added to a methanolic solution of DPPH (100 µM). The mixture was kept in the dark for 30 minutes. Vitamin C and Rutin were used as standard controls, and three replicates were made. The absorbance (A) was measured at 518 nm, and the percentage antioxidant activity was calculated using the following equation: % Scavenged [DPPH] = [(Ao − A1)/Ao] × 100, where Ao is the absorbance of the control and A1 is the absorbance of the extract and standard.

Xanthine oxidase superoxide (XOD) scavenging activity

The scavenging activity of the *B. glabra* flower methanol extract against superoxide free radical anions was evaluated using a spectrophotometric assay as previously described by Vimala et al [16]. The assay protocol was performed in accordance with the methodology outlined in the aforementioned study.

Ferric Reducing Antioxidant Power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) assay was executed using the methodology reported by Musa et al [17]. The methanol extract of the plant in distilled water were mixed with 2.5 ml of a 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1% w/v). The mixture was then incubated at a temperature of 50°C for 20 minutes. Afterwards, 2.5 ml of tri chloro acetic acid (10% w/v) was added to the mixture. The solution was centrifuged at 3000 rpm for 10 minutes. From the supernatant, 2.5 ml was mixed with an equal volume of distilled water and 0.5 ml of $FeCl_3$ (0.1% w/v) was added. The absorbance was measured at wavelength of 700 nm.

Result and Discussion

Phytochemical Analysis

Secondary metabolites play a significant role in the biochemistry of plants. The results of phytochemical screening of the extracts revealed presence of various secondary metabolites including tannins, steroids, flavonoids, Phlobatannin, quinones, phenols, caumarins and alkaloids (Table. 1).

Table 1 Phytochemical constituents of flower extract of B. glabra

S.No.	Phytochemicals	Flower extract	Test
1.	Alkaloids	+	Mayer's test
2.	Flavonoids	+++	Ethyl acetate test
3.	Glycosides	++	Borntranger's test
4.	Terpenoids	+++	Salkowski test
5.	Saponins	+	Frothing test
6.	Steroids	+++	Liebermann–burchard test

7.	Tannin	++	Ferric chloride
8.	Quinones	+	Sulphuric acid
9.	Phenols	+++	Ferric chloride test
10.	Coumarin	-	Sodium hydroxide
11.	Anthocyanin	+++	Sodium hydroxide

Heavily present, + + +; Slightly present, ++; Present, +; Absent, –

The present study highlights the potential of various phytochemicals as antioxidants. Alkaloids are secondary nitrogenous compounds, have antioxidant properties and utilized in traditional medicine [18, 19]. Terpenoids predominantly present in plants, reported to protective effects against oxidative stress-induced diseases [20, 21]. Saponins antioxidant properties reported to induce apoptosis in tumor cells [22]. Tannins found in medicinal products and beverages, possess antioxidant properties and act through the donation of hydrogen and chelation of metal ions such as Fe (II), Zn (II), and Cu (II) [23]. Glycosides including quercetin monoglycosides, diglycosides, and flavonol glycosides have strong inhibitory effects on lipid peroxidation [24]. Plant-derived quinones shown to exhibit superior antioxidant activity compared to synthetic antioxidants and possess superoxide scavenging activity. The anti-tumor, antiparasitic, and cytotoxic activities of quinones are ability to engage in redox cycling of free radicals. Anthraquinones have various biological activities, including antimicrobial, antioxidant, anti-inflammatory, and anticancer properties [25]. Steroids as electron donors, act upon free radicals and convert them to more stable compounds, thus terminating the chain reaction [26].

Antimicrobial activity

The efficacy of flower extract against the microorganism was evaluated using the in-vitro assay. The antimicrobial properties of the extracts were determined by measuring the growth on nutrient agar amended with flower extract at concentrations of 25mg/ml. Results showed that microbial growth was lower in flower extract compared to the control and methanol at 12, 24, 36 and 48 hrs after inoculation (Figure.1). These results suggest that flower extract have antimicrobial properties that can be utilized for controlling *Staphylococcus aureus, Bacillus subtilis, Pseudomonas putida, Escherichia coli, Candida albicans*. *S. aureus* showed highest sensitivity with zone of inhibition ranging from 17-22 mm, while *C. albicans* showed lowest sensitivity with a zone of inhibition range of 0-6 mm. Additionally, the sensitivity and susceptibility of microbes to the plant extracts varied. The presence of peroxidases and various phytochemicals in the extracts may contribute to their greater antimicrobial activity. The mechanism of antimicrobial activity could be related to the dimerization of phenols by peroxidase oxidative activity, leading to the production of toxic products for the pathogen.

