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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING OF AQUEOUS EXTRACT OF *FICUS BENGALENSIS* LEAF BUD

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ABSTRACT

Biologically active compounds present in plants are called phytochemicals. Phytochemicals are produced by plants as part of their defense mechanisms against various stresses, such as pathogens, predators, or environmental factors. These can be derived from barks, leaves, flowers, roots, fruits and a seed, knowledge of the chemical constituents of plants is desirable because they are used as direct sources of medicinal agent and such information will be valuable for synthesis of complex chemical substances. *Vata* (*Ficus bengalensis*) is such a herbal drug which is widely available and is having many mentioning in the classics for its effectiveness in *Stree Vandhyatwa*. Its vegetative buds are used in the treatment of *Vandhyatwa*. This paper mainly deals with the collection of leaf bud of *Ficus bengalensis,* and qualitative screening of the aqueous extract for the available phytochemicals. The extract of leaf bud was tested for the presence of tannins, flavonoids, phenols, glycosides, proteins, saponins and steroids by using standard protocols.

INTRODUCTION

Ficus bengalensis, also known as the banyan tree, is a species of fig that is widely used in traditional medicine for various ailments. Several studies have reported the phytochemical screening of different parts of *Ficus bengalensis*, such as fruits, leaves, and stem, using different solvents, such as methanol, chloroform, aqueous, etc. The results showed that *Ficus bengalensis* contains various phytochemicals, such as tannins, flavonoids, steroids, saponins, phenolics, etc., in varying amounts depending on the solvent and the plant part used. These phytochemicals may be responsible for the pharmacological effects of *Ficus bengalensis*, such as antioxidant, antimicrobial, anti-diabetic, etc.

According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of Individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, scientific research and countless other areas. Active constituents from plants are isolated and being used for diagnosis, treatment, mitigation, and prevention of various diseases, but many crude drugs are also in use. *Ficus bengalensis* L. is one of the most important plants of traditional medicines and is still in use, to treat various diseases, particularly diabetes, reproductive system disorders, inflammatory conditions and abscesses. Because of its importance in traditional medicines, its quality control parameters are established by pharmacognostic studies and various phytochemicals have also been isolated and

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identified. Pharmacological studies on various parts of the plant have verified its use in traditional medicines. Many aspects of this plant are to be uncovered, for example, toxicity studies, proper dose for a particular disease when the plant is used in crude form, isolation of further phytoconstituents, synergistic studies, drug-drug interactions etc. Phytochemicals have attracted considerable attention in recent years due to their potential health benefits, such as antioxidant, antimicrobial, anti-inflammatory, anti-cancer, etc. However, the phytochemical composition and activity of plants may vary depending on several factors, such as plant species, plant part, solvent used for extraction, etc. As a part of another clinical study, preliminary phytochemical screening of plant extract was done to identify the presence and quantity of various phytochemicals and to evaluate their biological activities.

The phytochemical profile and activity of *Ficus bengalensis* leaf bud, which is a young and tender part of the leaf, have not been explored yet. Therefore, the aim of this study was to perform preliminary phytochemical screening of aqueous extract of *Ficus bengalensis* leaf bud.

MATERIALS AND METHODS

Collection of Leaf Bud

The vegetative bud of *Ficus benghalensis* were collected from Gurukula Kangri (Deemed to be University) campus and Rishikula Ayurvedic Govt. College, Haridwar.

Authentication of Drug

Taxonomical identification and authentication of the plant were done by Dr.D.C.SINGH (H.O.D of Dravyaguna) Rishikul Campus on 14/07/2022 as per Reference No. DG/RC/UAU-86 of the herbarium section of Rishikul Ayurveda Govt College, Haridwar, India.

Preparation of Drugs

The leaves were washed, cleaned and shade-dried for three weeks. Thereafter, they were ground to coarse powder using an electrical grinder.

Place of Study

Under the guidance of Dr. M. K. Malik, Ph. D, AMRSC, Asst. Prof., Department of Chemistry, Gurukula Kangri (DTU), Haridwar.

