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A CONCISE STUDY ON MICROSCOPICALLY, PHOTOCHEMICAL AND PHARMACOGNOSTICAL PROFILE OF *BAUHINIA VARIEGATE LINN* (ROOT)

Padmani Shukla[1], Rakesh Kumar[2], Riya Mathur[3]*, Rashmi Singh[4], Sakshi Sagar[4], Akanksha[4] and Shatrughana Chaudhary[4]

[1]Associate Professor, Department of Pharmacognosy, Pharmacy Department, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Uttar Pradesh.

[2]Director, Department of Pharmacy, CSS Pharmacy College, Etawah, Uttar Pradesh.

[3]M. Pharm Research Scholar, Department of Pharmaceutical Chemistry, Advance Institute of Biotech and Paramedical Sciences, Kanpur, Uttar Pradesh.

[4]M. Pharm Scholar, Department of Pharmacy, ISF College of Pharmacy, Moga, Punjab.

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***Corresponding Author**
Riya Mathur
M. Pharm Research Scholar, Department of Pharmaceutical Chemistry, Advance Institute of Biotech and Paramedical Sciences, Kanpur, Uttar Pradesh.

ABSTRACT

The present study deals with the megascopic and microscopical studies of angiospermous tree Linn., stem. Some distinct and totally different characters were ascertained with section of stem. The anatomy of the stem was studied by taking transversal section. Periderm and cortex were seen distinctly. Secondary vascular tissue was wide and continuous cylindrical, it consisted of skinny and slender straight rays, 3 or four cylinders of discontinuous lots of fibres and every which way distributed sieve elements. Secondary vascular tissue was diffuse porous and it enclosed vessels, fibres, xylem rays and xylem parenchyma. Vascular tissue fibres had thick hard walls or some had jellylike walls. Vascular tissue parenchyma cells were bumper within the xylem. vascular tissue rays were one cell wide; they were straight and consisted of radially elongated thick walled hard walls. Calcium-oxalate crystals are preponderantly prismatic crystals and druses type. Powder microscopical examination showed presence of fibres, parenchymatous cells, periderm and vessel elements. Histochemical analysis of stem showed presence of protein, tannin, polymer and cellulose. Chemical science parameters and preliminary phytochemical studies of the stem powder were conjointly carried out. this study on pharmacognostical investigation of angiospermous tree

L., stem could be helpful to supplement info in respect to its identification parameters assumed considerably in the manner of satisfactoriness of seasoning medication in gift situation lacking restrictive laws to regulate quality of herbal drug.

KEYWORDS: Angiospermous, periderm, microscopial, phytochemical, pharmacogonistical.

INTRODUCTION

Bauhinia variegata Linn commonly known as Kachnar (mountain Ebony). The plant is widely used by the tribal throughout India and popular in various indigenous system of medicine like Ayurveda, unani, and homeopathy *Bauhinia variegata*, its 250 species grow in the tropical regions of the world. It includes shrubs, trees and vines that are frequently planted for their showy flowers and ornamental foliage. *Bauhinia variegateLinn*is native to south eastern Asia and grows throughout India and China.[1] It is distributed throughout India which include Punjab, central and south India ,sub Himalayan tract ,outer Himalayas' upon an altitude of 1300m. It is widely planted in the tropics and warm region of the world. It is native to SouthAsia and Southeast Asia, from southern China, Burma, India, Nepal, Pakistan, and Sri Lanka.[2]

The treatment of various ailments like asthma, ulcer leprosy, piles, snake bite and liver complaints are used in traditional medicine by the help of all parts of kachnar plants and its extracts have been found to have antibacterial and antifungal activity. It is also used in Fever, diarrhea, dysentery, hemorrhoids, piles, edema, skin diseases, wound healing, obesity, stomatitis, dyspepsia, flatulence ,as tonic, astringent, laxative, anthelmintic ,antileprotic, anti goitrogenic, antitumor, carminative.

Fig. 1: Plant and Root of *Bauhinia variegate.*

For the smooth conduction of the experiment work on *Bauhinia variegate linn*plant. Ihave gone through various books available in library, internet sites, journals, e-books etc and found the following contents from various source which are mentioned below:

Kachnar plant has following activities reported.[3]

1. Anti-oxidant activity

Sosa *et al* investigated the *B. variegate* and reportedquercetin, rutin, apigenin and epigenin 7-O- glucoside16. Flavonoid and quercetin are potent antioxidants and known to modulate the activities of various enzyme systems due to their interaction with biomolecules.

