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PHOTOCHEMICAL SCREENING OF PLANTS *MUCANA PRURITA, MESUA FERREA, PUNICA GRANATUM*

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ABSTRACTS

India has a rich culture of medicinal herbs and spices, including Ayurvedic, Unani, Siddha and other traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value. According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements. Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. As far as population growth is concerned India will be the leading country within few years of time span. Current pandemic population explosion demands an immediate betterment of new potential contraception. Thus, there is growing need to look for aphrodisiac more from natural plant or herbal origin as opposed to synthetic compounds which are known to cause severe unwanted side effects. In this regard, we have taken the plants Mucuna prurita, Mesua ferrea, punica granatum that has been used traditionally as antifertility agent in women and aphrodisiac, so that the couples without issue may be benefited for better acceptance in society and they may have better psychological health. In these plants so many secondary metabolites are present i.e carbohydrates, glycocides, alkaloids, tannins, phytosterol etc. All these three plants have pharmacological activity like Astringent, anthelimentic, nervine tonic, aphrodisiac, diuretic, vermifuge and stimulant, anodyne, antidotal, psychedelic, leucorrhea, spermatorrhea, facial paralysis and powerfully aphrodisiac, leprosy, scabies, skin diseases, pruritus, haemorrids, ulcers, depsia, impotency, leucorrhoea, haemoptysis, cephalalgia, fever and cardiac debility.

KEYWORDS: haemorrids, ulcers, depsia, impotency, leucorrhoea, haemoptysis, cephalalgia.

INTRODUCTION

PHYTOCHEMICAL STUDIES

1. Introduction of Phyto-chemical extraction

The phyto-chemical investigation of a plant may involve authentification and extraction of the plant material; separation and isolation of the constituents of pharmaceutical Interest, characterization of the isolated compound; investigation of the biosynthetic pathway to particular compounds and the quantitative evaluation. The choice of extraction procedure depends on the nature of the plant materials and the components to be isolated. The size reduction of the dried plant materials is an important factor for extraction. If the particle size are too fine a solid cake may be produced, which will affect the flow of menstrum and will result in the formation of 'dry pockets' within the body of the material. If the materials is too coarse then interstics are formed, which leads to a speedy percolation of menstrum this can even lead to an incomplete extraction with a need of excessive volumes of metnstrum to exhaust the marc. The composition of the drug or the nature of the drug i.e. hard or soft, thick or thin will affect the degree of comminution need not to great. If the materials used are hard and woody, then the sized is required to be greatly reduced while some of the substances likes aloes or gum resin need only to be crushed. Materials to be powdered roots, rhizomes, bark, corms, woods. There are five officials grade of powder. The medium course or the moderately fine powders are most suitable for the purpose of extraction.

Most often used mesh size for extraction purpose are 25 and 45 mesh mesh sizes. The degree of commination also depends upon the solvent to be used for the extraction. The crude materials which are used in the dried state should not contain moisture above 4 to 5%. The proper condition of the drug would lead to the rapid penetration of the solvent (water or a dilute alcohol) through the plant tissue, while use of strong alcohol would shrink the tissue as the water with in the tissue will be pulled to the surface. Water immiscible solvent like light petroleum is used in the extraction of essential oil, fixed oils and steroids and solvent like ether and chloroform are used in the extraction of alkaloids, quinines.

The importance of solvent in the herbal therapeutic is so precise that could not have advanced so far without it. There are various kinds of solvents widely used thought industry and households. Solvent differ from each other, not only in differing boiling points, but also on

how they react or affect other substances in which they come in constant. In order to maintain the synergy of the herbal preparation, it is crucial that the plant compounds do not decompose or dissociate or form complex when it is in contact with a solvent. There are several method of extraction as follows

- **Expression:** It is the physical process in which pressure is applied to squeeze the oil out of the materials or juices from plants. This was usually achieved with a tincture press.

- **Infusion:** Infusion are prepared by soaking a drug in water for a specified period of time. The process can be either hot or cold, depending upon the types of the ingredients present as decomposing may occur at higher temperatures. Infusion is generally prepared for immediate use, as preservatives are absent. Infusion are used for soft tissues like petals, leaves etc.

