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Phytochemical and Pharmacognostical investigations on aerial roots of *Ficus lacor* Buch. Ham

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Abstract

To evaluate Phytochemical and pharmacognostical investigations on the aerial roots of *Ficus lacor* Buch. Ham. The investigations as per WHO Guidelines and other standard parameters (Morphological studies, Microscopical studies, Histochemical colour reaction Extractive values, Ash values, Swelling index , bitterness value, foaming index haemolytic activity, tannin content and phytochemical investigations) The aerial roots are typical roots, fibrous and slightly bitter. The microscopy of the powder revealed the presence of annular xylem vessel, lignified fibre, parenchymatous cell and cork cells. Total ash, acid insoluble ash, water insoluble ash and sulphated ash were 14.15%, 8.57%, 10.75%, 6.00% respectively. The extractive values i.e. petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract were 5.7%, 10%, 5.5%, 4.5%, 10.5%. The fiber content was 9.45%. The plant can be used as bitter as its bitterness was found to be 1.9 unit/g. The foaming index was 124.6. The plant possesses haemolytic activity. The plant extracts were good to be free of microbial contamination. The tannin content was 22. The preliminary phytochemical screening of Petroleum Ether extract, Ethyl acetate extract, Chloroform extract, Ethanol extract, Aqueous extract was performed. The presence of flavonoids, carbohydrates, saponins, phenolic compounds, and sterol in various extracts were observed. The alcoholic and aqueous extracts were screened for presence of amino acid and carbohydrates. The TLC profile of extracts showed the presence of three amino acid viz. alanine, methionine, ornithine and tyrosine and three carbohydrates i.e. galactose, lactose and sucrose. This is first ever phytochemical and pharmacognostical study carried out on the aerial roots of *Ficus lacor* Buch. Ham. useful for future standardization of *Ficus lacor* aerial roots.

Keywords: Phytochemical, pharmacognostical, *Ficus lacor*, aerial roots, extracts.

Introduction

India has a rich cultural heritage of traditional medicines which chiefly comprised the two widely flourishing systems of treatments i.e. Ayurvedic Siddha, Unani systems etc. and since ancient times. The plant crude drugs are available easily in abundance, they are comparatively cheaper. They have negligible side effects and are frequently prescribed to patients of all age groups. The multiple therapeutic action and uses of these drugs are described in classical literature on indigenous medicines in many medicinal plant books and pharmacopoeias[1,2]. Ficus genus consists of over 800 species and about 40 genera of the mulberry family. Ficus genus also known as fig genus. All Ficus species possess latex-like material within their vasculatures, affording protection and self-healing from physical assaults[3]. Ficus aspera, Ficus auriculata, Ficus benghalensis (Indian banyan), Ficus binnendykii, Ficus carica, Ficus deltoidea, Ficus elastica (Indian rubber tree), Ficus lacor (pakur tree), Ficus lingua Ficus lyrata, Ficus macrophylla, Ficus microcarpa (Chinese banyan), Ficus pseudopalma, Ficus pumila, Ficus religiosa, Ficus saussureana, Ficus subulata etc [4]. Ficus species contains alkaloids, flavanoid, steroids, glycosides, phenolic acids, steroids, saponins , coumarins, tannins, triterpinoids etc [5]. Pharmacognostical study is the groundwork in the standardization of herbal drugs. The detailed pharmacognostical evaluation gives valuable information regarding the morphology, microscopical and physical characteristics of the crude drugs. Pharmacognostic studies have been done on many important drugs, and the resulting observations have been incorporated in various pharmacopoeias[6]. There are numeral crude drugs where the plant source has not yet been scientifically identified. Hence pharmacognostic study gives the scientific information regarding the purity and quality of the plant drugs [7]. There is no information in the litrature regarding the pharmacognistical evaluation of *Ficus lacor* aerial roots. The present study includes morphology, microscopy, powder study, ash values, extractive value, bitterness value, haemolytic activity, paper chromatography and phytochemical screening of the aerial roots of *Ficus lacor*.

Materials and Methods

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The plant of *Ficus lacor* aerial roots were collected during the month of the July 2009 from Panchkula Sector-17 (Haryana), North India. The plant material was taxonomically identified and authenticated by Dr. H.B. Singh, Head, Raw materials Herbarium and Museum division, with ref.no.NISCAIR/RHMD/Consult/2010-11/1638/236. The voucher specimen has been deposited in the herbarium section of the Phytochemistry and Pharmacognosy Division, Chitkara College of pharmacy, Chitkara university, Panjab for further reference. The root was dried under shade, sliced into small pieces, pulverised using a mechanical grinder and stored in an air tight container for further use.