Ethanolic and aqueous extracts of *B. variegate* root produced significant antioxidant activity carried out by *in-vitro* scavenging of free radicals using 1, 2-diphenyl1-2-picrylhydrazyl (DPPH), nitric oxide and superoxide. It may be the flavonoids and other phyto chemicalspresent in the plant extracts. Ethanolic extract produced significantly greater antioxidant activity than other extracts. Invitroantioxidant and free radical scavenging potential are of methanolic extracts of *B.variegata*. Different parts of *B. variegate* like leaf, bark and flowers have free radical scavenging activity by hydroxyl radical scavenging method. All extracts have different level of antioxidant activity. Amongst all extracts methanol was found to be good solvent for extraction and having good antioxidant activity. IC50 value of *B. variegate* leaf, stem bark and floral buds are 17.9, 19.5 and 17.2 µg/ml, respectively. The Reducing power of extracts was carried out with ascorbic acid as a standard reducing agent51. The percent free radical scavenging activity gradually increases with increasing concentrations of *B. variegate* extracts in DPPH radical scavenging assay. Dose dependent antioxidant activity pattern was also observed in phosphor molybdate assay. Antioxidant activity was directly correlated with the amount of total phenolic contents in the extracts. *B. variegate* in L-dopa extract has shown the highest FRAP values.[4] **Sharma *et al*.** studied that both ethanolic and aqueous extracts of root of *B. variegate* possesses significant antioxidant activity by scavenging of various free radicals such as 1,2- di phenyl 1-2 picrylhydrazyl (DPPH), superoxide, nitric oxide. Its observed the various extract of the plant product significant DPPH (1,2- di phenyl 1-2 picrylhydrazyl) is a lipophilic free radical from a stable diamagnetic form with electron or hydrogen radical. Pandey*et al*.observed antioxidant activity by inhibiting of TBARs. Methanolic extract of *B.variegata*possesses significant free radical scavenging, hydroxyl radical scavenging and antioxidant activity in *in vitro*. They confirm the presence of phenolic compound flavonoids, tannin etc.[5]

2. Anti-diabetic activity

Pandey et al. investigated the presence of insulin like protein in leaf, stem bark of *B. variegate* is widely utilized in popular medicine, as an anti-diabetic agent. The leaves and stem-bark of *Bauhinia* used in different Phyto preparation to lower blood glucose levels noted the anti- diabetic potential of the plant *Bauhinia* in mammal. Ethanol extract leaves of *B. variegate* show the hypoglycemic activity as well as anti-diabetic activity.[6]

Lino et al reported that the aqueous and organic solvent ethanol and hexane extract of *Bauhinia* in a model of alloxan-induced diabetes in rats caused reduced glucose, triglycerides, total cholesterol and high density lipid (HDL) cholesterol levels. Morikawa also found in his observation that aqueous extract of *Bauhinia* leaves hypoglycemic activity in normo glycemic mice, he suggested that this action may be related to the presence of glycosyl flavonoids and several natural flavonoids exhibit an anti-diabetic activity. The presence of insulin-like molecule was demonstrated in the leaves, where a 'chloroplast protein' was found that has a partial amino acid sequence identical to that of Bovine insulin. This protein found to act as hypoglycemic activity when it is injected in alloxan induced diabetic mice.[7]

Patel DK et al reported the anti-diabetic activity. It was noticed a major metabolite of the ethanolic extract of leaves; roseoside, demonstrates insulin inotropic activity toward pancreatic ßcells of the INS-1 cell line and may act in conjunction with the chloroplast protein to contribute to the overall anti-diabetic properties.[8]

Dewanganet al. screened and isolated, identified a bioactive carbohydrate D-pinitol(3-o-methyl D-chlroinositol) from the ethanolic extract of *B. variegate* leaves. The compound pinitol is belonging to group of cyclic polyol. It is natural product of cyclic polyol group was responsible for hypoglycemic activities**.**[9]