- **Decoction:** It is a similar process of infusion but the ingredients are boiled with that of water for a specified period of time or till a definite volume is attained. The decoction is used when the preparation is prepared using hard plant parts likes root, bark, wood etc.

- **Maceration:** It is a process differed from the above two processes in a way that the drug is left in contact with the menstrum, (usually alcohol and sometimes water), for a longer periods of time. The usual procedure follows is that the liquid is added to the drug in a closed vessel for seven days with occasional shaking, the menstrum is strained, the marc is pressed to obtain the remaining liquid, the two solutions are mixed and clarifying by filtering or by standing.

- **Hot percolation:** It is a soxtlet method, applied for continuous extraction of partially soluble solids using volatile solvent. The solvent taken in the round bottom flask, it is heated to boiling the vapor rise through the outer chamber and into the condenser where it gets condensed into liquid and fall back in to the bottom of soxtlet chamber. When the distilled solvent rise in the chamber, it pass through the permeable cellulose extraction thimble and the solvent extract the compounds of interest and solid mass is left behind. This is followed by the rise in level of the solvent which forces the solution through the small inner tube due to siphoning effect. The solvent that reaches the flask, takes with it the soluble component along and then the solvent 7is redistilled from the solution in the flask and gets condensed in the chamber. This process can be repeated as many times as necessary depending upon the requirement.

- **Cold percolation**: It is one of the simplest and the traditional method of extraction used by herbalist throughout the world. A cone and tube is suspended above a flask or vessel. The bottle of the tube consists of perforated base which holds in it the powdered drugs. Solvents is poured into the top of the tube consist of performed base which holds in it the powdered drug. Solvent is poured into the top of the tube where it soaks the drugs leaching out the extract and then falling out into the flask. The extraction can be facilitated by wrapping the percolation tube in heating tape.

- **High pressure – supercritical fluid extraction:** Phytoconstituents present in plant can be extracted for medicinal purpose, by using a nontoxic CO_2 as a solvent. This process is called supercritical fluid extraction. Supercritical fluids extraction can be an alternative technique for soxtlet extraction with lower consumption of solvent and a lower working temperature. it also help in selecting the fluid polarity and density letting the adjustment of the solvating power of the fluid, enabling a class –selective extraction.

1.2 Purification of Solvents

(a) Petroleum Ether: The petroleum ether was distilled and the fraction boiling between 60-80°C was collected and used for extraction and chromatographic studies.

(b) Chloroform: The chloroform was shaken well with equal volume of distilled water twice to remove water soluble impurities and separated by using separating funnel. It was further dried over anhydrous calcium chloride for 24 hours, filtered and dried subsequently with anhydrous potassium carbonate for 24 hours. This was decanted and distilled; the fraction boiling at 64°c was collected and stored in an amber colored bottle.

(c) Ethanol: A dry round bottom flask was fitted with a double surface condenser and a calcium chloride guard tube. Dry magnesium turnings (5gm) and iodine (0.5gm) were placed in the flask followed by 50-75ml of commercial absolute alcohol. The mixture was warmed until the magnesium is converted to ethanolate, then 900m1 of commercial absolute alcohol was added and refluxed for 30 minutes. The ethanol is directly distilled into vessel and used.

1.3 Preparation of Plant material for extraction: The collected plant materials i.eof shade dried aerial part of ***Mucuna prurita, Mesua ferrea, punica granatum*** was washed, cut into small pieces and were allowed to dry in the shade, then they are pulverized in mixer grinder to coarse powder and passed through mesh size 40 sieve.

Preparation of Plant Extracts

- **Requirements**

(a) Dry coarse powder of aerial part of ***Mucuna prurita, Mesua ferrea, punica granatum***

(b) Large cork-stoppered glass bottle with wide mouth

(c) Petroleum ether (60-80^{0}C)

(d) Ethanol

(e) Aqueous (Water).

- **Procedure**

1.3.1 Preparation of Petroleum ether extract: The shade dried, coarse powder of the aerial part of ***Mucuna prurita, Mesua ferrea, punica granatum*** (300gm) was packed well in a soxhlet apparatus and extracted with petroleum ether (60-80°C) until the extraction was completed which was confirmed by the color of the siphoned liquid. The extract was filtered while hot and the resulting extract was distilled in vacuum in order to remove the solvent completely and subsequently dried in a dessicator. The extract was weighed and calculated the percentage yield in terms of air dried material.