Morphology

The crude drug was evaluated for organoleptic properties shape, size. colour, odour, taste, fracture and texture were noted [Plate no.1].

Plate no.1 Morphology of roots

Microscopy of Root

Microscopy of plant material is performed to distinguish it from the allied drugs and adulterant. The dried root was soaked overnight in water to make it smooth enough for transverse section. Paraffin wax embedded specimens were sectioned using the rotatory microtom (Weswox Optik). The thickness of section was 10-12 μm. The very fine section was selectively subjected to staining reaction with staining reagent safranin (1%) and light green (0.2%). Slides were cleared in xylol and mounted in DPX mountant. Photomicrographs were taken using trinocular microscope (Olympus) [8]. [Plate no. 2]

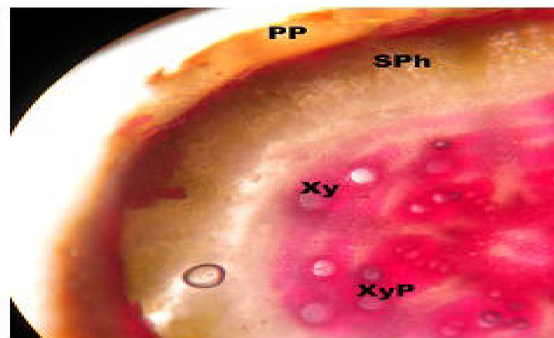

Plate no. 2 : Microscopiy of roots powder
Pp: primery phloem ; sp: secondry phloem ;xy;xytem vessels; xyp: xylem parenchyma

Histochemical Colour Reaction

Presence of different organic compounds in root of the plant is confirmed by using various histochemical tests. Care was taken to ascertain relative concentration of these chemicals by degree of colour produced in different tissues. The transverse section of fresh root was treated with different chemical reagents for colour tests viz. phloroglucinol, millon's reagent, iodine solution followed by sulphuric acid, dragendroff's reagent, wagner's reagent, sulphuric acid solution, libberman-burchard reagent, acetic anhydride and sulphuric acid solution, ferric chloride, iodine solution, caustic alkali, aqueous potassium hydroxide, chloroform with sulphuric acid, aniline sulphate and sulphuric acid [9] [Table no. 1].

Table 1 : Behaviour of transverse section with different chemical reagents

Sr.No.	Reagents	Test for	Nature of change in histochemical zone	Degree of change
1.	Phloroglucinol+HCl	Lignin	Xylem vessels become pink	+
2.	Millon's reagent	Proteins	Yellow colour	+
3.	Iodine solution followed by H_2SO_4	Cellulose	Cellulose wall become violet	+
4.	Dragendroff's reagent	Alkaloid	Brown colour	-
5.	Wagner's reagent	Alkaloid	Dark yellow colour	-
6.	H_2SO_4 solution	Sterol	Red colour	+
7.	Libberman – Burchard reagent	Terpenes	Pink colour	+
8.	Acetic anhydride and H_2SO_4 solution	Strerol	Black colour	++
9.	$FeCl_3$ solution	Tannins	Dark green to black colour	-
10.	Iodine solution	Starch	Light bluish	+
11.	Caustic alkali+HCl	Calciumoxalate	No change	-
12.	Aqueous. KOH solution 10%+ H_2SO_4	Suberin	Light brown	-
13.	Chloroform+ H_2SO_4	Sterol	Red colour	+

++ High; + Moderate; - Absent

Powder studies

Microscopic study

The shade dried root was mechanically pulverized to coarse powder and sifted through 40 mesh sieve. To study the ingredients of powder, a pinch of powder was taken on slide and mounted with phloroglucinol, hydrochloric acid and glycerine. The slide was observed under microscope [10][Plate no. 3].

Plate no.3 T.S of aerial root
1: parenchymatus cells; 2;spirel; 3: pitted cessles ;4 ; fiber

Colour reactions

To study the behavior of root powder with different chemical

reagents, a pinch of powder was treated with different chemical reagents as 1N hydrochloric acid, sodium hydroxide, acetic acid, 5% ferric chloride, picric acid, nitric acid with ammonia solution, 5% iodine, 1N nitric acid and powder as such were performed, change in color was observed [11] [Table no. 2].