3) Anti-microbial activity

Vikas Kumar et al. screened the *B. variegata*plant that could be utilized as an alternative source of antimicrobial drugs. Methanolic, chloroform and aqueous extracts of *B. variegate* fractions shows antibacterialactivity against both gram positive and gram negative bacteria namely- *Bacillus cereus, Staphyllococcus aureus, Klebsiella pneumonia, Escherichia coli,* and *Pseudomonas pseudo alcaligenes* .Antibacterial activity in methanol extract is more potent than aqueous extract. The alcoholic extract of leaves of *B. variegate* showed maximum antimicrobial activity compared with other organic solvent extracts. The methanol extract

from both *in vivo* and invitrogenerated plants of *B. variegate* were tested against a number of microbes, only*Escherichia coli and Pseudomonas aeruginosa*were found to be resistant at a concentration of 50 µg/ml2. Ethanolic extract of *B. variegate* stem bark act as antimicrobial activity against *B. subtilis, S. typhy, S. dysenterial, S. aureus, P. aeruginosa and Vibrio cholera.*[10]

4) Anti-tumour / Cytotoxic activity

Srilatha.M et.al Investigated noted the anti-tumor activity of ethanolic extract *B. variegata*againstdalton'sasceticlymphoma (DAL) on Swiss albino mice .Ethanolic extract of stem bark of *B.variegata*shows the chemoprevention and cytotoxic effect against N nitrosodiethylamineinduced liver tumour in rats and human cancer cell lines at a dose of 200 mg/kg. Oral administration of ethanolic extract of *B. variegate* effectively suppressed liver tumour induced by nitrosodiethyl amine induced elevated level of serum glutamate pyruvate transaminase(SGPT), serum glutamate oxaloacetate transminase (SGOT), alkaline phosphatase(ALP), total bilirubin, gama glutamate transpeptidase (GGTP), lipid peroxidase LPO), glutathione peroxidase (GPX), glutathione-s-transferase (GST)44. Ethanolic extract of bark, seed and leaf has been noted to be cytotoxic against human epitheliallarynx cancer and breast cancer.[11]

5) Anti-arthritic

Srilatha.M et.al. investigated the anti-arthritic activity of ethanolic extract of *B. variegatea*by the oral administration of ethanolic extract at the tested dose level of 250 mg/kg on complete Freund's adjuvant (CFA) induced arthritis in rat for 15 days. At the end of 15 days, the rats were sacrificed, their blood was collected and then serum was separated. After that various parameters such as alanine amino transferase (ALT), alkaline phosphatase (ALP), total cholesterol and triglycerides were estimated. In the level of various antioxidant enzymes were also evaluated in liver and kidney of normal, arthritic control and extract treated rats such as catalase, glutathione peroxidase (GPx), superoxide dismutase (SOD) and lipid peroxidase (LPO). The result of these Studies shows that administration of this significantly Paw Edema volume in rat and altered the biochemical parameters and also level of various antioxidant enzymes which got affected in arthritic rats. From this study, it was concluded that the ethanolic extracts of this plant showed significant antiarthritic effect in rats.[12]

6) Anti-Obesity

Balamurugan and Muralidharan et.al investigated the anti-obesity effect of methanolic extracts of stem and root bark of *B. variegate* by oral administration of methanolic extracts at the tested dose level of 200 and 400 mg/kg in female rats fed with hyper caloric diet for 40 days. At theend of 40 days, various parameters was evaluated such as body weight (BW), feed intake, high density lipoproteins (HDLP), low density lipoproteins (LDLP), triglycerides, total cholesterol, brain serotonin level. The results of these studies showed that administration of this extract significantly brought down the increased level of was total cholesterol, triglycerides, LDLP and there was increased in the level of HDLP, brain serotonin level. This was attributed to the presence of β-sitosterol in stem and tendency to release the serotonin level in the brain. Thus this finding showed a significant anti-obesity activity.[13]

7) Anthelmintic activity

Singh, A Aqueous and Chloroform extract of bark of *B. variegate* were investigated for their anthelmintic activity against *Pheretimaposthuma*and*Ascardiagalli*. All extracts exhibited a dose dependent (25, 50 and 100 mg/ml) inhibition of spontaneous motility (paralysis) and time of death of the worms. Extract obtained from bark not only killed the *Pheretimaposthuma*but also killed the *Ascardiagalli*. The observations were comparable with standard drug piperazine citrate at a concentration of 20 mg/ml and distilled water as control. Maximum vermicide activity was shown by both extract at the concentration of 100 mg/ml. From the experiment performed, it can be said that the aqueous and chloroform extract of bark of *B. variegate* bearing a potential anthelmintic activity.[14]

8) Insecticidal activity

Singh et al. Plant extract act as an effective measure for controlling insect pest like *Plutellaxylostella. B. variegata*var. candida is a promising source of edible wild vegetable flowers with plenty of nutrients. This plant may serve as a potential source for low cost proteins.