1.3.2Preparation of Ethanol extract: The marc left after petroleum ether extract was dried completely in hot air oven below 50°C and then packed well in soxhlet apparatus and extracted with ethanol until the extraction was completed. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure to remove the solvent completely and dried in a dessicator. Weighed the obtained extract and calculated its percentage yield in terms of air dried powdered crude material.

1.3.3Preparation of Aqueous (Water) extract: The marc left after ethanol extraction was dried in hot air oven below 50°C and packed well in soxhlet apparatus and extracted with distilled water until the completion of the extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely and dried in a dessicator. Weighed the extract and calculated its percentage in terms of air dried powdered crude material.

I.4 FLUORESCENCE STUDIES

Evaluation of different extracts based on the fluorescence in day light is likely to be unreliable due the weakness of the fluorescence effect. Some extracts may show fluorescence when the sample is exposed to ultraviolet radiation. Hence, the crude extracts were examined for the characteristic fluorescence and reported as in **Table I.2**

I.5 Phytochemical Analysis of Petroleum ether, Ethanolic and Aqueous extract of *Mucuna prurita, Mesua ferrea, punica granatum* the crude extracts were examined for the characteristic photochemical analysis fluorescence and reported as in **table 1.3.**

I.6 CHROMATOGRAPHIC ANALYSIS

The term ***"chromatography"*** is derived from greek, ***chroma*** meaning *"**color"*** and ***graphein*** meaning "***to write***". The chromatographic technique is now widely used for the separation, identification, and determination of the chemical component in complex mixture. it is a separation process applicable to essentially molecular mixture and relies on distribution of mixture between an essentially to dimensional or thin phase and one or more bulk phase which are brought into contact in a differentials counter current manner. Chromatography is a non-destructive procedure for resolving a multi-component mixture of trace, minor, or major constituents into its individual's fraction. Chromatography may be defined as a method of separating a mixture of component into individual component through equilibrium distribution between two phases the technique of chromatography is based on the differences in the rate at which the component of a mixture move through a porous medium called stationary phase, under the influence of some solvent or gas called moving phases. Chromatography is one of the widely used physiochemical method of separation of inorganic and organic substances related in their composition and properties. in general, chromatography is an effective method of separating element by their non-uniform distribution between a mobile and stationary phase. The mobile phase can be a gas (gas chromatography) or liquid (liquid chromatography), while the stationary phase can be liquid or a solid. the chromatography method of analysis are classified by different features, namely by the state of aggregation of the phase used, by the nature of sorbate- sorbent interaction (the separation mechanism), by the techniques used.

TLC analysis of various extracts of powder of whole plant *Mucunaprurita, Mesuaferrea, punicagranatum*is as follows

Procedure: 100gm of silica gel G was weighed and made into a homogenous slung with approx. 200gm of sufficient distilled water to form slurry. Then the slurry was poured into TLC glass plates by spreading technique and the thickness of silica gel layer on TLC plate was adjusted to about 0.25 mm thickness. The coated plates were allowed to dry in air and activated by heating in hot air oven at 100-105°C for 1 hour.The extracts were prepared with the respective solvent like petroleum ether, ethanol and water and made up

to 10ml in different test tubes. Then the extracts were taken in a capillary tube and it was spotted in glass plates coated with silica gel G. The plates were developed in TLC chamber previously saturated with different solvent systems.

I.6.3 Column chromatography

Principle: Each compound in a mixture will have a particular solubility in the solvent and a particular tendency to be absorbed by the solid adsorbent. Mostly no two compounds behave exactly alike in these respects. This principle is utilized in column chromatography.

Details of Column chromatography

Adsorbant : Silica gel (for column chromatography 60-120 #)
Eluent : Petroleum ether to distilled water in gradation
Length of column : 60 cm
Diameter of column : 3.5 cm
Amount of Ethanolic extract used: 5 gm
Length of column packed: 40 cm
Rate of Elution : 30 drops per minute
Fractions collected : Each of 10 ml

Procedure: The column with the cotton plug is filled with the sufficient silica gel (60-120 #) up to 40 cm in the given column height of 60 cm and 3.5 cm width. The column was carefully packed and uniformly filled with silica gel, by tapping the side of the column. Then the ethanolic extract of powder of whole plant of ***punica gratum*** was charged on column and eluted with different solvents ranging from non-polar to polar at the rate of 30 drops per minute. Each fraction was collected in the volume of 10 ml with different solvent ratio as given in Table II.7.