Table 2: Behaviour of aerial roots powder with different chemical reagents

Sr.No	Treatment	Color of powder
1	Powder as such	Brown
2	Powder + 1 N HCl	Dark brown
3	Powder + 1N NaOH	Light brown
4	Powder + Acetic Acid	Light pinkish
5	Powder + 5% Ferric chloride	Cream
6	Powder + Picric acid	Greenish yellow
7	Powder + HNO_3 + Ammonia solution	Brown
8	Powder + 5% Iodine	Black brown
9	Powder + 1N HNO_3	Brown green

Fluorescence behavior of powder

Many herbs show fluorescence behaviour when cut surface or powder is exposed to UV light and this can help in their identification. To study the fluorescence nature of root powder, a pinch of powder was treated with different chemical reagents viz.1N hydrochloric acid, 1N sodium hydroxide, 1N sodium hydroxide in methanol, picric acid, 1N nitric acid, acetic acid, acetone, 50% sulphuric acid, nitric acid in ammonia solution and observed under day light, long UV (365 nm) and short UV light (254 nm) [12] [Table no. 3]

Table 3: Fluorescence nature of root powder under UV and visible radiations

Sr.no.	Treatment	Long UV (365 nm)	Short UV (254 nm)	Visible
1	Powder as such	Parrot brown	Light green	Light brownish
2	Powder + 1N HCl	Dark green	Light brown	Light brown
3	Powder + 1N NaOH	Light brown	Light brown	brown
4	Powder + 50% HNO3	Dark brown	Light brown	Brown
5	Powder + Acetic acid	Yellowish	Light yellow	Light brown
6	Powder + Picric acid	Light brown	Dark brown	Light brown
7	Powder + 1N NaOH in methanol	Light greenish	Dark brown	Light brown
8	Powder + $FeCl_3$	Creamish	Light brown	Light brown
9	Powder + 1N NaOH in methanol + Nitrocellulose in amyl acetate	Light yellowish	Light greenish	Light brown
10	Powder + 1N HCl + Nitrocellulose in amyl acetate	Light greenish	Brown	Light brown
11	Powder + 1N NaOH + Nitrocellulose in amyl acetate	Light greenish	Dark green	Light brown

Ash Values

Total ash

Total ash is produced by incinerating the drug at the temperature possible to remove all of the carbon. A higher temperature may result in the conversion of carbonates to oxides. The total ash usually consists of carbonates, phosphates, silicates and silica which includs both physiological ash, which is derived from the plant tissue itself and non-physiological ash which is the residue of the adhering material to the plant, e.g., sand and soil. About 2 g of air-dried powdered drug was accurately weighed and taken in a silica crucible and incinerated at a temperature not exceeding 450 C until free from carbon. The crucible was cooled and weighed. The percentage of total ash was calculated with reference to the air dried drug [13]. [Table no. 4]

Table 4: Ash values

Sr. No.	Ash	Percentage
1	Total ash	14.15%
2	Water soluble ash	8.57%
3	Acid insoluble ash	10.75%
4	Sulphated ash	6.00%

Water soluble ash

Water-soluble ash is that part of the total ash content which is soluble in water. The total ash obtained was boiled for 5 min with 25 ml of water, the insoluble matter was collected in an ashless filter paper, incinerated at a temperature not exceeding 450 C, subtracted the weight of the insoluble matter from the weight of the ash and calculated the percentage of water soluble ash with reference to the air dried drug. [Table no. 4]

Acid-insoluble ash

Acid insoluble ash is determined by treating the total ash with dilute hydrochloric acid and weighing the residue. This limit particularly indicated contamination with siliceous materials such as earth and sand by comparison with the total ash value for the same sample differentiation can be made between contaminating material and in the natural ash of the drug. The total ash obtained was boiled with 25 ml of 2 N hydrochloric acid for 5 min, the insoluble matter was collected in an ashless filter paper, washed with hot water, ignited, cooled in dessicator and weighed. The percentage of acid-insoluble ash with reference to the air dried drug was calculated. [Table no. 4]

Suphated ash

About 1 g of air dried powder drug was treated with dilute sulphuric acid before ignition in a tared silica crucible to a constant weight. The ash obtained was weighed. Percentage of sulphated ash was calculated with reference to the air-dried drug [Table no. 4].

Extractive Value

Extractive value is used as a means of evaluating crude drug which are not readily estimated by other means. It is employed for that material for which no suitable chemical or biological assay method exist [13].

Petroleum ether extractive

Accurately weighed 5 g of the air dried powdered drug was macerated with 100 ml of petroleum ether (60-80°) in a stoppered flask for 24 h. The mixture was vigorously shaken at regular intervals. After 24 h the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100 C till it was completely dried and weighed. The percentage of petroleum ether soluble extractive was calculated with reference to air dried material [Table no. 5].

