THE PHYTOCHEMICAL AND ANTIBACTERIAL SCREENING OF THE SPECIES OF *BEGONIA*

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Summary

The antibacterial activity of the leaves extract of *Begonia albo-coccinia* (Hook.)*, B. cordifolia* (Wight) Thwaites*, B. dipetala* R.Grah.*, B. fallax* DC., and *B. floccifera* Bedd., were determined by disk- diffusion method against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa.* The best results of *B. fallax* against *Staphylococcus aureus* and *Pseudomonas aeruginosa* were observed and also similar results showed in *B. floccifera*. The antibacterial activity was observed might be due to the active constituents of alkaloids and anthraquinones found to be in the all species of *Begonia*.

Keywords: Begoniaceae; Begonia; leaves; extract; phytochemicals; antibacterial activity

Introduction

According to world health organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs. An average of 25% of prescription drugs sold in the USA during the period 1959-1973 contained active principles extracted from higher plants [1]. Many of these are derived from the same source as those used in traditional medicine. On a global scale, 74% of these chemicals have similar or related uses in traditional medicine [2]. The phytochemical research based on tribal and traditional knowledge is considered an effective approach in the discovery of new several effective drugs from higher plants [3].

Begonia L. (Family: Begoniaceae) is a large genus of about 1,400 species widely distributed in tropical and subtropical areas, especially in Central and South America [4-5]. The leaves of *Begonia* species are used for the treatment of respiratory tract infections, diarrhoea, blood cancer, and skin diseases and anti HIV activity [6-7]. There is no report in the literature about the phytochemical and pharmacological activities of *Begonia albo-coccinia* (Hook.)*, B. cordifolia* (Wight) Thwaites*, B. dipetala* R.Grah.*, B. fallax* DC., and *B. floccifera* Bedd. The present study is aimed to antibacterial activity and phytochemicals identification of the species of *Begonia*.

Materials and Methods

Collection of Plant Materials

Plant materials of *Begonia albo-coccinia* (Hook.)*, B. cordifolia* (Wight) Thwaites*, B. dipetala* R.Grah.*, B. fallax* DC., and *B. floccifera* Bedd., were collected from the Kalakad Mundanthurai Tiger –Reserve forest (KMTR), Tirunelveli District, Tamil Nadu,South India (Table-1).

Solvent Extraction

100 gms of dried and powdered leaves of *Begonia albococcinia, B. cordifolia, B. dipetala, B. fallax* and *B. floccifera* were separately extracted in a Soxhlet apparatus. 70% ethanol was used to extract the samples successively. Extracts obtained were concentrated by rotary vacuum evaporation at 50°C, then further dried under vacuum at ambient temperature using a vacuum oven. Dried extracts were stored in sealed vials at 4°C until used for further analysis.

Determination of Alkaloids

5ml of the test solution was treated with a few drops of Dragendroff's reagent (Mix 2g of bismuth subnitrate, 25mL of acetic acid and 100 mL of water (a). Dissolve 40g of potassium iodide in 100mL of water (b). Mix 10 mL of (a) and 10 mL of (b), 20 mL of acetic acid and 100 mL of water). Another aliquot (1 mL) was treated with a few drops of Mayer's reagent (Dissolve 1.36g of mercuric chloride in 60 mL of water. Also prepare a solution of 5g potassium iodide in 20 mL of water. Mix both solutions and make up to 100mL with water). The formation of a precipitate was seen as indicating the presence of alkaloids [8-9].

Test for Phenolic Compounds

One millilitre of test solution was treated with 10% ethanolic ferric chloride. Phenolic compounds were considered present when a colour change to blue green / dark blue was observed [8-9].

Test for Flavonoid

Four milliliters of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.

Test for Anthraquinones

The Borntrager Test was used for the detection of anthraquinones. Two millilitre of the test sample was shaken with 4 mL of hexane. The upper lipophilic layer was separated and treated with 4 mL of dilute ammonia. If the lower layer changed to violet to pink was indicated the presence of anthraquinones [8-9].

Test for Steroids

One millilitre of the respective plant extract was treated with three drops of acetic anhydride and one drop of concentrated sulphuric acid. A colour change from deep green turning to brown to dark brown, indicated the presence of sterols [8-9].

Cardiac Glycosides

5mL of sample solution was mixed with 1ml of glacial acetic acid then treated with one drop of 5% ethanolic ferric chloride solution. 1ml of concentrated sulphuric acid (H_2SO_4) was carefully poured down the side of the test tube. The appearance of a brownish ring between the two formed layers with the lower acidic layer turning blue green upon standing indicated the presence of cardiac glycosides [8-9].

Test for Saponins

1ml aliquots of the various plant extracts were combined with 5 ml of 60^{o}C water, then shaken for 2min. As saponins are known to possess frothing activity, the volume of froth produced in this experiment was observed and recorded every 10 min for a total period of 30 min.

