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Original Research Article

Evaluation of Phytochemical Parameters of Herbal Formulation of *Ficus Benghalensis* and *Panax Ginseng*

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ABSTRACT

Panax ginseng belongs to the family Araliaceae. Panax is derived from the Latin word panacea, which refers to its historical usage for many conditions. *Ficus benghalensis* is commonly known as the Banyan tree. This tree is considered to be sacred tree in India. The evaluation of physico chemical parameters of *Panax ginseng* root and *Ficus benghalensis Linn* bark extract was carried out by using different standard methods like determination of ash values and extractive values. Whereas phytochemical investigation was carried out to estimate the presence of sterols, tannins, saponins, and reducing sugars. Results revealed the presence of carbohydrates, glycosides, tannins, saponons, sterols and alkaloids. The petroleum ether extract of the formulation showed the presence of flavonoids which is determined by Shinoda test. The present investigation will be helpful in assessing the quality and purity of a crude drug and laying down pharmacopoeial standards for *Panax ginseng* and *Ficus benghalensis Linn.*

Keywords: *Panax ginseng, Ficus benghalensis Linn,* phytochemical evaluation and physicochemical evaluation.

INTRODUCTION

The plant kingdom is a virtual goldmine of potential drug targets and other active molecules waiting to be discovered. During the last decade, use of traditional medicine has expanded globally and gained popularity. Plants are used as medicine since time immemorial. Plant based drugs are having a revived interest now-a-days because of the awareness of the deleterious effects of modern synthetic drugs. Natural products can play a very crucial role in the pharmaceutical industry as a drug or as drug carrier or bio enhancers or excipients.

It has been estimated that only 10-15% of around 7, 50,000 existing species of higher plants have been surveyed for biologically active compounds. Modern herbal research is focused mainly on an activity guided isolation or bioassay of the phytoconstituents from crude drugs. Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. Medicinal plants can be important source of previously unknown chemical substances with potential therapeutic effects. [1]

Scientific studies on a good number of medicinal plants indicate that promising herbal drugs can be developed for many health problems. Herbalism is a traditional medicine or folk medicine practice based on the use of plants and plant extracts. [2]

The importance of the present study is to assess the purity and quality of the crude drug with the help of the determination of the physico chemical and phytochemical parameters. The physico chemical include determination of ash values and extractive values. Then phytochemical investigation includes estimation of the presence of carbohydrates,

alkaloids, sterols, tannins, saponins, and flavonoids etc.

PANAX GINSENG:

Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs. [3] *Panax ginseng*, used medicinally for thousands of years in China, Korea, and Japan is well known as an adaptogen and a restorative tonic that is widely used in traditional Chinese medicine [4] (TCM) and Western herbal preparations. [5] The common names of *Panax ginseng* is American ginseng, Asiatic ginseng, Chinese ginseng, five-fingers, Japanese ginseng, jintsam, Korean ginseng, ninjin, Oriental ginseng, schinsent, seng and sang, tartar root, Western ginseng. [6] It is used in many conditions like infertility, liver disease, amnesia, colds, menopause, and erectile dysfunction. [7]

It has been used to increase physical endurance and lessen fatigue, to improve the ability to cope with stress, and to improve concentration. It is also used during anemia, diabetes, gastritis, neurasthenia, erectile dysfunction, impotence and male fertility, fever, hangover, and asthma. Panax ginseng is also used for bleeding disorders, loss of appetite, vomiting, colitis, dysentery, cancer, insomnia, neuralgia, rheumatism, dizziness, headache, convulsions, disorders of pregnancy and childbirth, hot flashes due to menopause, and to slow the aging process. It may also improve your overall being. [8]

FICUS BENGHALENSIS LINN:

Ficus benghalensis is commonly known as the Banyan tree. The genus *Ficus* include 750 species of plants occurring in most tropical and subtropical forests throughout the world. The genus is remarkable for the large variation in the habits of its species. [9] Many plants of this genus are used in medicine for the treatment of skin diseases, enlargement of liver and spleen, dysentery, diarrhoea, diabetes, leprosy, lung diseases, leucorrhoea, heart diseases, cough, asthama, piles, ulcers, gonorrhea and rheumatism. [10,11] Several species belonging to the genera of Ficus were reported to contain furanocoumarins which is an important plant phototoxin. [12,13] The plant is a large evergreen tree distributed all over India from sub Himalayan region and in the deciduous forest of Deccan and south India. It is a grown in gardens and road sides for shades. [14,15]

The bark, leaves and fruits of this group are used as astringent, haemostatic, anti-septic, anti-inflammatory, antioxidant and anticancer agent and also in the treatment of diarrhoea, dysentery and in the treatment of skin diseases, ulcers, vaginal disorders, leucorrhoea, menorrhagia and deficient lactation. In the traditional system of medicine, the plant is used for various health problems and diseases. [16-20]

Figure 1: *Panax ginseng* root and leaves [21]

Figure 2: *Ficus benghalensis* stem [22]

MATERIALS AND METHODS

Preparation of extracts:

The dry powder of the stem bark (2.5kg) of *Ficus Benghalensis Linn* was macerated at room temperature, in Hexane for 24 h. The extract was suctioned and filtered using Whatmann filter paper. This was repeated for two more days and similar extracts were pooled together and concentrated at 40^0C under reduced pressure using Buchi R-153 Rotavapour. The residual plant material was extracted successively with chloroform and methanol, petroleum ether and water in the same manner as followed for hexane.

The dry powder of *Panax ginseng* root (2.5 kg) was macerated at room temperature, in Hexane for 24 h. The Extract was suctioned and filtered using Whatmann filter paper. This was repeated for two more days and similar extracts were pooled together and concentrated at 40^0C under reduced pressure using Buchi R-153 Rotavapour. The residual plant material was extracted successively with chloroform and methanol, petroleum ether and water in the same manner as followed for hexane.

The percentage extract were calculated.

Preparation of the Formulation:

5g of the ethanolic extract of *Ficus benghalensis Linn* bark extract and *Panax ginseng* root extract were mixed together in 25ml of tween 80. The mixture was homogenized and stirred for 2 hours and further 25 ml of the tween 80 was added and stirred continuously for 2 hours to obtain a uniform suspension of the formulation.

Each ml of the formulation contains 500mg of the extract. GMP standards and specifications have been used in the manufacture of formulation.

This is formulation designated as PF.

Phytochemical Screening:

The different qualitative tests were performed for establishing profile of the given extract for its chemical composition. The following tests were performed on extracts to detect various phytoconstituents present in them.

1) Detection of Alkaloids [23]

0.5 g. of extract was taken and it was dissolved in 10 ml of dilute 0.1 N HCL and then filtered. The filtrate was used to test the presence of alkaloids.

a) Dragendorff's Test

To the 2 ml of filtrate, Dragendorff's reagent (2-3 drops) was added. Appearance of reddish brown colored precipitate indicates the presence of alkaloids.

b) Hager's Test

To the 2 ml of filtrate add Hager's reagent which gives yellow colored precipitate indicates the presence of alkaloids.

c) Mayer's Test

To the 2 ml of filtrate, 2-3 drops of Mayer's reagent were added, this leads to formation of cream colored precipitate indicates the presence of alkaloids.

d) Wagner's Test

To the 1 ml of the extract, add 2 ml of Wagner's reagent. Appearance of reddish brown precipitate indicates the presence of alkaloids.

