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Thin Layer Chromatography, Extraction and Phytochemical Investigations of Celastrus Paniculatus

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***Abstract*—**The present studies show the phytochemical screening and the medicinally active constituents present in petroleum ether extract obtained from seeds of celastrus paniculatus wild. It is a useful medicinal plant which gives benefit in different field of medicines as well as pharmacological. The present research work is aimed to investigate and focus the light on the chemical-constituents of seeds of valuable medicinal plant C. paniculata wild. The preliminary phytochemical studies show the presence of terpenoids, steroids, saponins, flavonoids, carbohydrates, glycoside etc. The result of the study could be useful for identification and preparation of monograph of the plant Extraction, TLC.

***Keywords*—**Celastrus paniculatus; extraction, phytochemical screening; thin layer chromatography.

I. INTRODUCTION

Celastrus Paniculata is one of the most important medicinal plant of Celastraceae family locally is known as "Jyotishmati", Malkangni. It is used as a brain tonic to promote intelligence and to sharpen the memory. The green plants are the storage house of many chemicals which may act as a role of secondary metabolites "compounds which conduct metabolic activities". The seed oil is intellect promoting & used for curing epilepsy seeds yield as much as 52% oil by weight which is also useful in abdominal disorders headache, joint pain, leucoderma, paralysis, ulcer etc, oil stomachic tonic good for cough, asthmas and also used in leprosy. It is brain clearer and believed to promote intelligence.

Botanical Description of C. Paniculata

C. Paniculata is wild woody liane belongs to the Family celustraceae. The plant is commonly known as Malkangni, Black oil tree, Intellect tree, "jyothismathi" in the Ayurvedic system of medicine. It grows throughout India at the height of almost 1800-2000 meters. Because of its high medicinal values & destruction of habitat, this species has faced the stage threat and its abundance is very less in tropical forests of India and it has reached the stage of vulnerable.
Scientific Classification Kingdom: *Plantae* Family: *Celastraceae* Species: *Celastrus* Genus: *Paniculata*.

II. MATERIALS AND METHODS

Collection of plant materials: The fresh specimen and seeds of selected plant C. paniculatus having medicinal value were collected from the forest of Rahatgarh hills, Sagar district in Madhya Pradesh India.

Extraction and Preliminary Phytochemical Screening

1. Extraction of Seeds of C. Paniculata

The seeds were dried in shade and used for analytical work. About 100 gm of shade dried seeds were made into powder form by using electrical grinder. The powdered material was filled in the thimble of soxhlet apparatus and exhaustively extracted with petroleum ether (400c) for about 48 cycles. The solvent was distilled off at low temperature under vacuum and concentrated on water-bath to get thick syrup after extraction with Petroleum ether the material was refluxed with other solvents like Benzene Chloroform, alcohol and finally with water .

2. Phytochemical Screening

The phytochemical screening of the plant is carried out by testing of different class of compounds using standard methods to identify the compound showing in table I.

Test for Terpenoids

Libermann-Burchard test: Extract treated with few drops of acetic anhydride, boil and cool, concentrated sulphuric acid is added the side of test tube, shows brown ring at the junction of two layer and the upper layer turns green which shows the presence of sterols and formation of deep red colour indicate the triterpenoids.
Salkowski's test: Treat extract in chloroform with few drops of concentrated sulphuric acid, shaken well and allow to stand for some time, red colour appear in the lower layer indicate the presence of sterols and formation of yellow coloured lower layer indicate the presence of triterpenoids.

Test for Flavonoids

Shinoda test (Magnesium hydrochloride reduction test): To the test solution add few fragments of magnesium ribbon and concentrated hydrochloric acid drop wise, pink scarlet, colour appears after few minutes indicating the presence of flavonoids.
Ferric chloride test: To the test solution, add few drops of ferric chloride solution, intense green colour was formed to show the presence of flavonoids.

Test for Carbohydrate

Molisch test: Treat the 2 ml of test solution with few drops alcoholic α-napthol solution in a test tube and then 1 ml of

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concentrated sulphuric acid was added carefully along with side of the test tube. Formation of vateiolet ring at the junction indicates the presence of carbohydrates.

Fehling's test: Equal volume of Fehling solution A and Fehling solution B are mixed and few drops of sample is added and boiled, a brick red precipitate indicate the presence of reducing sugar.