Chloroform extractive

Accurately weighed 5 g of the air dried powdered drug was macerated with 100 ml of chloroform in a stoppered flask for 24 h. The mixture was vigorously shaken at regular intervals. After 24 h the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100 C till it was completely dried and weighed. The percentage of chloroform soluble extractive was calculated with reference to air dried material [Table no. 5].

Ethyl acetate extractive

Accurately weighed 5 g of the air dried powdered drug was macerated with 100 ml of ethyl acetate in a stoppered flask for 24 h. The mixture was vigorously shaken at regular intervals. After 24 hthe solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100 C till it was completely dried and weighed. The percentage of benzene soluble extractive was calculated with reference to air dried material [Table no. 5].

Ethanol extractive

Accurately weighed 5 g of the air dried powdered drug was macerated with 100 ml of ethanol in a stoppered flask for 24 h. The mixture was vigorously shaken at regular intervals. After 24 h the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100 C till it was completely dried and weighed. The percentage of ethanol soluble extractive was calculated with reference to air dried material [Table no. 5].

Table 5: Extractive values

Sr. No.	Solvent	Extraction period (h)	Colour of extract	Extractive value (%) w/w
1.	Petroleum ether (60-80 C)	24	Light brown	5.7
2.	Chloroform	24	Light brown	10
3.	Ethyl acetate	24	Yellowish brown	5.5
4.	Ethanol	24	Brown	4.5
5.	Aqueous	24	Dark brown	10.5

Water extractive

Accurately weighed 5 g of the air dried powdered drug was macerated with 100 ml of water in a stoppered flask for 24 h. The mixture was vigorously shaken at regular intervals. After 24 h the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100 C till it was completely dried and weighed. The percentage of water soluble extractive was calculated with reference to air dried material [Table no. 5].

Determination of Crude Fiber Content

2 g of powdered drug was extracted with diethyl ether and added 200 ml of boiling dilute sulphuric acid (1.25%) to the ether exhausted marc in a 500 ml flask. The mixture was refluxed for 30 min, filtered through filter paper and the residue was washed with boiling water until the effluent washing was acid free. Rinsed the residue and placed back into the flask with 200 ml of boiling sodium hydroxide solution (1.25%) and refluxed the mixture again for 30 min., filtered through ashless filter paper and washed the residue with boiling water until the last washing was neutral. It was then dried at 110°C to constant weight and then ignited to constant weight. The ash was cooled in dessicator, weighed [15].

Calculations as follows

$$\% \text{ Crude Fibre} = \frac{\text{Weight of the ash obtained}}{\text{Weight of the drug sample}} \times 100$$

The results are represented in Table no. 6.

Loss on Drying

This parameter is used to determine the amount of moisture present in a particular sample. The powder drug (2 g) sample was placed on a tared evaporating dish. The tared evaporating dish was dried at 105 $\pm$ 1 C until constant weight and weighed. The drying was continued until two successive readings match each other [13][Table no. 6]

Determination of Swelling Index

Swelling properties of many medicinal plants shows specific therapeutic or pharmaceutical utility e.g. gums, pectin, or hemicellulose. One g of plant material was accurately weighed, placed into 25 ml glass stoppered measuring cylinder. 25 ml water was added and shaken the mixture thoroughly in every 10 min for one h, allow standing for 3 h at room temperature. Measured the volume in ml occupied by plant material calculated the mean value of individual determination, related to one gm of plant material [15] [Table no. 6].

Table 6: : Crude fiber content, Loss on drying, Swelling index, Foaming index, Tanins contents, Bitterness value, Heamolytic value.

Parameter	Observation
Crude fiber content	9.45 %
Loss on drying	14 %
Swelling index	No significant result
Foaming index	124.6
Tannins	22
Bitterness value	1.9 unit / g
Haemolytic activity	23.45 %

Determination of Foaming Index

The medicinal plant materials contain saponins that cause the persistent foam formation when an aqueous decoction is shaken. The foaming ability of plant material and their extract is measured in term of foaming index. 1 g of powdered root was accurately weighed and transferred in to a 500 ml conical flasks containing 100 ml water and boiled for 30 min, cooled and filtered into 100 ml volumetric flask and made the volume with water. The decoction was poured into 10 stoppered test tubes in successive portion of 1 ml, 2 ml, 3 ml, etc up to 10 ml and adjusted the volume of each test tube with water to 10 ml and shaken them in lengthwise motion for 15 sec. Allowed to stand for 15 min and measured the height of the foam. The results were assessed as follows:

If height of foam in every tube was less than 1 cm the foaming index was considered less than 100. If height of the foam was more than 1cm in every tube the foaming index was over than 1000. In such case repetitions was done by using a new series of dilutions of decoction in order to obtain the result. If height of foam in any test tube was 1 cm, the volume of the plant material decoction in that tube (a) was used to determination of index.