I .7 SPECTRAL STUDIES

(a) UV ABSORPTION SPECTROSCOPY

The measurement of absorption of ultraviolet and visible radiation provides a convenient means for the analysis of numerous inorganic and organic species. The wavelength in UV region is usually expressed in nm that is 200 - 400nm.

Instrument used : Varian Cary 5E
Solvent : Methanol
Wavelength : 200 – 400 nm
Speed : Fast

The UV spectra of isolated fraction 1 and fraction 2 were done. The sample solution was prepared in benzene and the same benzene was used as blank. UV scan was done between 200 - 400 nm and the speed of instrument scanning was set as fast. The peak absorbance obtain for both fractions are tabulated as follows

Sample	λ max. (nm)	Absorbance
Fraction 1	280.00	0.26738
Fraction 2	275.00	1.78985

(b) FTIR SPECTROSCOPY

The IR region (4000-450 cm^{-1}) is of great importance in studying organic compounds. Since the IR spectrum contains a large number of bonds, no two bonds will have the same IR spectrum (except the Optical isomers). Thus the IR spectra can be regarded as the finger print of the molecule.

Fourier Transform Infra Red Spectroscopy of the selected two fractions i.e **fraction 1 and fraction 2** of the ethanolic extract of powder of whole plant of ***Mucunaprurita, Mesuaferrea, punicagranatum*** obtained from the coloum chromatography was investigated for its characteristic functional groups. All the peaks shown by IR spectroscopy are shown below

I.8 HPTLC ANALYSIS

I.8.1 HPTLC Tool for standardization: Standardization manufacturing procedures and suitable analytical tools are required to establish the necessary frame work for quality control in herbals. Among those tools separation techniques include high performance liquid chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC) and capillary electrophoresis are most widely used methods to establish reference fingerprints of herbals, against which raw material as well as finished products can be assayed.

HPTLC also known under the name of planner chromatography is a modern powerful analytical technique with better resolution, performance and reproductability superior to

classics TLC. Based on the use of HPTLC plates with small particle size and precise instruments for each steps of the chromatographic p[procedure (Sample application, Chromatograph Development and Chromatograph Evaluation). HPTLC provides the means for demanding quantitative determination. Instruments can be easily validated and are fully compliant with GMP.

For the analysis of herbals, HPTLC offers a number of advantages. The technique is especially suitable for comparison of samples based on fingerprints. Finger print analysis by HPTLC is one of the most powerful tools to link the botanical identity to the chemical constituent profile of the plant. From constituent profile, a number of marker compounds can be chosen, which might further describe the quality of herb ort the herbal preparation. HPTLC can also be employed for quantitative determination of such marker compounds.

➢ PROCEDURE FOR HPTLC ANALYSIS

STEP I

Sample Application: The samples to be chomatographed were applied on the precoated HPTLC plates of silica Gel F^{254} Volume precision and exact positioning was ensured by the use of suitable instrument i.eCamagNanomat ,Camag Capillary Dispenser , CamagLinomat IV, Camag Automatic sampler.

STEP II

Chromatographic Development: The conventuional way to develop a planner chomatograph is to immerse the plate with its lowest edge in the developing solvent contained in the tank. The solvent rises in the; layer by capillary action whereby sample components are separated. When the solvent front had reached the desired length the run is terminated.

Various developmental modes are available i.eCamag Flat BottomChamber ,CamagLKight Weight Developing Chambers, Camag Twin trough Chambetr, CamagAutomative Developing Chamber (ADC) . In this process the samples are separated into fractions.

STEP III

Chromatogram Evaluation: The track was scanned in densitometer with a light beam in the visible or ultra violet range of spectrum. Absorbance or fluorescence is measured by diffused reflectance i.eCamag TLC scanners with CATS software. CATS stand for CAMAG TLC

software.Alternatively to classical densitometry, the chromatogram can be evaluated by video technology i.e video scan and video store. It has been developing software for densitometric evaluation of thin layer chromatogram and electrophoresis.