The tree is susceptibleto ‘Brown Root Rot’ caused by *Phellinusnoxius.* The abundance of phytophagousmites is higher, being *Lorryia Formosa* Cooreman the dominant species.[14]

9) Molluscicidal activity

Singh et al. reported that *Bauhinia variegate* leaf is a potential source of molluscicides

against snail *Lymnaea*and*Indoplanorbisexustus.* Theses nails are the intermediate host of liver fluke *Fasciola hepatica* and *Fasciolagigantica*, which causes 94% fascioliasis in the buffalo's population of Eastern Uttar Pradesh in India. The active molluscicidal component of *Bauhinia variegate* leaf is soluble in organic solvent such as acetone, carbon tetra chloride, chloroform, ether and ethanol. The toxicity of ethanolic extract of *Bauhinia variegata*leaf powder is higher than other organic solvents.

Singh et al. characterized that quercetin is the active component present in *Bauhinia variegate* leaf by column chromatography and thin layer chromatography. Toxicity of 96h exposure period of column purified fraction of *B.variegata*leaf (LC50 -5.98 mg/l) against *L.acuminata*is lower than the values of synthetic molluscicides- carbaryl (14.40 mg/l) 84, phorate (15.0 mg/l) formothion(8.56 mg/l) 84 and aldicarb (11.50 mg/l) . Plant molluscicides of *Thujaorientalis*fruit powder (255.12 mg/l), Areca *catechu* powder (36.59 mg/l), *Termanaliachebula*fruit powder (93.59 mg/l), *Morusnigra*fruit powder (166.92 mg/l) *M. oleifera*leaf powder (602.75 mg/l), respectively. Further, treatment of sub lethal concentration (40% and 80% of 96h LC50) in vivoof column-purified fraction of *B. variegate* leaf and their active component quercetin inhibit The acetylcholinesterase (AChE), acid and alkaline phosphatase (ACP and ALP) activities in the nervous tissue of *L.acuminata*. AChE activity was more inhibited than ACP and ALP in snail exposed to column purified fraction of *B. variegate* leaf and their active componentquercetin.[15]

10) Antihyperlipidemic activity

Wahab et al. Ethanolic and aqueous extracts of the stem bark and root of *B. variegate* shows antihyperlipidemic activity against albino rat. It showed significant ($p > 0.05$) reduction in cholesterol and triglyceride level. The very low density lipids (VLDL) level was also significantly reduced, with a significant increase in high density lipid (HDL).[16]

11) Immunomodulatory activity

Ghaisas MM et al reported that the ethanolic extract of stem bark of *B. variegate* shows the Immunomodulatory activity on the primary and secondary antibody responses by humoralantibody response for specific immune response. Phagocytic activity test and neutrophil activation test were evaluated by the carbonclearance and neutrophil adhesion test for a nonspecific immune response respectively. Increase in phagocytic index and percentage neutrophil adhesion at the doses of 250 and 500 mg/kg has been noted45. The acetone water, aqueous extracts and isolated compound (tannin) of *B. variegate* stem bark were screened for

their possible immune-modulatory activity by assessing nitro blue tetrazolium test, phagocytosis of killed *Candida albicans*, candidacidal assay, neutrophil locomotion and chemotaxis46. All the extracts were tested at concentrations viz. 10µg/ml, 20µg/ml, 50 µg/ml, 100 µg/ml and 1000 µg/ml. The acetone: water and isolated compound of *B. variegate* stem bark showed predominantly significant activity on *in vitro* human neutrophils.[17]

12) Hepatoprotective activity

Bodakhe Surendra H etal The some experimental studies have revealed that *B. variegate* showing antiasthematic and hepatoprotective effect. Ethanolic extract of stem bark of *B.variegata*exhibited significant hepatoprotective activity against carbon tetra chloride induced hepato toxicity in Sprague Dawleyrats at the dose of 100 and 200 mg/kg. Oral administration of ethanolic extract decreases the level of allenin-stransferase, alkaline phosphatase (ALP), gamma glutamate transferase, total lipids and increase the level of total protein which increases during the hepato toxicity and decrease the level of total protein47. Ethanolic extract of stem bark of *B.variegata*exhibit highly significant reduction (p> 0.001) in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin. The ethanolic extract has decreasing the extent of centrilobular necrosis, fatty changes and congestion of sinusoids when compared to CCl4.[18]