2) Detection of Carbohydrates [24]

100 mg of extract was dissolved in 10 ml of water and filtered. The filtrate prepared was used to test the presence of proteins and amino acids.

a) Molisch's Test

To the 1 ml of filtrate add 2 drops of Molisch's reagent in a test tube and add 2 ml of concentrated sulphuric acid carefully along the sides of the test tube. Formation of violet color at the interface of two liquids indicates the presence of carbohydrates.

b) Fehling's Test

To the 1 ml of filtrate add 4 ml of Fehling's reagent (2 ml Fehling A and 2 ml Fehling B solutions) in a test tube and heated for about 10 minutes in a water bath. Formation of red precipitate indicates the presence of reducing sugar.

c) Barfoed's Test

1 ml of Barfoed's reagent is heated with 5 drops of filtrate in a test tube on water bath. Formation of a brick-red precipitate within 5 minutes indicates the presence of monosaccharides. Disaccharides generally don't give any reaction even for ten minutes.

3) Detection of Glycosides [25]

0.5 g of extract was hydrolyzed with 20 ml of dilute 0.1 N HCL and then filtered. The filtrate obtained was used to test the presence of glycosides.

a) Borntrager Test

To the 1ml of filtrate add 2 ml of 1% ferric chloride solution in a test tube and heated for about 10 min in boiling water bath. The mixture was cooled and was shaken with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Appearance of pink colour in the ammonical layer indicates the presence of glycosides.

b) Legal Test

To 1 ml of filtrate add three ml of sodium nitropruside in pyridine and methanolic alkali (KOH) in a test tube. Appearance of blue colour in alkaline layer indicates the presence of glycosides.

4) Detection of Saponins [26]

Foam Test

To 1 ml of extract add 20 ml of distilled water and it was shaken in a graduated cylinder for about 15 min. Formation of 1 cm layer of the foam in test tube indicates the presence of saponins.

5) Detection of Proteins and Amino acid [27]

100 mg of extracts were dissolved in water (10 ml) and then it was filtered. The filtrate was used to test the presence of proteins and amino acids.

a) Millon's Test

2 ml of filtrate was treated with 2 ml of Millon's reagent in a test tube and it was heated in a water bath for about 5 min, cooled and few drops of NaNO2 solution were added to the test tube. Formation of white precipitate and it turns to red upon heating indicates the presence of proteins and amino acids.

b) Biuret Test

To the 2 ml of filtrate add 2 ml of 10% sodium hydroxide solution in a test tube and heated for about 10 min, to the above solution, add a drop of 7% of copper sulphate. Formation of violet colour confirms the presence of proteins.

c) Ninhydrin Test

To the 2 ml of filtrate add 2-3 drops of Ninhydrin reagent in a

Test tube and boiled for about 2 min. Formation of deep blue colour indicates the presence of amino acids.

6) Detection of Phytosterols [28]

To 0.5 g of extract add 10 ml of chloroform and it was filtered. The filtrate was used to test the presence of phytosterols and triterpenoids.

Libermann's Test

To the 2 ml of filtrate in hot alcohol in a test tube to that add few drops of acetic anhydride. Formation of brown precipitate indicates the presence of sterols.

7) Detection of Fats and Oils [29]

Oily Spot Test

One drop of the extract was placed on the filter paper and then the solvent was allowed to evaporate. Appearance of oily stain on the filter paper indicates the presence of fixed oil.

8) Detection of Flavonoids [30]

Shinoda Test

To the extract (100 mg) in a test tube add few fragments of magnesium metal. To the test tube add 3 to 4 drops of conc HCL. Formation of magenta colour or light pink colour indicates the presence of flavonoids.

9) Detection of Phenolic Compounds and Tannins [31]

100 mg of extract mixed with 1 ml of water and then it was boiled and filtered. The filtrate was used for the following test.

a) Ferric Chloride Test

Take 2 ml of filtrate in a test tube to that add 2 ml of ferric chloride solution (1%). Formation of bluish to black colour indicates the presence of phenolic nucleus.

b) Lead Acetate Test

To the 2 ml of filtrate in a test tube add 2 to 3 drops of lead acetate solution. Appearance of yellowish precipitate indicates the presence of tannins.

c) Alkaline reagent test:

An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicated the presence of flavonoids.

10) Detection of gum and mucilages:

The extract (100mg) was dissolved in 10ml of distilled water and to this 25 ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilages.