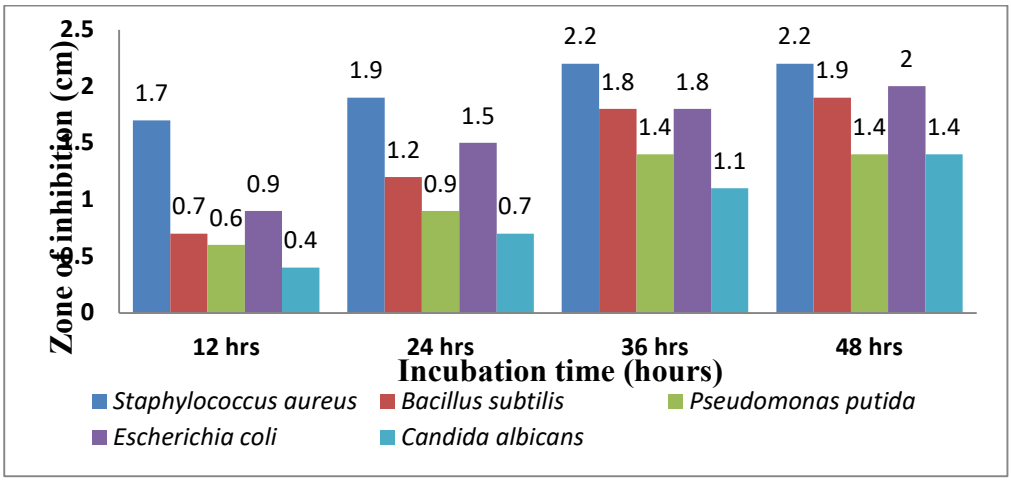

Figure 1 Antibacterial activity of flower extracts of B. glabra after 12, 24, 36 and 48 hrs days of inoculation

Antioxidant activity

The antioxidant properties of *B. glabra* flower extract were evaluated using three bioassay as shown in Table 2. The *B. glabra* flower extract displayed high antioxidant activity in all three pathways, with an IC50 of 0.064 mg/mL for DPPH free radical scavenging activity, 0.97 mg/mL for superoxide radical scavenging activity, 134.71±0.97 mg TE/100 g of extract for FRAP. These results demonstrate the high natural antioxidant potential of *B. glabra* flower as a plant source.

Table 2 Phytochemical composition and antioxidant activity of B. glabra flower extract

Assay	Phytochemical composition
Total phenol content (mg GAE/g)	727.44± 2.43
Total flavonoid content (mg QE/g)	262.30 ± 24.49
Total Betacyanins content (mg/L)	11.98 ± 0.24
Total Betaxanthins (mg/L)	7.12 ± 0.89
Antioxidant activity	
DPPH radical scavenging activity (IC50mg/mL)	0.064±0.32
Xanthine oxidase superoxide scavenging activity (IC50 µg/mL)	0.97±0.34
Ferric reducing antioxidant power (mg TE/100 g)	134.71 ± 0.97
Oxygen radical absorbance capacity M TE/100 g	201.40 ± 1.8

DPPH is a stable free radical and the free radical reducing activity of antioxidants is based on one electron reduction. The DPPH free radical scavenging activity of *B. glabra* flower reflects its effectiveness in preventing, intercepting and repairing injury in a biological system.

Superoxide anions are by-products produced during protein and cell metabolism and biochemical functions. Over production of superoxide anions can lead to cellular damage and various diseases and aging. The high superoxide anion scavenging activity of *B. glabra* flower can

be attributed to both neutralization of superoxide anion radicals via hydrogen donation and inhibition of xanthine oxidase by various phenolic compounds present in the extract.

FRAP measures the second line of antioxidant defense, where reductive antioxidants scavenge active free radicals before they can attack target molecules. The results showed that flower extract has potent FRAP, which help prevent oxidative stress-related tissue damage. The second line of antioxidant defense aims to prevent the generation of ROS, destroy potential oxidants, and scavenge ROS, thereby minimizing oxidative stress-induced tissue damage.

Conclusion

The study investigated the antioxidant and antimicrobial properties of methanolic extracts of flower of *B. glabra*. The results showed the presence of various phytochemical components in both extracts, with varying concentrations affecting their antioxidant and free radical scavenging activities. The correlation between phenolic contents and free radical scavenging activities was observed to be positive. In vitro assays indicated that the extracts had the potential to act as direct antioxidants through free radical scavenging or as indirect antioxidants through inducing enzyme systems responsible for antioxidant activity. Further research is needed to isolate and identify the active antioxidant and antimicrobial compounds present in the crude extract.

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