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Screening of Bioactive compounds and antimicrobial properties from plant extracts of Biscofia javanica

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Abstract

Phytochemicals are primary or secondary product of Plant's metabolism. They have many biological activities in the plant including role in plant growth and defense against competitors, pathogens or predators. These bioactive compounds of plants are the core of the traditional ethnic medicine practices among many tribes. Biscofia javanica, commonly known as Uriam is a tree in tropical areas of the world. The leaves of Uriam have been used by the people of Tai-Phake community of Assam against different stomach ailments. Leaves have been traditionally used for their daily meal and perceived to have therapeutic value among this community. Current investigation evaluated the bioactive compound present in the leaves Biscofia javanica. The leaves were collected from Nam-Phake village of Assam. Leaves were air-dried at 25°- 30°C for 2 weeks before grinded into powder. The smooth leaf powder was used to prepare the extract by using water, ethanol and the combination of both water and ethanol (50%) solvents. Presence of phytochemicals was tested using biochemical tests. Saponin, Steroids, Glycosides, Terpenoids, Phenols, Tannins, Flavonoids, Proteins, Carbohydrates were found to be present. Antimicrobial activities of different solvent extracts of Biscofia javanica were tested using Agar well diffusion method and highest zone of inhibition was measured against test organism. Our study was a very small attempt to validate their knowledge with scientific approach. Further studies will be able to reveal the mechanism of its therapeutic activity and its broad range antimicrobial properties against other human pathogen.

Keyword: Phytochemicals, Traditional knowledge, Tai-Phake community, Antimicrobial property

1. Introduction

The resistance of microorganisms to antimicrobial drugs is one of the world's current challenges in public health. Phytochemicals act as natural antimicrobials with antioxidant activities and often devoid of the many side effects associated with synthetic antimicrobials. Phytochemicals play a very important role in plant growth or defense mechanism. Phytochemical molecules can be vitamins, terpenoids, phenolic acids, lignins, tannins, flavonoids, quinones, coumarins, alkaloids, amines, and other metabolites, which are rich in antioxidant activity [1]. Medicinal plants contain a mixture of several chemicals that act synergistically while medicines contain one active substance. Medicinal plants also contain a large number of vitamins and minerals which are assimilated by the human body very easily. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities [2]. The use of plants as medicine is a traditional practice common to all the Asian communities. The basis of traditional ethnic

medicine is the bioactivity of Phytochemicals of the indigenous plants of a particular area.

1.1 The Plant-*Biscofia javanica*

Bischofia javanica or bishop wood, belongs to the family Phyllanthaceae. It was originated from West Africa. *Bischofia javanica* and *Bischofia polycarpa* are the two members of the genus Bischofia. The tree is commonly known as 'Uriam' in Assam and used by Tigers to scratch-mark their territory in the jungles of Assam. Several parts of the plant are used traditionally in the treatment of certain disease particularly stomach ailments. *B.javonica* is popular among members of Tai-Phake tribe for its many medicinal properties. Traditionally stem of *Bischofia javanica* is used in the treatment of diarrhea. The young stem is used against stomach ache. The leaves are used in the treatment of diarrhea. The leaves are also used to treat burns and ulcers. Young leaves and Buds are used in tonsilites and for the treatment of throat pain. This proposed study aims at documentation and scientific study of the phytochemical and antimicrobial properties of *Biscovia javanica* .

1.2 Current Investigation

There are no extensive reports on the presence of phytochemical compounds from the leaves of this plant. In this investigation, the in vitro antimicrobial effects of crude leaf extracts of this plant against the test organism found responsible for ailments aforementioned [3]. The proposed work is based on traditional knowledge of Tai-Phake community regarding the use of *Bischofia javanica* through a comprehensive approach that would consider the potential application of the phytochemicals in the formulation of drugs [4]. The screening of bioactive compounds was done using biochemical tests. Saponin, Steroids, Glycosides, Terpenoids, Phenols, Tannins, Flavonoids, Proteins, Carbohydrates were found to be present. Antimicrobial activities of different solvent extracts of *Biscofia javanica* were tested using Agar well diffusion method and the highest zone of inhibition was measured against test organisms.[5]