REQUIREMENTS

1) **TLC Plates :** Silica gel F 254
2) **Scanner :** CAMAG TLC SCANNER 3
3) **Chamber:** CAMAG twin through (20x10 cm)
4) **Applicator :** CAMAG Linomat IV
5) **DOCUMENTATION:** CAMAG REFROSAR 3 video documentation
6) **SOFTWARE :**-WIN CATS
7) **SOLVENT SYSTEM :** *(Toluene- ethyl acetate)* ***(9.5 :0.5)***
8) **SCANNING WAVELENTH :**256nm

PROCEDURE

1) Sample preparation
2) Standard preparation
3) Chromatographic condition
4) Sample application
5) Chromatograph Development
6) Chromatograph Evaluation and Estimation
7) Results

RESULT AND DISCUSSION

Percentage yields of extracted pet-ether, ethanol and aqueous extract were calculated and as are in Table I.1.

Table- I.1. Percentage yields of pet ether, ethanol andaqueous extract of *Mucuna prurita, Mesua ferrea, punica granatum*

S. No.	Extracts	% Yield
1	Petroleum ether extract	0.52
2	Ethanolic extract	3.43
3	Aqueous extract	8.1

Table I.2 Fluorescence Study of Various Extracts of Powder of Aerial Part of *Mucuna Prurita, Mesuaferrea, Punica Granatum*

S. No.	Extracts	Day Light	UV Light(254 nm)
1	Petroleum ether extract	Brown	Light Green
2	Ethanolic extract	Yellowish Brown	Dark Green
3	Aqueous extract	Yellowish orange	Light Green

I.5 Phytochemical Analysis of Petroleum ether, Ethanolic and Aqueous extract of *Mucuna Prurita, Mesua Ferrea, Punica Granatum*

The Constituents Present In Different Extracts Of Powder Of Whole Plant Of *Mucuna Prurita, Mesua Ferrea, Punica Granatum* Are Given In Table *I.3.*

Table I.3 Qualitative Phytochemical Analysis of Various Extracts of Powder of Whole Plant of *Mucuna Prurita, Mesua Ferrea, Punica Granatum*

Plant constituents	Petroleum Ether extract	Ethanol extract	Aqueous extract
Alkaloids	-	+	+
Saponins	-	-	-
Glycosides	-	-	+
Carbohydrate	-	-	+
Tanins & Phenolic compounds	-	-	+
Flavonoids	+	+	+
Phytosterols	+	-	-
Proteins & amino acids	-	-	-
Triterpenoids	-	-	-
Fixed oil & fats	+	-	-
Gums & Mucilage	+	+	+

(+) Present

(-) Absent

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Plant constituents	Petroleum Ether extract	Ethanol extract	Aqueous extract
Alkaloids	-	+	+
Saponins	-	-	-
Glycosides	-	-	+
Carbohydrate	-	-	+
Tanins & Phenolic compounds	-	-	+
Flavonoids	+	+	+

Phytosterols	+	-	-
Proteins & amino acids	-	-	-
Triterpenoids	-	-	-
Fixed oil & fats	+	-	-
Gums & Mucilage	+	+	+

(+) Present

(-) Absent

For isolation and resolution of spots of the three extracts the following solvent systems were selected by trial and error method.

- **FOR MONOTERPENES**

(Benzene- ethyl acetate) ***(8 : 2).***

- **FOR BETA SITOSTEROL**

(Toluene- ethyl acetate) ***(9.5 :0.5).***

- **FOR EUGENOL**

(N-hexane-ethyl acetate) ***(9 : 1).***

(Toluene- ethyl acetate) ***(9.3 : 0.7).***

- **FOR URSOLIC ACID**

(Carbon tetrachloride-ethyl formate-formic acid) ***(6.6 : 3.3 : 0.07).***

(Toluene- acetone- acetic acid) ***(9 : 0.3 : 0.7).***

- **FOR FLAVONES ACID**

(Chloroform- acetic acid- water) ***(5 : 4.5 : 0.5).***

(Toluene- chloroform- acetone) ***(4 : 2.5 : 3.5).***

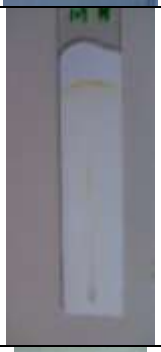
The different spots developed in each solvent system were identified by means of Iodine vapors and UV light and the R_F values were correspondingly calculated and presented in.