Formula used for calculation of foaming index = $\frac{1000}{a}$

a = Volume of decoction was used for preparing the dilution in tube where foaming height was 1cm measured. The results are represented in Table 6.

Determination of Tannins

Tannins are complex substances. They occur as mixture of polyphenols that are difficult to separate and crystallize. They are capable of turning animal hide into leather by binding proteins to form water insoluble substances that are resistant to proteolytic enzyme. Powdered root 2 g of each root was accurately weighed and placed into conical flask. Added 150 ml of distilled water and heated over boiling water for 30 min, cooled, transferred the mixture to 250 ml volumetric flask and diluted to volume with water. Allowed the solid material to settle down and filtered the liquid through filter paper, discarded the first 50 ml of filtrate. Evaporated 50 ml of extracts of root, to dryness, dried the residue in an oven at 105 C for 4 h and weighed (T1). Took 80 ml of root extract, added 2 g of hide powder and shaken for 1 h. Filtered and evaporated 50 ml of clear filtrate to dryness. Dried the residues in an oven at 105 C and weighed (T2). This is the amount of plant material that does not bind to hide powder. 2 g of hide powder was dispersed in 80 ml of water and shaken well for 1 h. Filtered and evaporated 50 ml of clear filtrate to dryness. Dried the residues in an oven at 105 C and weighed (T0).

Formula used for calculation of tannins percentage: = $\frac{[T1-(T2-T0)]\ 500}{w}$

Where w = the weight of the plant material 2 g. The results are given in Table no. 6.

Determination of Bitterness Value

Medicinal plant materials have a strong bitter taste act as appetizing agents. The bitter properties of plant materials are determined by comparing the threshold bitter concentration of an extract of the materials with that of a dilute solution of quinine hydrochloride.

Stock and diluted quinine sulphate solutions

Accurately weighed 0.1 g quinine hydrochloride (R) was dissolved in safe drinking water to produce 100 ml. 5 ml of this solution was further diluted to 500 ml with safe drinking water. This stock solution of quinine hydrochloride (S_q) contained 0.01 mg/ml. Nine serial dilutions were made each containing 0.042, 0.044, 0.046, 0.048, 0.050, 0.052 0.054, 0.0.54 and 0.058 ml solution of S_q and volume made up to 10 ml with safe drinking water and obtaining a concentration of 0.1, 0.20, 0.3 upto 1 mg/ml.

Stock and diluted solutions of the plant material

The stock solution was prepared of the concentration of 10 mg/ml in distilled water (S_T). 10 test tubes were used for serial dilution with 1 ml, 2 ml, 3 ml to 10 ml of (S_T) and final volume made up with safe drinking water to 10 ml.

Method

After rinsing the mouth with safe drinking water, 10 ml of the most dilute solution was tasted while swirling it in the mouth mainly near the base of tongue for 30 sec. After 30 sec the solution was spit out and it was ascertained for I min whether a delayed sensation for bitterness existed. Then mouth was rinsed with safe drinking water. The next highest concentration was not tasted until at least 10 min. The lowest concentration at which material continues to provoke a bitter sensation after 30 sec was referred the threshold bitter concentration. After a first series of test, rinsed the mouth thoroughly with safe drinking water until no bitter sensation remains, wait at least 10 min before carrying out second test.

Formula used for bitterness calculation = $\frac{2000 \times C}{A \times B}$

Where

A = Concentration of stock solution (S_q) mg/ml

B = Volume of (S_T) ml tube with threshold bitter concentration

C = Quantity of quinine hydrochloride (in mg) tube with threshold bitter concentration. The results are given in Table no. 6.

Determination of Haemolytic Activity

Haemolytic activity of plant material is carried out for detection of saponins. It is determined by comparison of plant material extract and reference material saponin which has activity of 1000 unit per g. The erythrocyte suspension was prepared by filling a glass stoppered flask to one tenth of its volume with sodium citrate (36.5 g/L). Sufficient volume of blood freshly collected from healthy rat was introduced to it and shaken immediately. 1 ml of citrated blood was further diluted with 50 ml phosphate buffer of pH 7.4. The reference solution was freshly prepared by dissolving 10 mg glycyrrhizinic acid, (Himedia) in phosphate buffer pH 7.4 to make 100 ml.