11) Detection of Triterpenoids

A. Salkowsky test: A small quantity of the extract in chloroform was treated with a few drops of concentrated sulphuric acid, the solution turned yellow, then to red.
B. Hishorn test: A small quantity of the test extract was heated with trichloroacetic acid, the solution turned yellow colour and finally changed to red.

Physicochemical Evaluation of the Extract of Herbal Formulation PF [32]

The formulations were subjected to physicochemical evaluation for the
Determination of Ash values.
Determination of Extractive values.

1) Determination of ash values of the formulation PF:

a) Determination of total acid ash value

About 4g of the formulation PF accurately weighed and placed, in a previously ignited and tared silica crucible. The formulation was spread in a even layer and ignited gradually increasing the heat to 500^0C until it was white, indicating the absence of carbon. Cooled in desiccators for 30 minutes and then weighed without any delay. The experiment was repeated thrice with three different batches of formulation.

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of the crude drug taken}} \times 100$$

b) Determination of acid insoluble ash

The ash obtained was boiled with 2 M HCl (25 ml) for five minutes and it was filtered using an ash less filter paper. Insoluble matter retains on the filter paper and it was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble ash was calculated with reference to the shade dried plant powder by using the following formula.

$$\% \text{ acid insoluble ash value} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

c) Determination of water soluble ash

The ash above obtained, was boiled with 25 ml of water for 5min, cooled and the insoluble matter was collected on an ash less filter paper. Paper was washed with hot water and ignited at a temperature not exceeding 450°C, for 15min in a muffle furnace. The difference in the weight of ash and the weight of water insoluble matter gave the weight of water soluble ash. The percentage of water soluble ash was calculated with reference to the shade dried plant powder by using the following formula.

$$\% \text{ Water soluble ash value} = \frac{\text{Weight of total ash-Weight of water insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

2) Determination of extractive values of the formulations pf:

a) Water soluble extractive:

Five grams of PF were added to 50 ml of water at 80^oC in a stoppered flask. It was shaken well allowed to stand for 10 minutes. It was cooled to 15^oC and 2g of Kiesulghur was added and filtered, 5 ml of the filterate was transferred to a tarred evaporating basin. The solvent was evaporated on a water bath, for ½ hours and then dried in steam for 2 hours and weighed. The percentage of water soluble extractive was calculated with reference to the air dried powdered plant material.

a) Alcohol soluble extractive:

Five grams of the formulation PF was macerated with 100 ml of 70% alcohol in a closed flask for 24 hours, shaken frequently during 6 hours and allowed to stand for 18 hours. It was filtered rapidly taking precautions against loss of alcohol and 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105^0C and weighed. The percentage of alcohol soluble extractive was calculated with reference to air-dried powdered formulation.

b) N-Hexane, Chloroform and Methanol Soluble Extractives:

Five grams of the formulation PF was placed in a soxhlet apparatus. 25 ml of hexane was taken in the round bottom flask and hot extraction was carried out for 24 hours. The extract in the round bottom flask was concentrated by distillation and the dry extract was weighed to get the hexane soluble fraction. The marc was used for successive extraction with chloroform and methanol. The percentage solubility in each case was calculated with reference to powdered formulation taken initially.

All the experiments for extractive values were repeated thrice with three different batches of the formulation.

RESULTS AND DISCUSSION

Ficus benghalensis Linn bark extract and *Panax ginseng* root extract were subjected to systematic physicochemical and phytochemical screening by extracting with various organic solvents in the order of increasing polarity to determine the soluble constituents in a given amount of plant material. The present work is analysing the quality and purity of the crude drugs. In this study the parameters used for the evaluation of *Ficus benghalensis Linn* bark extract and *Panax ginseng* root were moisture content, extractive values by different solvents (includes petroleum ether, methanol and water), ash values (total ash, water soluble and acid insoluble ash). On incineration, drugs leave an ash and it consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The determination of ash value is useful to detect the exhausted drugs, low-grade products and excess of sandy matter which is applicable to powdered drugs. Phytochemical analysis was performed on the petroleum ether, alcohol, hexane, chloroform and aqueous extracts of *Ficus benghalensis Linn* bark extract and *Panax ginseng* root.