13) Proteinase Inhibitor

Different species of *Bauhinia* seeds inhibit blood clotting enzymes, as well as other serine and cysteine proteinases. Two varieties *B. variegate* seeds, shown to possess through *B. variegate*trypsin inhibitors, viz. *Bauhinia variegate Candida* trypsin inhibitor (Bvc TI) and *B. variegateLilac*trypsin inhibitor (BvlTI) are proteins about 20,000, with four cysteine residues forming two disulphide bridges in one polypeptide chain88.The complete sequences have been determined by automated Edman degradation of the reduced and carboxy methylated proteins of the peptides resulting from *Staphylococcus aureus*protease and trypsin digestion.[19-20]

AIM, OBJECTIVE AND PLAN OF WORK

Aim: "Astudy on Microscopical, phytochemical and pharmacognostical profile of ***Bauhinia variegate Linn."(Root).***

Objective

- Collection of plant material.
- Cleaning and drying of plant material.
- Authentication of plant by taxonomistDr.PriyankaChaturvedi.
- Organoleptic evaluation of plant part {root}.
- Determination of foreign matter.
- Microscopy of the root. .
- Successive extraction using solvents of increasing polarity like n-Hexane, chloroform, Alcohol.
- Chromatographic study-TLC (Thin layer chromatography).
- Moisture content determination by LOD (Loss on drying) method.
- Determination of Extractive value.
- Chemical test for carbohydrate, saponin, flavonoids, proteins, Alkaloids.

METHODOLOGY

Methods adopted during the study was as follows-

- Plant collection
- Physical appearance
- Foreign matter
- Microscopy
- Extraction
- Thin layer chromatography
- Moisture content
- Extractive value
- Chemical test

1. Plant collection

The kachnar plant (root) was collected from the Botanical garden of Pharmacy College Saifai, Etawah UPUMS (Uttar Pradesh University Of Medical Science).

- **Drying**

Firstly the roots was cleaned properly in order to remove the excess sand from it and then the removal of foreign particles was done which includes the small stones pieces ,lumps of soils. When the roots were cleaned then it was shade dried for 3-4 days.

2. Physical Appearance (Organoleptic evaluation)

- Colour– Evaluated by the help of the naked eyes.
- Odour- Evaluated by the help of crushing the drug in between the fingers.
- Thickness- Evaluated by the help of ruler.
- Size- Evaluated by the help of ruler.

3. Foreign Matter

The roots 5gm was taken and spread on the white sheet. By the help of 10X lens foreign particles were picked out.

4. Microscopy of kachnar plant (Root)

The root was cleaned with the help of water. Root was boil till it get soften in order to get the section. Cut the Transverse section of the root. Stained the section in safranin solution for 2-3minutes.Few drops of glycerin was added to the section to avoid the dryness of the section. By the help of the coverslip it was covered. Section was observed under microscope under 10x objective lens.

Procedure

The root was cleaned with the help of water. Root was boil till it get soften in order to get the section. Cut the Transverse section of the root. Stained the section in pholoroglucinol solution for 2-3minutes.Few drops of glycerin was added to the section to avoid the dryness of the section. By the help of the coverslip it was covered. Section was observed under microscope under 10x objective lens.

5. Successive Extraction

After getting the coarse powered form of the root it was packed in the Soxhletappatrus for the extraction process. The thimble was prepared using the filter paper and it was put in the extractor and by the use of different solvent the extraction was done.Solvent used in the extraction are mentioned below

A} n-Hexane B} chloroform C} Alcohol.

Hot Percolation (soxhlet extraction)

It is considered the best technique where the continuous heating process is involved without the loss of substantial solvent. Soxhlet has been the technique used since a long and is the min reference to which the performance of other leaching methods is compared. After final

processing, the filtration and concentration is done as required.[36]

With n- hexane /Alcohol / Chloroform Procedure

Shade dried raw material (roots) was crushed to coarse powder form. According to the need of the crushed material was packed in thimble and add with solvent into raw material was fully soaked in the solvent. Maximum it take two cycle to completely soaked the raw material with the solvent (n- hexane , alcohol, chloroform) .The temperature was kept constant at 60˚c.The hot percolation was carried out for about 48hours till the extraction becomes clear in the thimble. Then percolate was filtered and finally subjected to air dry for concentration.