Extraction of Drugs

The *Ficus benghalensis* vegetative bud (FBVB) was cleaned thoroughly using double distilled water and semidried in shade. Semidried leaves were stored in an air tight container in deep freezer at -15°C. Aqueous extract of the semi-dried, upper layer peeled off *Ficus benghalensis* vegetative bud was prepared by soxhlet apparatus. The aqueous extract was collected and stored in air tight, sterilized reagent bottles in a refrigerator at 4°C till further used.

Preliminary Phytochemical Screening

Qualitative Screening

Qualitative screening of aqueous extract *Ficus bengalensis* bud was conducted using standard procedures. The extract was tested for the presence of tannins, flavonoids, phenols, glycosides, saponins and steroids using standard protocols. All chemicals used were of analytical grade and were used as received without any further purification and were obtained from Himedia and CDH India. All solutions were prepared with double distilled water.

Test for Tannins[1]

The test method suggested by Ejikeme 2014 and Ugochuhwu 2013 were used to confirm the presence of tannins in sample. Briefly, 10ml of bromine water was added to the 0.5 g FBVB aqueous extract. Decoloration of bromine water showed the presence of tannins. Further, 1ml of freshly prepared solution of 3% $FeCl_3$ was added to 1ml of the extract. Brownish green color development indicated the existence of the tannins.

Tests for Flavonoids[2] (Nanna 2013, Pamar 2012)

- **Shinoda Test:** To 1ml FBVB extract few pieces of magnesium ribbon was added followed by addition of 3 drops of concentrated hydrochloric acid development of pink color showed the existence of flavonoid.
- **Alkaline Reagent Test:** Alkaline reagent test was also performed to assess the presence of flavonoids in the FBVB aqueous extract. NaOH (2.0%, 2ml) was mixed with crude extract (1ml). Presence of intense yellow color on addition of alkaline reagent to aqueous extract indicates the presence of flavonoids.

Tests for Glycosides [3] (Jagessar 2017)

- **Liebermann's Test**: To 2ml of FBVB extract 2.0 ml of chloroform and 2ml of acetic acid was added. The aqueous suspension was heated and was then cooled. Further, concentrated sulphuric acid was added to the cooled solution slowly. Change in the color of the suspension to green showed the presence of glycosides.
- **Keller-Kiliani Test**: A solution of glacial acetic acid (1.5ml) with 1 drop of 5.0% freshly prepared $FeCl_3$ mixture was gently mixed with the aqueous plant extract (1ml) and 0.5ml H_2SO_4 concentrated was added to the side of the test tube.
  A positive test demonstrating the presence of glycosides, was indicated by the formation of a brown ring between the layers.

Test for Proteins[4] (Silva 2017)

- **Biuret Test:** 0.5ml of 2% copper sulphate solution, was added to 2ml of FBVB solution containing alcoholic potassium hydroxide solution. The suspension was mixed well and 2 drops of 1% copper sulphate solution was added. The change in the color of the alcoholic layer of the FBVB extract to pink indicated the presence of two or more peptide bond of proteins.
- **Ninhydrin Test**: 0.5ml of 0.1% freshly prepared ninhydrin solution (ninhydrin was dissolved in acetone) was added to 2ml of the neutralized FBVB aqueous extract solution. The mixture was boiled for a minute and was allowed to cool. A positive test demonstrating the presence of proteins was indicated by the change in the color of the solution to purple.
- **Xanthoproteic Test**: 0.5ml of concentrated nitric acid was added to 2ml of the FBVB aqueous extract solution. The contents were boiled and cooled. Appearance of yellow colour indicated the presence of nitro derivatives of aromatic amino acids. To this solution 40% of NaOH was added. A deep orange colour solution indicated the presence of sodium salts of nitro derivatives of aromatic amino acids.
- **Hopkins Cole Test**: 0.5ml of glacial acetic acid was added to 0.5ml of the FBVB aqueous extract and mixed well. To this 0.5ml concentrated sulphuric acid was added carefully along the sides of the test tube. The formation of violet ring in the junction of two liquids indicated the presence of indole group of tryptophan.