Preliminary test

The alcoholic and aqueous extract (1 g) of root 0.1 ml, 0.2 ml, 0.5 ml and 1ml were taken and adjusted the volume in each tube with phosphate buffer to 1 ml. In each tube 1 ml of 2% blood suspension was added. Gently inverted to mix the tubes, to avoid the formation of foam. Tubes were shaken after 30 min interval. Then allowed to stand for 6 h at room temperature. Examined the tubes and recorded the dilution at which total haemolysis had occurred, as indicated by clear, red solution. The alcoholic extract of root has shown haemolytic activity in highest concentration i.e. 1 ml. Therefore further dilutions were done as follows.

A serial dilution of alcoholic extract of root was prepared by using 13 test tubes in a concentration of 0.40, 0.45, and 0.50 up to 1 mg/ml and adjusted the volume in each tube with phosphate buffer

to 1 ml. I ml of 2% blood suspension was added in each tube. Tubes were observed for haemolysis after 24 h. A serial dilution of glcyrrhizinic acid was prepared in the same manner. Calculated the quantity of glcyrrhizinic acid (g) that produces total haemolysis.
Calculated the haemolytic activity of the medicinal plant material using the following formula :

$$\text{Haemolytic activity} = \frac{1000 \times a}{b}$$

Where 1000 = the defined haemolytic activity of saponin (R).
a = quantity of saponin (R) that produces total haemolysis.
b = quantity of plant material that produces total haemolysis (g).
The results are represented in Table no. 6.

Paper Partition Chromatography of Amino Acids

Amino acids are the basic units of proteins. The proteins are found in every living cell. The amino acids which can be synthesized by the living cells are called non-essential amino acids, while those which cannot be synthesized are called essential amino acids and must be supplied by diet. The essential amino acids are Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine and Valine. The Non-essential amino acids are Alanine, Aspartic acid, Cysteine, Glutamic acid, Glycine, Proline, Tryptophan, Glutamine, Asparagine, Serine and Tyrosine etc [16].
Carbohydrates are the most abundant organic molecule in nature. These are the carbon compounds that contain large quantities of hydroxyl groups. The simplest carbohydrates also contain either an aldehyde moiety or a ketone moiety. All carbohydrates can be classified as either monosaccharides, oligosaccharides or polysaccharides e.g. Ribose, Ribulose, Xylulose Glucose, Galactose, Mannose, Fructose, Erythrose.

Preparation of extract

Powdered (10 g) aerial roots of *Ficus lacor* was weighed and macerated with 100 ml of water and left overnight. The supernatant clear liquid was filtered. The extraction was repeated for three consecutive days so as to exhaust the root of all water soluble extractives. The combined filtrates were concentrated on a water bath and the proteins precipitated by addition of alcohol (95%) were washed with ethanol to remove unbound amino acids. The mother liquor obtained after removing the proteins was concentrated for detection of amino acids in free state and carbohydrates [17,18].

Paper chromatography

Chromatographic Whatman paper No.1 sheets (Qalligens) were used for paper chromatography. The starting line was marked two centimeter above from the base. To obtain the desired concentration of the extract on the paper, the spots were applied repeatedly at the same point. The spots were kept at a distance of two centimeter apart for the amino acid identification
The solvent systems used were n-butanol: glacial acetic acid: water (4:1:1) for amino acids and n-butanol: glacial acetic acid: water (4:1:5) for carbohydrates.
The chamber was saturated in 16 h prior the experiment with respective solvent systems. Care was taken so as not to touch the paper with fingers. The papers were developed in descending manner. Air dried chromatograms were sprayed with 0.2% w/v solution of Ninhydrin in acetone for amino acids and aniline hydrogen pthalate for carbohydrates, heated at 110^0C in oven. Pink to violet colour were visualized for amino acids and yellow to dark brown for carbohydrate (19) [Table no. 7, 8].

Table 7: Amino acids analyzed by paper chromatography

Sr.No.	Amino Acids	R_f Value	Amino acids detected
1.	Alanine	0.32	+
2.	2-Aminobutyric acid	0.70	–
3.	Arginine	0.15	–
4.	Aspartic acid	0.16	–
5.	Glutamic Acid	0.18	–
6.	Glycine	0.16	–
7.	Histidine	0.14	–
8.	Lysine	0.18	–
9.	Methionine	0.37	+
10.	Norleucine	0.10	–
11.	Ornithine	0.10	+
12.	Phenylalanine	0.25	–
13.	Tyrosine	0.47	+

+ve : Detected; –ve : Not detected
Solvent system : n-butanol :glacial actic acid : water *(4:1:1, upper phase)*
Spray reagent : Ninhydrin

