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Studies on Phytochemical Profiles and Anti Microbial Activity of Leaves Extract of Bauhinia Purpurea Linn. (Ceasalpinaceae)

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Abstract: *Present paper deals with the study of photochemical profiles and antimicrobial activity of leaf extract of Bauhinia Purpurea Linn. studied with the aim of determining the following pytochemical parameters for this species: Microscopical characters; Leaf constants; Physico-chemical constants; Extractive values; Colour; Consistency; Extractive values with different solvents; Micro chemical tests; Fluorescence characters of liquid extracts and leaf powder after treatment with different chemical reagents under visible and, Measurement of cells and tissues; Bulk density angle of repose; and, Power microscopy. Hopefully, the determination of these characteristics will aid future investigators in their pharmacological analyses of this species. Preliminary pytochemical studies on different extracts of the leaves were also performed. Leaf contents, photochemical content, about 6 extract (methanol, acetone, hexane, ethyl acetate, hexane-ethyl acetate, aqueous extraction) from the leaves of Bauhinia purpurea Linn. was extracted. Hexane-ethyl acetate was characterized by Gas Chromatography- Mass Spectroscopy. The antimicrobial activity of different extract was tested against human and plant pathogenic bacteria. Hexane-ethyl acetate extract showed significant role on inhibiting almost all tested pathogenic organisms*.

Keywards: *phytochemical analysis, antimicrobial activity, Microscopical, Chromatography, Spectroscopy,*

1. INTRODUCTION

This is an ornamental plant found throughout subtropical area in India, North and South America, Nepal, Australia, Africa and United Kingdom. The plant is Botanical name of this plant is *Bauhinia purpurea Linn.* Commonly known as Mandarin in Tamil and khairwal Kachnar in Hindi. This is a medicinal plant different communities of the country traditionally used this plant for curing different diseases. Traditionally used medicinal plants have recently attracted the attention of the biological scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations plants have limitless ability to synthesize aromatic secondary metabolites, most of which are phenols or their oxygen-substituted derivatives. Extraction methods involve separation of medicinally active fractions of plant tissue from inactive/inert components by using selective solvents and extraction technology. Solvents diffuse into the solid plant tissues and solubilize compounds of similar polarity *Bauhinia Purpurea Linn.* (Khairwal) is one of the most important tropical plants in the world. It grows in the tropical and subtropical regions and its parts are commonly used in folk medicine for a wide variety of remedies. Many phenolic compounds have been detected in khairwal Kachnar peels and seed kernels. Several pharmacological activities of khairwal Kachnar extracts have been reported including anti-inflammatory 6, antioxidant ant allergic and antihelmintic and antiamoebic. In the present study, certain works such as phyto-chemical characterization, antimicrobial activity of extract, histochemical studies, and cytotoxic effects of hexane-ethyl acetate extract against cell lines.

2. MATERIALS AND METHODS:- PLANT MATERIALS

The plant material leaves of *Bauhinia Purpurea Linn.* Was collected from tropical area from the Pachmarhi District Hoshangabad (M.P) In the month of October 2013-14. The plant was identified by local people of that village and authenticated by Dr. U. K. Chauhan, Professor, of

Biotechnology, APSU Rewa; A herbarium specimen of the plant was preserved in the Department of Environmental biology and biotechnology APS University Rewa (MP) of our Institute for further reference. The leaves were separated and dried under shade, pulverized by mechanical grinder, passed through 40 mesh sieves and stored in a closed vessel for further use. All the reagents used were of analytical grade obtained from Pinnacle Biomedical Research institute (PBRI). Bharat Scout and Guide Campus (Near Regional Science Centre) Shanti Marg, Shayamla hills Bhopal (m.p.) India 462003. The microscopical characters (size, shape, colour, odour, taste, surface, texture, venation, margin, base, and petiole) of the leaves were observed the leaf extract an amount of 5gm of fresh leaves was weighted and grain using mortar and pestle with 5ml solvent. Than the solution was kept for centrifugation at 5000rpm for 15minutes and the super nateant was collected and filtered through what man no. 1 filter paper and kept it under UV for 1 hours to prevent contaminated and then stored at 50c further used. The fluorescence characters of the various extracts and powdered leaf with different chemical reagents were observed under day light and UV light (254nm&366nm), by following procedure reported by Kokoshi et al. Measurements of the cells/ tissues were made with the help of micrometer under a compound microscope. Other extractive values were determined successively starting from petroleum ether (60-80°), chloroform, acetone, ethyl acetate, methanol and distilled water by using sox let extraction apparatus. For this purpose the powder (100g) was successively hot extracted with 300ml of above solvents for 72 h. Before switching over to the next solvent, the powder under extraction (marc) was dried to remove the traces of earlier solvent. The dried extractives were obtained after evaporation of solvent under reduced pressure. The angle of repose of powder was determined by the funnel method. The accurately weighed powder was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the powders. The powder was allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation, Tan q = h/r, Where h and r are the height and radius of the powder cone. For the determination of bulk density, 2 g of powder, previously lightly shaken to break any agglomerates formed, was introduced into a 10 ml, measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5 cm at 2 seconds intervals. The tapping was continued until no further change in volume was noted. Bulk density was calculated for dried powdered drug using following formula: Bulk Density = Mass of Powder/ Bulk volume.20 Preliminary phytochemical tests of different extracts were performed by using specific reagents through standard procedures. (Figure 1, 2).