Test for Detection of Phytosterols [5] (Singh 2017)

- **Salkowski's Test:** 1ml of the FBVB aqueous extract was mixed in 1ml of chloroform thoroughly. To 1:1 solution of aqueous extract of FBVB and chloroform, few drops of the concentrated sulphuric acid was added. The development of wine red colour in lower layer of the test tube indicated the presence of steroidal nuclei.

Test for Saponins [6] (Ezeonu 2016)

- **Foam Test:** Extract was shaken vigorously with distilled water in a test tube. Honeycomb like foam produced, persists for few minutes. It indicated the presence of saponin.

Test for Phenols[7] (Tiwari 2011)

- **Phenol Test:** When 0.1ml of $FeCl_3$ solution was added to 0.4ml of test solution.

  Result: Formation of an intense colour indicates the presence of phenols.

Test for Anthocyanins [8] (Obouayeba 2013)

- **Aqueous NaOH Test:** 1ml aqueous NaOH solution was added to 1ml FBVB aqueous extract solution. The color of the solution turns to violet. This indicated the presence of anthocyanins.
- **Conc. H_2SO_4 Test:** 1ml of conc. H_2SO_4 was added to the 1ml of test solution, formation of yellow to orange colour indicated the presence of anthocyanins.

RESULTS

During the exploration of candidate plant materials for pharmacological activities, the characterization of their chemical nature is essential. Phytochemical screening of the aqueous extract of FBVB showed the presence of several primary and secondary metabolites, or phytoconstituents, which are summarized in Table 1.

Table 1 Phytochemical analysis for the aqueous extract of the FBVB

S.No	Test	FBVB aqueous extract
1.	Test for tannins (a) Bromine water test	 ++
2.	Test for flavanoids (a) Shinoda test (b) Aqueous NaoH solution	 - -
3.	Test for Glycosides (a) Liebermann's Test (b) Keller-Kiliani Test	 + ++
4.	Test for Proteins (a) Biuret tests (b) Ninhydrin test (c) Xanthoproteic test (d) Hopkins cole test	 + + ++ +
5.	Test for detection of phytosterols (a) Salkowski's test	 -
6.	Test for saponins (a) Foam test	 -
7.	Test for Phenols FeCl3 test	 +
8.	Test for Anthocyanins (a) Aqueous NaoH test (b) Conc. H2SO4 test	 -

Explanatory note: '+' Indicate the presence, '-' indicates the absence and '++' indicates the presence with higher concentration.

DISCUSSION

Phytochemical components of plant extracts have been discovered as bioactive substances that may be responsible for the varied actions observed when herbs are used medicinally[9] (Alabri 2014, Aziz 2015). Phenolic chemicals are universally acknowledged as antioxidants and free radical scavengers against oxidative damage. The existence of these chemicals in FBVB extract, like tannins, flavonoids, and phenols may lend credence to its local use for the care of oxidative stress-induced disorders. Tannins have been utilized for centuries to treat diarrhoea, bleeding, and detoxification[10,11] (Afolayan 2010, Mbaebie 2012). Flavonoids are plant secondary metabolites that regulate lipid peroxidation, which is involved in atherogenesis, thrombosis, and carcinogenesis. It has been proven that the pharmacological impact of flavonoids is associated to their antioxidant potential[12] (Okwu 2006). The presence of many active phytochemicals, antioxidants advocate its ethno-pharmacological uses in traditional medicine.

CONCLUSION

It has been estimated that 80% of the populations of developing countries rely on traditional medicines i.e., mostly plant drugs. The experimental material selected for the study was *Ficus benghalensis* leaf bud. The present study was carried out to determine the qualitative phytochemical screening of aqueous extract of *Ficus bengalensis* leaf bud, constituents and the functional groups present in the *Ficus benghalensis* leaf bud extracts. The extract of FBVB, which has been claimed to be commonly employed in traditional systems of medicine, has a significant amount of phytochemicals, according to this study. Nonetheless, additional quantitative chemical experiments are required to isolate and define the structure of the active ingredients. Beside this, invitro and invivo analysis of the aqueous extract and its toxicity analysis is need of the era to establish the hypothesis of traditional use of the plant for *Vandhayatva* treatment. Again, genotoxicity study of this plant should be carried out for safety evaluation.

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