2. Methodology

The leaf of *Bischofia javanica* was collected from Nam-Phake village, the largest village of Tai-Phake at Naharkatia in Saraidew District of Assam. The leaves of *B. javanica* was dried under shade and pulverized into fine powder. About 300 g of the powdered form was extracted with 95% (v/v) ethanol in H_2O .Water and ethanol extract was prepared by mixing powder (10gm) of *Bischofia javanica'*s leaves in 100 ml of each solvent (distilled water and ethanol) under magnetic agitation for 8 hours at room temperature.[5] The homogenate was filtered two times on filter paper. Phytochemical screening was done with all three extracts (0.05 g/ml) following standard methods for detection of steroids, saponins, alkaloids, protein, carbohydrate, flavonoid, terpenoid, phenol and glycosides.

Steroids: 5mL of chloroform and 5 ml of Sulphuric acid were added to 500 µl of the plant extract. The presence of steroids was indicated by formation of a ring of blue/green. [6]

Saponins: 3 ml of plant extracts were added to 3 ml of distilled water and shaken vigorously. The presence of saponin was indicated by formation of a stable persistent froth. [7]

Alkaloids: A mixture of 3 ml of plant extract and 3 ml of 1% HCl was heated for 20 min. The mixture was then cooled and used to perform the following tests:

Mayer's test: 1 ml of Mayer's reagent was added drop by drop to the filtrate in test tube. The formation of a greenish coloured or cream precipitate indicated the presence of alkaloids.

Dragendoff's test: 1 ml of Dragendoff's reagent was added drop by drop to the filtrate in test tube. The formation of a reddish-brown precipitate indicated the presence of alkaloids.[8]

Wagner's test: 1 ml of Wagner's reagent was added drop by drop to the filtrate in tube. A reddish-brown precipitate indicated the presence of alkaloids.

Protein: Xanthoproteic test: To 2 ml of plant extracts, few drops of nitric acid were added. Presence of protein was indicated by a colour change to yellow.

Carbohydrates: Fehling Test: Dilute HCl was added to 2 ml of each plant extract and neutralized with alkali. Then the mixture was heated with Fehling's solution A and B. Formation of a red

precipitate indicated for the presence of a reducing sugar.[9]

Flavonoid

Alkaline reagent test: For flavonoid test, 3 ml of sample plant extract was treated with 1 ml of 10% NaOH solution. The formation of an intense yellow colour indicated the presence of flavonoids.[9]

Terpenoids

A mixture of 2 ml of chloroform and 3 ml of H_2SO_4 were added to 5 ml of plant extracts. Formation of reddish-brown coloration indicated presence of terpenoids.

Phenols and tannins: Ferric chloride test:

To 1 ml plant extract, 2 ml of 2% solution of $FeCl_3$ were added. Formation of black or blue-green colour indicated the presence of tannins and phenols. [10]

Test for Glycosides: Liebermann's Test-2ml of sample extract is taken in test tube and mixed with 2ml of $CHCl_3$ (Chloroform) and 2ml of Acetic acid (CH_3COOH).

Appearance of violet to blue or green coloration shows the presence of glycosides.

Antimicrobial Tests: Antimicrobial activity of each plant extract was determined using Agar disc diffusion method [11]. The antibacterial activity of three extracts was studied on gram positive and gram negative bacterial strains, *Staphylococcus aureus*, and *Escherichia coli*. The inhibition zone was then measured from the diameter of the clearing zone in millimeters. Under aseptic condition, four perforations were prepared on petri dish which was previously inoculated with the culture of test organism. 25 µl of *Bischofia javanica*'s extract solution was pipetted in the hole. Inoculated petridishes were kept for 20 minutes at room temperature before incubation at 37°C for 24 to 48 hours. After the incubation period, the dishes were examined for inhibitory zones. All three solvent extracts were used for the determination of antibacterial activity. Standard antibiotics, Penicillin (10 µg/ disc), Ciprofloxacin (10 µg/disc,) served as positive controls for antimicrobial activity. Filter discs impregnated with 10 µl of distilled water were used as a negative control. [12]

Result and Discussion

Presence and absence of the bioactive compounds in the leaf extracts of *B.javanica* were recorded as given in the Table no: 1. Phytochemical Tests for Glycosides, Tannins, Sapponins and Flavonins were found to be positive in the extract prepared with ethanol. Steroid, Saponins, Phenol, Flavonids and Tarpenoids , Tannins ,Glycosides were found to present in the Water and Ethanol extract along with protein and carbohydrate.