R_f values for each spot was calculated

$$R_f = \frac{\text{Distance travelled by the solute from the start}}{\text{Distance travelled by the solvent from the start}}$$

Table 8: Carbohydrates analyzed by paper chromatography

Sr. No.	Carbohydrates	R_f Value	Carbohydrate Detected
1.	Galctose	0.646	+
2.	Maltose	0.60	-
3.	Lactose	0.622	+
4.	D-Ribose	0.525	-
5.	Sucrose	0.629	+
6.	Fructose	0.666	-
7.	D-Xylose	0.711	-
8.	L-Arabinose	0.666	-

+ve : Detected; –ve : absent
Solvent system : *n*-butanol : glacial acetic acid : water (4:1:5)
Spray reagent : Anisaldehyde in sulphuric acid

Preliminary Phytochemical screening

Preparation of the extract

About 20 g of air dried powdered root was extracted with ethanol in a soxhlet extractor for 72 h. the aqueous extract was prepared by maceration with distilled water for 24 h to obtain the aqueous extract. Concentrated ethanol and aqueous extract in rotary vaccum evaporator and crude ethanol extract was fractioned viz. petroleum ether, ethyl acetate, chloroform, ethanol and water. The extracts were screened for the presence of various phytoconstituents [Table no. 9].

Test for alkaloids

Stirr a small portion of the solvent free petroleum ether, chloroform, ethyl acetate, alcohol and water extracts separately with a few drops of dilute hydrochloric acid and filter. The filtrates were tested with various alkaloidal reagents such as mayer's reagent (cream precipitate), dragendorff's reagent (orange brown precipitate), and wagner reagent (reddish brown precipitate). Mayer's reagent: Few drops of mayer's reagent were added in each extract and observed formation of the white or cream colored precipitates.
Dragendorff's reagent: Few drops of dragendorff's reagent were added in each extract and observed formation of the orange yellow or brown colored precipitates.
Wagner reagent: Few drops of wagner reagent were added in each extract and observed formation of the reddish brown precipitates.

Test for carbohydrates

Dissolve small quantities of alcoholic and aqueous extracts, separately in 4 ml of distilled water and filter. The filtrate may be subjected to varios tests to detect the presence of carbohydrates.
Molisch's Test: To about 2 ml of extract few drops of -naphthol (20% in ethyl alcohol) were added. Then about 1 ml of concentrated sulphuric acid was added along the side of the tube. Reddish violet ring appeared at the junction of two layers. Indicates the presence of carbohydrates.
Fehlings Test: 1ml of fehling's reagent (copper sulphate in alkaline conditions) was added to the filtrate of the root extract in distilled water and heated in a steam bath. Brick red precipitates appeared which confirm the presence of carbohydrates.

Test for glycosides

Hydrolysed another small portion of the extract with dilute hydrochloric acid for few hours in water bath and subjected the hydrolysate with liebermann-burchard's, keller-killani, and borntrager's tests to detect the presence of different glycosides.
Keller-Killani Test: 1ml of glacial acetic acid containing traces of $FeCl_3$ and 1 ml of concentrated H_2SO_4 was added to the extract carefully. Colour appeared which confirm the presence of glycosides in the root extracts.
Borntrager's test: 1ml of benzene and 0.5 ml of dilute ammonia solution were added to the extract. A black brown colour was obtained which show the presence of glycosides in the root extracts.

Table 9: Prelimenry phytochemical screening

Sr.no.	Plant Constituents Test / Reagent	Pet. Ether extract	Ethyl acetate extract	Chloroform extract	Ethanol extract	Aqueous extract
1.	**ALKALOIDS** Mayer's reagent Dragendroff's reagent Wagner's reagent	 - - +	 - + +	 - + -	 + + +	 - - -
2.	**GLYCOSIDES** Killer-Killani test Sodium nitropruside test Borntrager test	 + - +	 - - -	 - - -	 + + +	 - - -
3.	**CARBOHYDRATES** Molisch's reagent Fehling solution	 + +	 - -	 - -	 + +	 + +
4.	**STEROLS** Liebermann-Burchard's test Salkowski test Hesses reaction Hersch reaction	+ + + - -	- - - - -	+ - + + -	+ - + + +	- - - - -
5.	**SAPONINS** Foam test Sodium bicarbonate test	 - -	 - -	 + +	 + +	 - -
6.	**PHENOLIC COMPOUNDS & TANNINS** Ferric chloride solution Lead acetate solution	 + +	 + +	 - -	 + +	 - -
7.	**PROTEINS & AMINO ACIDS** Biuret test Millon's reagent Ninhydrin reagent	 - - +	 + - +	 - - -	 + + +	 - + +
8.	**FLAVANOIDS** Shinoda/Pew test Ammonia test	 + +	 + +	 - -	 + +	 + +