Figure 1. *Bauhinia Purpurea Lnn.* Tree

Figure2. *Bauhinia Purpurea Lnn. Eaf And Flower*

3. HISTOCHEMICAL STUDIES OF BAUHINIA PURPUREA LINN.

The uses for Fluorescence microscopy and histochemical works, fresh plant materials are collected and serial hand sections were produced. To this thin hand sections specific fluorescent and histochemical reagents were added and observed under the fluorescent microscope. By the appearance of specific colour change of storage and cellular chemicals, the presence of various histochemicals will be identified. The histochemical reagent such as Phloroglucinol-HCl, Dragendorff reagent was used for the localization of lignin and alkaloids respectively. Fluorescence studies on extracts revealed different shades of green fluorescence under UV light at 254nm and 365nm. After successive extraction with each solvent by using the soxhlet apparatus , the percentage of dry extract was calculated in terms of air-dried weight and reported: Petroleum ether (6.68 %); Chloroform (1.72%); Acetone (3.24%); Ethyl acetate (2.69%); Methanol (17.93%) ; Water (18.58%), etc. The chloroform extract was minimum whereas the water extract showed the maximum extractive value, thus indicating the presence of more polar constituents in the leaf extract. Also noted were the colour and consistency of the extracts (Table 1).

Table 1. *Fluorescence analysis of different extracts of Bauhinia purpurea Linn.. Colour Developed under UV light and percentage of dry extract.*

Extract	Long (366 nm)	Short (254nm) percentage
Petroleum ether yellow	Light green	Greenish
Chloroform	Greenish brown	Dark 1.72%
Acetone	Dark green	Light green 3.24%
Ethyl acetate	Blackish green	Green 2.69%
Methanol	Yellowish green	Green 17.93%
Water	Greenish buff. Colour solution	Dark green 18.58%

4. RESULTS AND DISCUSSION

Macroscopic as well as microscopical studies of any phytodrug are the primary steps to establish its botanical quality control before going to other studies. As per WHO norms, botanical standards are to be proposed as protocol for the diagnosis of the herbal drug. The above mentioned parameters are helpful for the future in the present study, an amount of 500gm *Bauhinia purpurea Linn.* Of leaf and solvents such as methanol, acetone, hexane, ethyl acetate, hexane-ethyl acetate and water were used for the extraction. From each sample, ~10ml extracts were collected. In this 20 % was the yield of methanol, acetone, hexane, ethyl acetate, hexane-ethyl acetate and aqueous extract of leaf. Analysis of Hexane-ethyl acetate extract analysis indicated that the hexane-ethyl acetate extract contained about 10 peaks.. Terpinyl acetate (5.80%) and phytol isomer (5.12%) are as the major constituents and this leaf extract contains the minor constituents like oxirane (3.57%), sabinene (3.24%), beta-pinen (3.34%), beta-myrcene (3.23%), cymene (3.68%), alpha-limonene (2.82%), eucalyptol (1,8-cineo (4.71%), 1,3-benzodioxole, 5-(2-, (3.68%). The phenolic profile of ethyl acetate fraction of *Bauhinia Purpurea Linn.* Khairwal Kachnar leaves and a total compounds including benzoic acid, pyrogallol, p-hydroxybenzoic acid, vanillic acid, syringic acid ferulic acid, ethyl gallate and Gallic acid were tentatively identified on the basis of spectral data and standard chemicals. The relative percentages are given in below table 2

Table2. Percentage, Composition Of *Bauhinia Purpurea Lnn. Leaf* Hexane-Ethyl Acetate Extract:-

Number of Peaks	Retention Time (minutes)	Compounds	Abundance (%)
1.	9.807	Oxirane	3.37
2.	9.005	Sabinene	3.24
3.	9.514	Beta-pinen	3.34
4.	10.725	Beta-myrcene	3.23
5.	10.896	Cymene	3.68
6.	11.073	Alpha-limonene	2.82
7.	20.223	Eucalyptol (1,8-cineo 1,3-)	4.71
8.	49.340	Benzodioxole , 5-(2- 3)	3.68
9.	52.647	Terpinyl acetate	8.80
10.	62.524	Phytol isomer	5.12