6. Thin layer chromatography (TLC)

TLC is a method based on adsorption chromatography. It is at present an important analytical tool for qualitative and quantitative analysis of a number of natural products .The absorbent such a silica gel G or C is coated to a thickness of 0.3mm on clean TLC plates using commercial spreader, the plates are activated at 105˚ for 30 min. and used. The selection of mobile phase.

Depend upon type of constituents to be analyzed. After the development of chromatogram by ascending technique, the resolve spots are revealed by spraying suitable detecting agent.[37]

Solvents for n- hexane extract - (Chloroform: Ethtylacetate: Formic acid) **(**10:8:2) **Solvents for chloroform extract -**(Chloroform: Methanol)(16:4)
Solvents for alcoholic extract-(Toulene: Ethylacetate: Glacial acetic acid) (5:4.2:1.6)

Procedure

The TLC plate was prepared by using the silica gel G. Activate the plate by placing it in hot air oven for 30 minutes. Solvent system was prepared. Saturate the chamber by the solvent. Put the extract by using the capillary tube on the TLC plate. Put the plate into the TLC Chamber. The solvent run over the plate and constituent will separate according to it. Calculate the Rf value of the spot.

7. Moisture content

The percentage of active chemical constituent in crude drugs is mentioned on air dried basis. Hence, the moisture content of a drug should be determined and should also be controlled. The moisture content of a drug should be minimized to prevent decomposition of crude drug

either due to chemical change or microbial contamination.

Procedure

Weighed the 3 empty petri plates A, B, C (Initial weight), Take 5 gm of the kachnar Root powder in the petri plate. Weighed the petri plates again with the drug and the value is noted. Now put the petri plates in the oven at 105degree centigrate for 30 min. After 30 min take out the petri plates and put it in dessicator till it cools. Then weighed it again note the value(final weight). Take the 3 or till the final weight become constant, then take the average of the final weight. Using the formula Calculate the Loss on drying value(LOD).

8. Extractive value

The extract obtained by exhausting crude drugs are indicative of approximately measures of the chemical constituent. Taking into consideration the diversity in chemicals nature and properties of contents of drugs, various solvent are used for determination of extractive. The solvent used for extraction is in the position to dissolve appreciable quantities of substance desired.[37]

Extractive value

1. Water soluble extractive
2. Alcohol - soluble extractive 3 .Ether-soluble extractive **Procedure**

Take 5 gm of drugs and dissolve it in 100ml of solvent (water/alcohol/Di ethyl ether), shake it for a 15 minutes continuously. Vessel should be covered to avoid evaporation, occasionally shaking is done for 6hrs. Keep it aside for 18 hrs and then next day it was filtered. Take 25 ml of extract and dry it in petri plate, the weight of Petri plate initial (empty) and final (with dried extract + petri plate) should be noted. Percentage of extractive value can be calculated by using the formula.

Formula

$$\%\text{Extractive value} = (B\text{-}A) \times 4 \times 100/W$$ **Where,**

W= Drug taken in grams

B= Petri plate weight +dried extract weight

A= Empty dish weight

9. CHEMICAL TEST

A. Test using the chloroform extract

1. FLAVONOIDS

a) Shinoda test: To the test solution add few magnesium turning and concentrated Hydrochloric acid drop wise a pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

b) Alkaline reagent test: To test solution ad few drop of sodium hydroxide solution, intense yellow colour which turn to colorless on addition of few drop of dilute hydrochloric acid it give red colour after few minute.

2. Carbohydrates

a) Molisch's test – To the test solution add few drops of alcoholic a naphtha, add few drops of concentrated sulfuric acid through side of test tube purple to violet ring appears at the junction.

b) Barfoed's test- 1ml of test solution is heated with 1ml of barfoed reagent on water wath if red cupric oxide is formed monosaccharide is present. Disaccharides on prolonged heating may also cause reduction owing to partial hydrolysis to formed monosaccharide.

c) Benedict test- To the test solution add the benedict reagent and boil it in water bath for 2 minutes then it forms green colour.

3) Protein

a) Biuret test – To the solution add biuret reagent violet colour indicates the presence of protein.

b) Warming test- Heat the test solution in boiling water wath, protein get coagulated.