+ve: Present; -ve: Absent

Test for phenolic compound and tannins

Take small quantities of alcohol and aqueous extracts separately in water and test for the presence of phenolic compounds and tannins with dilute ferric chloride solution (5%) and lead acetate test.
Ferric chloride test: On addition of ferric chloride solution (5%), colour was observed in all the three portions due to the presence of phenolic compounds. colour appeared which show the presence of phenolic compound.
Lead acetate test: Few drops of lead acetate solution (5%) were added to the alcoholic extract of the root. White precipitate was appeared which confirm the presence of phenolic compounds.

Test for flavonoids

Ammonia test: Filter paper strips were dipped in the alcoholic and aqueous solutions of the extract and ammoniated. The filter paper changed its colour to yellow which indicates the presence of flavonoids.
Pew test for flavonoids: To 1ml of the each extracts, a piece of metallic magnesium/zinc was added followed by addition of 2 drops of concentrated hydrochloric acid. A brownish colour confirmed the presence of flavonoids in all the extract.

Test for proteins and free amino acids

Added a few ml of alcoholic and aqueous extracts in a few ml of distilled water and subjected to million's, biuret and ninhydrin tests.
Millon's test: To 2 ml of filtrate, 5-6 drops of million's reagent (solution of mercury nitrate and nitrous acid) was added. A red colour precipitate appeared which confirms the presence of proteins and free amino acids.
Biuret test: To the ammoniated alkaline filtrate 2-3 drops of 0.02% copper sulphate solution was added. A red colour was obtained which confirms the presence of proteins and free amino acids.
Ninhydrin test: To each of the filtrate, lead acetate solution was added to precipitate tannins and filtered. The Filtrate was spotted on a paper chromatogram, sprayed with ninhydrin reagent and dried at 110° C for 5 minutes. Violet spots were seen which confirm the presence of proteins and free amino acids.

Test for saponin

Foam test: Dilute 1 ml of alcoholic and aqueous extracts separately with distilled water to 20 ml and shake in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicates the presence of saponin.
Sodium bicarbonate test: To the few milligrams of extract few drops of sodium bicarbonate were added and shaken well. Formation of honey comb like frothing indicates positive test for saponins.

Test for phytosterol and triterpenes

Liebermann-Burchard's test: The hydro-alcohlic extract was shaken with chloroform and few drops of acetic anhydride were added chloroform extract along with a few drops of concentrated sulphuric acid from the side of the tube. The appearance of blue to brick red colour indicates the presence of sterol and triterpenes.
Hesse's reaction: The residue was dissolved in chloroform (4 ml) and an equal quantity of concentrated sulphuric acid was then along the side of the tube. The formation of the pink colored ring, which is on shaking diffused in both the layers, indicating the presence of sterols in the extract [20,21] [Table no. 9].

Results

The phytochemical and pharmacognostical investigations i.e. chemical and morphological study of the aerial root of the plant was performed. The aerial roots are typical roots and in transverse section it shows the features of a dicot root. The microscopy of the powder revealed the presence of annular xylem vessel, lignified fibre, parenchymatous cell and cork cells. Total ash, acid insoluble ash, water insoluble ash and sulphated ash were 14.15%, 8.57%, 10.75%, 6.00% respectively. The extractive values i.e. petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract were 5.7%, 10%, 5.5%, 4.5%, 10.5%. The fiber content was 9.45%. The plant can be used as bitter as its bitterness was found to be 1.9 unit/g. The foaming index was 124.6. The plant possesses haemolytic activity. The tannin content was 22. The alcoholic and aqueous extracts were screened for presence of amino acid and carbohydrates. The extracts showed the presence of three amino acid viz. alanine, methionine, ornithine and tyrosine and three carbohydrates i.e. galactose, lactose and sucrose. The preliminary phytochemical screening of Pet. Ether extract, Ethyl acetate extract, Chloroform extract, Ethanol extract, Aqueous extract was performed. The presence of flavonoids, carbohydrates, saponins, phenolic compounds, and sterol in various extracts were observed.

Discussion

The present studies carried out on the aerial roots of *Ficus lacor* Buch. Ham. On the basis of litrature survey, this is first ever phytochemical and pharmacognostical evaluation of aerial roots. So we selected aerial roots and standardized according WHO guidelines and other s parameters which are useful for future standardization and isolation of new phytoconstituents from aerial roots.

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