Table 1: Preliminary phytochemical screening of the bark of *Ficus benghalensis Linn*

Extractive	Reducing sugar	Flavonoid	Alkaloid	Sterols	Taninns	Saponins
Hexane	-ve	-ve	-ve	+ve	-ve	-ve
Alcohol	-ve	-ve	-ve	+ve	+ve	+ve
Chloroform	+ve	-ve	-ve	-ve	-ve	+ve
Water	+ve	-ve	-ve	-ve	+ve	+ve
Petroleum ether 60-80	-ve	-ve	-ve	+ve	+ve	+ve

Table 2: Preliminary Phytochemical screening of the root of of *PANAX GINSENG*

Extractive	Reducing sugar	Flavonoid	Glycosides	Sterols	Taninns	Saponins
Hexane	-ve	-ve	-ve	+ve	-ve	-ve
Alcohol	-ve	-ve	-ve	+ve	+ve	+ve
Chloroform	+ve	-ve	-ve	-ve	-ve	+ve
Water	+ve	-ve	-ve	-ve	+ve	+ve
Petroleum ether 60-80	-ve	-ve	-ve	+ve	+ve	+ve

Table 3: Preliminary Phytochemical Screening of the Formulation PF

Extractive	Reducing sugar	Flavonoid	Alkaloids	Sterols	Tanins	Saponins	Protein
Hexane	-ve	-ve	-ve	+ve	-ve	-ve	-ve
Alcohol	-ve	-ve	-ve	-ve	+ve	+ve	-ve
Chloroform	+ve	-ve	-ve	-ve	-ve	-ve	+ve
Water	-ve	-ve	-ve	+ve	+ve	+ve	+ve
Petroleum ether 60-80	-ve	+ve	-ve	+ve	+ve	+ve	-ve

DETERMINATION OF ASH VALUES:

Determination of ash values:

The total ash value of the formulation, PF = 14.26± 0.35 w/w

The acid insoluble ash value of the formulation, PF= 1.39± 0.14w/w

The water soluble ash is the difference in weight between in weight between the total ash and the residue after treatment of total ash with water.

The water soluble ash values of the formulation, PF= 12.2±0.46w/w.

Determination of extractive values:

The water soluble extractive value for PF = 12.5±0.32w/w.

N-hexane, chloroform and methanol soluble extractives:

The n-hexane soluble extractive for PF = 15.483±0.42w/w.

The methanol soluble extractive for PF = 18.786±0.32w/w.

The alcohol soluble extractive for PF = 25.475±0.42w/w.

CONCLUSION

Herbal based remedies serve as the important means of therapeutic medical treatment. The people are turning to usage of medicinal plants and phyto-chemicals in health care. India has one of the oldest cultural traditional uses of its herbal plants since from Vedic period. Ayurveda, Unani, Siddha and other traditional systems of medicine are the ancient systems of medicine and utilize numerous numbers of medicinal plants. Phytochemical screening, biological screening of randomly collected plants and their phytochemical examination have proved to be helpful in discovering the new drugs.

In order to standardize the extract and to determine the quality and purity of the formulation the Total ash, insoluble ash, acid insoluble ash and water soluble ash were determined in the three different batches of the formulation.

The present study concluded that the plant *Ficus benghalensis Linn* bark extract and *Panax ginseng* root contains variety of phytoconstituents. The physicochemical evaluation of *Ficus benghalensis Linn* bark extract and *Panax ginseng* root revealed that the standard quality and purity of drugs. Phytochemical studies on the extracts of *Ficus benghalensis Linn* bark extract, *Panax ginseng* root and their formulation showed presence of sterols, tannins, saponins, and reducing sugars, where as its formulation showed the presence of similar constituents, but in addition showed the presence of flavonoids. This information may be further useful for isolation of various compounds for treatment of diseases in human beings.

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