Table I.4 for Petroleum ether extract of powder *Mucuna prurita, Mesua ferrea, punica granatum*

Table I.5 for ethanolic extract of powder of *Mucuna prurita, Mesua ferrea, punica granatum*

Table I.6 for Aqueous extract of powder of *Mucuna prurita, Mesua ferrea, punica granatum*

Table I.4 TLC OF Petroleum ether extract of powder of *Mucunaprurita, Mesuaferrea, punicagranatum*

S. No.	TLC for Phytoconstituent	Solvent system	No. of spots	Colour of spots	R_f values	Picture
1	**MONOTERPENES**	*(Benzene- ethyl acetate)* ***(8 : 2)***	2	Light blue Blue	0.24 0.52	
2	**BETA SITOSTEROL**	*(Toluene- ethyl acetate)* ***(9.5 :0.5)***	1	Bluish green	0.82	
3	**FOR EUGENOL**	*(N-hexane-ethyl acetate)* ***(9 : 1)***	3	Blue Dark blue Dark blue	0.20 0.62 0.81	
4.	**FOR EUGENOL**	*(Toluene- ethyl acetate)* ***(9.3 : 0.7)***	1	Bluish green	0.52	
5.	**FOR URSOLIC ACID**	***(****Carbon tetrachloride-ethyl formate-formic acid)* ***(6.6 : 3.3 : 0.07)***	1	green	0.56	

6.	**FOR URSOLIC ACID**	*(Toluene- acetone- acetic acid)* ***(9 : 0.3 : 0.7)***	2	Blue Dark blue	0.37 0.41	
7.	**FLAVONES ACID**	*(Chloroform- acetic acid- water)* ***(5 : 4.5 : 0.5)***	NO SPOT	-	-	-
8.	**FLAVONES ACID**	*(Toluene- chloroform- acetone)* ***(4 : 2.5 : 3.5)***	2	Bluish green Dark blue	0.77 0.45	

Table I.5 TLC OF Ethanol extract of powder of *Mucunaprurita, Mesuaferrea, punicagranatum*

S. No.	TLC for Phytoconstituent	Solvent system	No. of spots	Colour of spots	R_f values	Picture
1	**MONOTERPENES**	***(****Benzene- ethyl acetate****)*** ***(8 : 2)***	1	Light blue	0.52	
2	**BETA SITOSTEROL**	***(****Toluene- ethyl acetate****)*** ***(9.5 :0.5)***	1	Bluish green	0.85	

3	**FOR EUGENOL**	***(****N-hexane-ethyl acetate****)*** ***(9 : 1)***	2	Dark blue Dark blue	0.21 0.60	
4.	**FOR EUGENOL**	*(Toluene- ethyl acetate)* ***(9.3 : 0.7)***	1	Bluish	0.50	
5.	**FOR URSOLIC ACID**	***(****Carbon tetrachloride-ethyl formate-formic acid****)*** ***(6.6 : 3.3 : 0.07)***	1	Bluish green	0.57	
6.	**FOR URSOLIC ACID**	*(Toluene- acetone- acetic acid)* ***(9 : 0.3 : 0.7)***	2	Blue Dark blue	0.35 0.42	
7.	**FLAVONES ACID**	*(Chloroform- acetic acid- water)* ***(5 : 4.5 : 0.5)***	NO SPOT	-	-	-
8.	**FLAVONES ACID**	*(Toluene- chloroform-acetone)* ***(4 : 2.5 : 3.5)***	NO SPOT	-	-	-

Table I.6 TLC OF Aqueous extract of powder of *Mucunaprurita, Mesuaferrea, punicagranatum*