Table 1: Phytochemical screening of crude leaf extract of *Bischofia javanica*

Sl.no	Compounds	Test performed/Reagent used	Observation	*Result
1)	Carbohydrates	Fehling's test	Yellow colour observed	+
2)	Steroids	Sulphuric acid	colour change from violet to blue or green.	+
3)	Phenol	Ferric chloride test	black or blue-green	+
4)	Glycosides	Liebermann's test	violet to blue or green colorati on	+
5)	Saponnins	Foam test	Turbidity obtained	+
6)	Terpenoids	Salkowski test	reddish-brown colorati on	+
7)	Flavonoids	Alkaline reagent test	formatio n of an intense yellow colour	+
8)	Tannins	Ferric chloride test	Dark blue to grrenish colour	+
9)	Protein	Xanthoprotei c test	Reddish black not seen	+

*Sign + indicates presence and – indicate absence

The antimicrobial test was done where *E. coli* (ATCC-10536.) and *Staphylococcus aureus* (ATCC-BAA-1026) strains were used to screen the antimicrobial properties of leaf extracts of

B.javanica. It was seen that zone of inhibition was directly proportional to concentration of the extract. It was also observed that plates with ethanol extract had highest zone of inhibition. The zone of inhibition was checked by measuring the radius of the zone with a measuring scale in each plates and it was found that the plate with 100 µl extract in the agar well has radius of 20.15 mm which was highest than others.

Table 2: Measurement of zone of inhibition for screening of Antimicrobial activity

Sl. no	Name of the compounds and their concentrations	Antibacterial inhibition zone(mm)	
		E.coli	*Staphylococcus aureus*
1	Standard antibiotic I(Ciprofloxacin)	37	-
2	Standard antibiotic II(Penicillin)	-	40
3	Plant extract 25µl	11.2	12.8
4	Plant extract 50µl	13.91	18.05
5	Plant extract 100µl	19.20	20.45

Current study showed that Bioactive compounds such as steroids, saponnins, terpenoids, phenols flavonoids, proteins, tannins, glycosides, and carbohydrates were present in leaf extracts of *B.javanica.*(Table no.1). As it is a common forest tree, it is very important to make people aware about its therapeutic properties and popularize its use among non consumers and initiate a conservation process. Phytochemicals which were found in leaf extracts of *B.javanica* viz alkaloids, saponin, steroides, terpenoids contribute to its therapeutic properties against a wide range of microorganisms. Both Gram-positive and Gram-negative bacterial strains were selected for screening antimicrobial impact. [14] Results of this study show that the ethanolic extracts of the leaf of *B.javanica* produced well defined zone of inhibition against both Gram-positive bacteria and Gram-negative bacteria in agar well diffusion method. The practical exhibition and explanation of antimicrobial activity against both Gram-positive and Gram-negative bacteria is an indication of presence of broad spectrum antimicrobial compounds in the extracts .We can conclude that ethanolic leaf extracts of *B.javanica* have an influencing antimicrobial activity.

Conclusions

The present study justifies the claimed uses of leaves of *B.javanica* in the traditional system of Tai-Phake community to treat various stomach ailments. Future studies to determine other pharmacologically active compound by preparing plant extracts with other solvents should be carried out. Also studies on its effect on causative organism of various diseases will be beneficial .The Bioactive compounds present in *Biscovia javanica* suggests that the plant has therapeutic value. The quantitative analysis of these bioactive compounds needs further studies.The use of other plant parts viz roots and bark need further investigation to exploit the potential biomedical applications of *B.javanica*. This plant species is not much known to the outside world as a source of therapeutics yet. In the current investigation ethanolic extracts of *B.javanica* has been selected after among water, ethanol and water: ethanol extracts for better results. The ethanolic extracts of *B.javanica* were found to be active on test organisms as compared to standard drugs. Detailed studies are required to evaluate the potential effectiveness of the crude extracts of this plant as the antimicrobial agents.

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