Test for Tannins

Ferric chloride test: Some amount of extract was dissolved in distilled water to this solution 2 ml of 5% ferric chloride solution was added. Formation of blue green indicates presence of tannins.

Lead acetate test: Some amount of extract a few drops of lead acetate solution was added. Formation of precipitate indicates presence of tannins.

Test for Saponins

Foam test: The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Test for Glycoside

Borntrager's test: To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene was added and shake it welled. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red colour in ammonical layer indicates presence of anthraquinone glycoside.

Keller-Killiani test: To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue colour in the acetic acid layer indicates the presence of Cardiac glycosides.

Separation of Chemical Constituents

The purity of each eluted sample was tested by using TLC method. It is a technique used to separate wide range of compounds of biochemical interest. It can be utilized to quantitative as well as qualitative and preparatory work [Stahl, 1965]. The petroleum ether extract was subjected to thin layer chromatography about 0.1-0.2 ml of conc. Pet. ether extract was loaded on the plate by using capillary tube. During spotted plates were carefully dried and used for elution purpose. Initially various solvents such as benzene, pet ether, chloroform ethanol were tested alone. Later different combinations of solvents were tested depending on polarity basis. The spotting was done at the centre of plate three spots were appeared on the plate. The spotting plate was carefully dried and used for elution purpose. Different solvent systems ranging from lower polarities to higher polarities were tested for the separation of bioactive components. The TLC plates were observed under UV light and the separated spots were marked.

a) Development of chromatogram: The eluted spotted plates were dried at room temperature and they were placed in iodine chambers for the development of chromatogram. The Rf values of cleared sport were calculated & proper solvent system was identified Rf. values determined are shown in table no. II.

b) Column chromatography: 50 ml of concentrated petroleum ether were dissolved in 10 ml of benzene. The activated silica gel H is added slowly to benzene solution and absorbs pet ether extract. The chromatograms are allowed to develop Elution was started after, the formation of complete bands and it was adjusted to 12-15 drops per mm. Nearly 10 ml of eluted solvent was collected in a clean bottle of 50 ml capacity and was labeled by given number 5.

Table I. Preliminary Phytochemical screening of Extract of seeds of C. paniculata.

S. No	Name of phytoconstituents	Presence/Absence
1	Terpenoides	+ + ve
2	Flavonoids	- ve
3	Carbohydrates	+ ve
4	Tannins	+ ve
5	Alkaloids	+ ve
6	Saponins	+ ve
7	Oil	+ ve
8	Volatile oil	- ve
9	Steroids	+ ve

++ = Higher presence of Phytoconstituents + = Lower presence of Phytoconstituents.

Table II. Results of TLC of petroleum ether extract of C. Paniculata.

S. No	Name of class of compound	Rf value of fraction
1	Terpenoids	0.74
2	Carbohydrates	0.36
3	Alkaloids	0.23

Solvent system: Chloroform: n Haxen: Dimethyl formamide (DMF) (5:3:2).

Stationary Phase: Silica gel. 60-120 mesh size (Merk).

Thin layer chromatography

III. RESULTS AND DISCUSSIONS

Table No. I of the results of preliminary phytochemical screening of petroleum ether extract seeds of *C. Paniculata* of shows that the seed extract was rich in chemical know as

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Phytoconstituents, such as terpenoids, tannins, saponins and steroids petroleum ether extract was subjected to TLC in order of separate and identify the bioactive compounds present in the seeds of C. penticulata in the present research work the most suitable T.C.L. system for analysis was shown to be terpenoids, saponins, tannins and steroids with the largest discriminating power TLC plates shown in the fluorescence light under UV at 254-365 nm wavelength and find these active spots in TLC plate with following Rf values (0.74, 0.36, 0.23) these values indicates the presence of terpenoids.

IV. CONCLUSION

The petroleum ether extracts obtained through solvent extraction by Soxhlet apparatus from Celastrus paniculata. Seeds of C. Paniculata plant have been raw material for the synthesis of many drugs and thus remain an important source of new therapeutic agent. It is found that C. paniculata oil has a beneficial effect on the learning and memory process in mentally retarded children. The pet ether extract obtained from C. paniculata though successive solvent extraction in order of prove that the ethno pharmacological applications of the plant in Indian folk medicines. Phytochemical screening of C. paniculata is preliminary and important aspect. It is concluded from the data that petroleum ether extracts of Celastrus paniculatus seeds exhibited significant role in medicinal chemistry for formulation of life saving drugs.

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