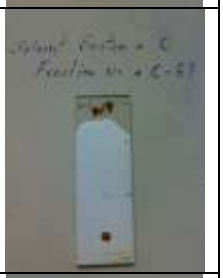
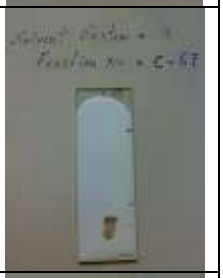
S. No.	TLC for Phytoconstituent	Solvent system	No. of spots	Colour of spots	R_f values	Picture
1	**MONOTERPENES**	*(Benzene- ethyl acetate)* ***(8 : 2)***	NO SPOT	-	-	-
2	**BETA SITOSTEROL**	*(Toluene- ethyl acetate)* ***(9.5 :0.5)***	NO SPOT	-	-	-
3	**FOR EUGENOL**	*(N-hexane-ethyl acetate)* ***(9 : 1)***	1	Dark blue	0.81	
4.	**FOR EUGENOL**	*(Toluene- ethyl acetate)* ***(9.3 : 0.7)***	1	Bluish green	0.53	
5.	**FOR URSOLIC ACID**	*(Carbon tetrachloride-ethyl formate-formic acid)* ***(6.6 : 3.3 : 0.07)***	NO SPOT	-	-	-
6.	**FOR URSOLIC ACID**	*(Toluene- acetone- acetic acid)* ***(9 : 0.3 : 0.7)***	NO SPOT	-	-	-
7.	**FLAVONES ACID**	*(Chloroform- acetic acid-water)* ***(5 : 4.5 : 0.5)***	NO SPOT	-	-	-
8.	**FLAVONES ACID**	*(Toluene- chloroform-acetone)* ***(4 : 2.5 : 3.5)***	NO SPOT	-	-	-

Table I.7 COLUMN CHROMATOGRAPHY OF ETHANOLIC EXTRACT OF POWDER of whole plant of *Mucuna prurita, Mesua ferrea, punica granatum*

S. No	Solvent Fraction	No. of Spots	R_f Value of Spots	Colour of Spots
1	Petroleum ether (100)	0	-	-
2	Pet. Ether : Benzene(75:25)	0	-	-
3	Pet. Ether : Benzene(50:50)	0	-	-
4	Pet. Ether : Benzene(25:75)	0	-	-
5	Benzene (100)	0	-	-
6	Benzene : Chloroform (75:25)	1	0.62	Blue
7	Benzene : Chloroform (50:50)	1	0.61	Light Blue
8	Benzene : Chloroform (25:75)	1	0.64	Dark Blue
9	Chloroform (100)	1	0.61	Light Blue
10	Chloroform : Ethyl acetate (75:25)	0	-	-
11	Chloroform : Ethyl acetate (50:50)	0	-	-

12	Chloroform : Ethyl acetate (25:75)	0	-	-
13	Ethyl acetate (100)	0	-	-
14	Ethyl acetate : Methanol (75:25)	1	0.82	Bluish Green
15	Ethyl acetate : Methanol (50:50)	1	0.81	Bluish Green
16	Ethyl acetate : Methanol (25:75)	1	0.82	Bluish Green
17	Methanol (100)	0	-	-
18	Methanol : Water (75:25)	1	0.21	Light Blue
19	Methanol : Water (50:50)	0	-	-
20	Methanol : Water (25:75)	0	-	-
21	Water (100)	0	-	-

TLC FOR various fractions (RANDOM)

Fraction No.	Solvent System	Detector	Rf (detected)	Rf (Standard)	Picture
70	Benzene-Metenol (10 : 1)	Iodine vapor	0.63	0.75	
69	benzene- ethyl acetate (8 : 2)	Iodine vapor	0.80	0.72	
68	benzene- ethyl acetate (8 : 2)	Iodine vapor	0.56	0.72	
67	toluene- ethyl acetate (9.3 : 0.7)	Iodine vapor	0.43	0.33	
64	toluene- ethyl acetate (9.3 : 0.7)	Iodine vapor	0.30	0.33	

Fraction No.	Solvent System	Detector	Rf (detected)	Rf (Standard)	Picture
68	Toluene-ethyleacetate (9.3 : 0.7)	Iodine vapor	0.26	0.72	
68	Benzene-ethylacetate (8 : 2)	Iodine vapor	0.56	0.72	
67	Benzene-ethylacetate (8 : 2)	Iodine vapor	0.43	0.72	
67	Toluene-ethyleacetate (9.3 : 0.7)	Iodine vapor	0.37	0.33	
66	Benzene-methenol	Iodine vapor	0.30	0.75	
66	Toluene-ethyleacetate (9.3 : 0.7)	Iodine vapor	0.64	0.72	