4) Cardiac glycosides

a) Killer killani test- Extract the drug with chloroform evaporates to dryness. Add 0.4ml of glacial acetic acid containing trace amount of ferric chloride. Transfer to asmall test tube add carefully 0.5ml conc. Sulfuric acid. By the side of test tube. Acetic acid layer show blue color.

b) Bali jet's test – Treat the test solution with picric acid or sodium picrate, orange colour is formed.

5) Alkaloid

a) Hager's reagent –Alkaloids gives yellow precipitate with Hagerreagent (saturated solution of picric acid)

CALCULATIONS

1. **TLC(Thin layer chromatography)-** Total distance covered by the solvent -12.5cm Total distance travelled by solute (spot)-5.9cm

RF =Distance travelled by the solute /distance travelled by the solvent =5.9/12.5 =0.47

2. **Moisture content**

Calculations of loss on drying of kachnar Root Weight of 3 empty petri plate (initial weight) namely A, B, C A- 89.65gm

B- 84.58gm C- 84.47gm

A Weight of petri plate with 5gm drug A- 94.67gm

B- 89.59gm C- 89.47gm

Weight of petri plate after 30 min (final weight - 1) A- 94.66gm

B- 89.57gm C- 89.46gm

Weight of petri plate after again 30 min (final weight - 2) A- 94.56gm

B-89.50gm C- 89.34gm

Weight of petri plate after again 30 min (final weight - 3) A- 94.55gm

B- 89.50gm C- 89.32gm

LOD of petri plate

Average Final weight of Petri plate A- 94.66+94.56+94.55 = 283.77/3 =94.59

Loss on drying of petri plate A=Initial weight - final weight

1. **LOD of petri plate A**= 94.67-94.59

=0.08

%LOD = 1.6%

LOD of petri plate B =Average Final weight of Petri plate B=89.57+89.50+89.50

=268.

= 89.5

2. **LOD of petri plate B=Initial weight - final weight**

=89.59-89.52

=0.07

%LOD =1.4%

LOD of petri plate C

Average Final weight of Petri plate C =89.46+89.34+89.32

=268.12/3

=89.37

LOD of petri plate C=**Initial weight - final weight**

=89.47-89.37

=0.1

%LOD =2%

3. Extractive value

- Extractive value of water Empty petri plate weight = 86.61gm

Weight of petri plate after drying 25ml of solvent (water) =85.80gm

%water extractive value= (B-A) ×4×100/W

=86.83-86.61×4×100/5

=17.6%

- Extractive value of alcohol Empty petri plate weight = 87.56gm

Weight of petri plate after drying 25ml of solvent (alcohol) =87.61gm

% Alcohol extractive value= (B-A) ×4×100/W

=87.61-87.56×4×100/5 =**4%**

- Extractive value of Di ethyl ether Empty petri plate weight = 85.71gm

Weight of petri plate after drying 25ml of solvent (Diethyl ether)=85.80gm

% Diethyl ether extractive value=(B-A) 4×100/W

=85.80-85.71×4×100/5

=7.2%

RESULT AND DISCUSSION

1. Plant Collection –Plant material was collected from the Herbal Garden of Pharmacy College Saifai, Etawah and the plant part were shade dried.

2. Physical Appearance (Organoleptic evaluation)

- Colour – Outer surface (brownish to dark brownish)

Inner Surface (white to creamish)

- Odour- odourless
- Thickness-0.4cm
- Size-32cm
- Foreign matters-Foreign particle in 5gm of root was found to be 0.08gm

3. Microscopy

- Microscopy of kachnar root by the Safaris staining TS Section.
- Microscopy of kachnar root by the phloroglucinol staining TS Section.

4. Extraction

Successive extraction was performed using n-hexane

Chloroform Alcohol

5. TLC(Thin layer chromatography)

SNO.	EXTRACT OF ROOT	RF VALUE
1	n- hexane extract	-
2	Chloroform extract	0.47
3	Alcoholic extract	-

Fig. 2: TLC of Chloroform Extract.