Detection of Anti-bacterial Activity

Staphylococcus aureus, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were used in the study. All cultures were from the collection of IMTECH, Chandicharh, India. 0.1ml of 1g/L agar media containing 30ml of overnight culture of each microorganism was overlaid on the plates containing 20ml of sterilized nutrient agar. The agar disk-diffusion method was used to detect anti-bacterial activity. Plates injected with bacteria were incubated at 37±1°C for 24h) after this period; inhibition zones were measured and recorded in mm.

Results and Discussion

The extracts yields expressed in relation to dry weight plant material are shown in Table -1. The phytochemical constituents of the plants are seen in - Table 2. The highest yield of ethanolic extract found in *B. floccifera* (4.52% w/w) and lowest in *Begonia albococcinia* (2.24% w/w). From the phytochemical screening, more amounts of alkaloids and anthroquinone were found in *B. floccifera* and *B. fallax*; and glycosides in *B. albococcinia*, *B. cordifolia.* Steroids were trace amount observed in *B. albococcinia, B. cordifolia, B. dipetala, B. fallax* and *B. floccifera*. Flavonoids were found in *B. cordifolia* and *B. dipetala*. Glucosides were found in all the *Begonia* species but saponins are complete absent (Table-2).

The results show an antibacterial effect of the extract of *Begonia albococcinia, B. cordifolia, B. dipetala, B. fallax* and *B. floccifera* on the microorganisms (Table-3). All the extracts were active against the zone of inhibition ranges from <6 to 10 mm, 11 to 15 mm and >16mm above for resistant, intermediate and sensitive bacteria. *B. floccifera* and *B. fallax* showed strong activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Table-3). The present study was observed that zones of inhibition ranging from 6-10mm for the leaves extract of *B. albococcinia, B. dipetala* and *B. floccifera* against *Bacillus subtilis,* while *B. cordifolia* and *B. cordifolia* were active against *Staphylococcus aureus*. Leaves extract of *B. albococcinia, B. cordifolia* and *B. dipetala* were inhibit the growth of the gram-negative *E. coli*. This shows that the sensitivity of the bacteria to most of the active extracts could be considered as intermediate, with high sensitivity to two extract, *B. floccifera* and *B. fallax*.

Table-1: Extractive values and collection of locality of *Begonia* species

Plant Name	Extracts (%)	Collection Locality
B. albococcinia	2.24	Kannikatti
B. cordifolia	3.25	Kannikatti
B. dipetala	2.84	Inzhikuzhi
B. fallax	4.12	Monjolai
B. floccifera	4.52	Monjolai

Table - 2: The phytochemical identification of the species of *Begonia*

Active constituents	Plant Name				
	B. albococcinia	*B. cordifolia*	*B. dipetala*	*B. fallax*	*B. floccifera*
Alkaloids	+	+	+	+++	+++
Phenolic compounds	+	+	++	+	+
Flavonoids	+	++	++	+	+
Anthraquinones	++	+	+	+++	+++
Glycosides	++	++	++	++	++
Steroids	+	+	+	+	+
Saponins	-	-	-	-	-

+ trace level; ++minor level; +++ major level

Table-3: Antibacterial activity of leaves extract of the species of *Begonia*

Tested bacteria	Plant Name				
	B. albococcinia	*B. cordifolia*	*B. dipetala*	*B. fallax*	*B. floccifera*
Staphylococcus aureus	++	+	+	+++	+++
Bacillus subtilis	+	++	+	++	+
Escherichia coli	+	+	++	+	++
Pseudomonas aeruginosa	++	++	+	+++	+++

"+ " Resistant 6-10mm; "++"intermediate11-15mm; "++ +" more sensitive 15 mm above

The main active constituents of luteolin, quercetin and *β*-sitosterol were isolated from the leaves of *B. malabarica* [6] and also cucurbitacin D, hexanorcucurbitacin D, cucurbitacin B and dihydrocucurbitacin B isolated from the roots of *B. parviflora* L.[10]. Begonanline, nantoamide, methyl (S)-glycerate, cucurbitacin B, dihydrocucurbitacin B, cucurbitacin E, dihydrocucurbitacin E, cucurbitacin I, and (-)-auranamide; 3β,22α-Dihydroxyolean-12-en-29-oicacid; indole-3-carboxylic acid, 5,7-dihydroxychromone, and (-)-catechin [7] have been found from different species of *Begonia*, which shows that this genus contains a broad range of different antimicrobial compounds.

Conclusion

In view of these results, it is concluded that good antibacterial activity was observed the leaves of *Begonia* species. The observed activity might be due to the alkaloids and anthraquinones found to be in the *Begonia* species. It is important to point out that work is in progress to isolate and to characterize the active compounds present in leaves extract of the species of *Begonia*.

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