5. LEAF EXTRACT OF BAUHINIA PURPUREA LINN. AND ANTIMICROBIAL ACTIVITY

In the present study, the antimicrobial activity of different extract of. *Bauhinia Purpurea Linn.* Leaves was tested against nine bacteria (*Salmonella typhi, Klebsiella pneumoniae, Enterobacter aerogens, Mycobacterium*

tuberculosis, Streptococcus pyrogens, Pseudomonas aeuroginosa, Proteus vulgaris, Escherichia coli, and Staphylococcus aureus). In antimicrobial activity, the methanol extract showed maximum zone of inhibition against *Entrobacter aerogens* (1.3 cm). The acetone extract showed a maximum zone of inhibition against *Salmonella typhi* (3.0cm). The hexane extract showed maximum zone of inhibition against *Mycobacterium tuberculosis* (0.5 cm). The ethyl acetate extract showed maximum zone of inhibition against *Enterobacter aerogens* (1.9 cm). The hexane-ethyl acetate extract showed maximum zone of inhibition against *Streptococcus pyrogens* (2.6 cm) and also against *Salmonella typhi* (2.5 cm). It was clear from the present results, that hexane- ethyl acetate extract exhibited pronounced activity against all the tested bacteria. The presence of phytoconstituents in the leaf extracts may be responsible for the antibacterial activity of the plant. It has been documented that different solvents have diverse solubility capacities for different phytoconstituents .The difference in activities among the solvents recorded in this study may be associated with the presence of oils, wax, resins, fatty acids or pigments, which had been reported to be capable of blocking the active ingredients in the plant extract , thus, preventing the plant extract from accessing the bacterial cell wall. The result was represented in table 3.

Table 3. *Leaf extract of Bauhinia Purpurea Linn. and Antimicrobial activity against 9 bacterial strains by Kirby Bauer method (Zone of inhibition in 10 µl sample (cm).*

Microorganisms Methanol extract	Acetone	extract	Hexane extract	Ethyl acetate extract	Hexane ethyl acetate extract	Aqueous extract
Salmonella typhi	3.0	0.2	1.2	2.7	2.5	0.3
Klebsiella pneumoniae	1.0	1.3	1.2	1.9	1.5	0.4
Enterobacter aerogens	1.3	2.2	0.5	0.1	0.2	1.9
Mycobacterium tuberculosis	1.0	1.1	0.5	1.1	1.5	0.2
Streptococcus pyrogens	0.2	1.0	0.5	1.1	2.6	0.6
Pseudomonas aeuroginsa	0.8	1.4	0.1	1.2	2.3	1.0
Proteus vulgaris	0.6	1.9	0.1	0.2	0.4	0.3
Escherichia coli	0.5	1.7	0.0	1.3	1.0	0.6
Staphylococcus aureus	0.6	1.9	0.0	0.4	0.2	0.4

6. ANALYSIS OF HISTOCHEMICAL ALAIGNIN CONTENT AND ALKALOID

The stains localize specific histochemicals. Localizations include lignin content and alkaloids (Figure 3, 4). In general, parenchymatous cells showed less amount of lignin because infection with any pathogenic agent might have delayed the process of lignification in cortical and pericycle region. Localization of lignin content. and localization of alkaloids.

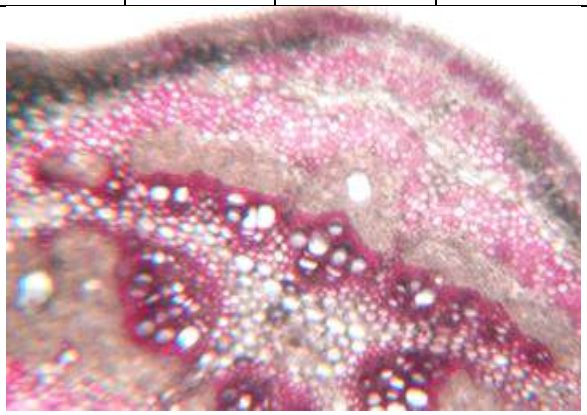

Figure 3. *Localization Of Lignin Content*

Figure 4. *Localization of Alkaloids*

7. CONCLUSION

This present study can be concluded that the Macroscopic as well as microscopical studies of any phytodrug are the primary steps to establish its botanical quality control before going to other studies the hexane-ethyl acetate leaf extract of of *Bauhinia Purpurea Linn.* Khairwal Kachnar exhibited pronounced activity against all the tested bacteria. The presence of phytoconstituents in the leaf extracts may be responsible for the antimicrobial activity. The use of medicinal plants to cure diseases has been extensively applied by people. Data from the literature as well as our results reveal the great potential of plants for the therapeutic treatment and have not been completely investigated. The above mentioned parameters are helpful for the future identification and authentification of the plant in the herbal industry and in factories. The physico-chemical standards, such as ash values, extractive values, crude fiber content and fluorescence analysis, will be useful to identify the authenticity of the drug even from the crushed or powdered plant materials. It will serve as a standard data for the quality control of the preparations containing this plant in future. The leaf constants can be included as microscopical standards in Indian herbal pharmacopoeia. Phytochemical study is also useful to isolate the phytochemical active principles present in the drugs additional studies would be need future to evaluate the potential of this leaf extract as antimicrobial agents.

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