6. Moisture Content – (By loss on drying method)

Sno.	Petriplates	Initial wt. Empty petriplate	With 5gm	Avg. wt. after 30 min	Final wt.	LOD	LOD %
1.	Petriplate A	89.65	94.67	94.59	94.59	0.08	1.8
2.	Petriplate B	84.58	89.59	89.52	89.52	0.07	1.4
3.	Petriplate C	84.57	89.47	89.37	89.37	0.01	2

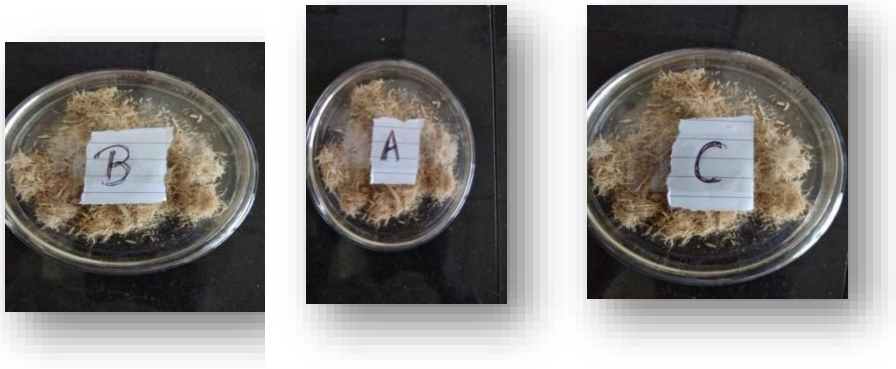

Fig. 3: Petri plate B, A, C Contaning 5gm of kachnar root powder.

8. Extractive value

S.no	Solvent system	Extractive value
1	Water	17.6%
2	Alcohol	4%
3	Diethyl ether	7.2%

Sno.	Extract	Test	Result
1	**Chloroform extract**		
a)	Flavonoid	Shinoda test	+
		Alkaline reagent test	+
b)	Carbohydrate	Molish test	-
		Barfoed test	+
		Benedict test	-
c)	Protein	Biuret test	-
		Warming test	-
d)	Cardiac glycoside	Killer killani test	-
		Baljit test	-
e)	Alkaloid	Hagers test	-
2.	**Water extract**		
a)	Saponin	Froath formation test	+
b)	Cardiac glycoside	Baljit test	+
c)	Carbohydrate	Barfoed test	-
d)	Alkaloid	Hagers test	+
3.	**N- hexane extract**		
a)	Flavanoid	Shinoda test	-

		Alkaline reagent test	-
b)	Carbohydrate	Molish test	-
		Barfoed test	-
		Benedict test	-
c)	Protein	Biuret test	-
		Warming test	-
d)	Cardiac glycoside	Killer killani test	-
		Baljit test	-
e)	Alkaloid	Hagers test	-
Observation Table of Chemical Test			

Fig.4: Chemical test of plant.

DISCUSSION

The Study on 'Pharmacognostical, Microscopical, and phytochemical Profile of ***Bauhinia variegata Linn*** (Root)' was done and various parameter was studied and are listed below:

1. Plant collection form herbal garden Pharmacy College Saifai UPUMS.
2. Physical appearance was done which includes the external nature, colour, and odour.
3. Foreign particles -0.08gm in 5gm of kachnar.
4. Successive extraction.
5. Thin layer chromatography RF value –0.47 in chloroform extract.
6. Moisture content using Loss on drying method: 1.8 petri plate A. 1.4 petri plate B. 2 petri plate C.
7. Extraction value was found to be: Water- 17.6% Alcohol-4% Diethyl ether -7.2%
8. Chemical test was performed and the presence of Flavonoids, carbohydrates, saponin, cardiac glycosides, alkaloids.

CONCLUSION

*Bauhinia variegata*Linn commonly known as "kachnar" is a small sized deciduous tree with dark brown root found in India, South Asia, Japan, China etc. The plant is reported for its various activites like anti-inflammatory, anti-oxidant, anthelmintic, hepatoprotective, antidiabetic, antimicrobial, anti tumour, anti-obesity, anti lipidimic, protease inhibitor etc.

In the present work, the phytochemical studies of this plant was performed for the identification of various phytoconstituents. The detailed phytochemical analysis of kachanar plant (root) reveals that it contains flavonoid, saponin, Alkaloid, cardiac glycoside etc. The microscopical study also shows its various internal structures. From the phytochemical screening the various phytoconstituents identified in the plant are responsible for various pharmacological activities like Fever, Diarrhea, Dysentery, Hemorrhoids, Piles, Edema, Wound healing, Obesity, Stomatitis , Dyspepsia etc.This study may help the researchers for further investigation on different models of activities. It also help in isolation of various chemical constituents which are responsible for the different pharmacological activities. Therefore, it will be helpful scientists to provide efficient, safe and cheap medication especially for traditional system of medicine.

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