Fraction No	Solvent System	Detector	Rf (detected)	Rf (Standard)	Picture
65	Benzene-methenol	Iodine vapor	030	0.75	
65	Toluene-ethyleacetate (9.3 : 0.7)	Iodine vapor	0.56	0.33	
65	Benzene-ethylacetate (8 : 2)	Iodine vapor	0.35	0.72	
64	Benzene-ethylacetate (8 : 2)	Iodine vapor	0.30	0.72	
64	Toluene-ethyleacetate (9.3 : 0.7)	Iodine vapor	0.65	0.33	

Fraction No.	Solvent System	Detector	Rf (detected)	Rf (Standard)	Picture
63	Benzene-methenol	Iodine vapor	0.45	0.75	

63	Toluene-ethyleacetate (9.3 : 0.7)	Iodine vapor	0.60	0.33	
63	Benzene-ethylacetate (8 : 2)	Iodine vapor	0.41	0.72	
62	Benzene-ethylacetate (8 : 2)	Iodine vapor	0.50	0.72	
61	Toluene-ethyleacetate (9.3 : 0.7)	Iodine vapor	0.61	0.33	
61	Benzene-methenol	Iodine vapor	0.60	0.75	

TLC for specific Fraction 1 and 2 (Selective)

TLC FOR MONOTERPENES

Solvent System	Detector	Rf (detected)	Picture
Benzene- ethyl acetate (8 : 2)	Iodine vapor	0.60	

Benzene- ethyl acetate (8 : 2)	Iodine vapor	0.68	

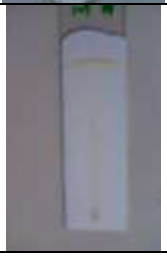
Result- The given sample may contain monoterpenes as per TLC profile.

TLC FOR ROSEMARINIC ACID:

Solvent System	Detector	Rf (detected)	Picture
Toluene- ethyl acetate (9.5 : 0.5)	Iodine vapor	0.54	
Toluene- ethyl acetate (9.5 : 0.5)	Iodine vapor	0.55	

Result- The given sample may contain rosemarinic acid as per TLC profile.

TLC FOR EUGENOL

Solvent System	Detector	Rf (detected)	Picture
N-hexane- ethylacetate (9 : 1)	Iodine vapor	0.54	
toluene- ethyl acetate (9.3 : 0.7)	Iodine vapor	0.53	

Result- The given sample may contain Eugenol as per TLC profile.

TLC FOR URSOLIC ACID

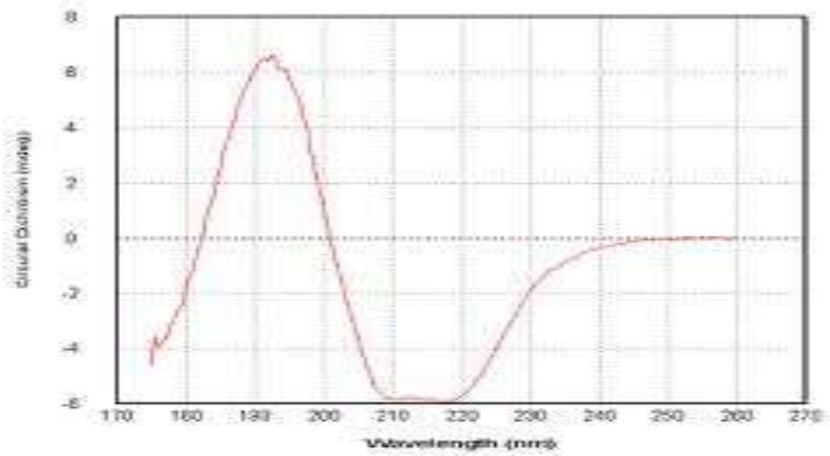
Solvent System	Detector	Rf (detected)	Picture
Carbon tetrachloride-ethyl formate-formic acid (6.6 : 3.3 : 0.07)	Iodine vapor	0.57	
toluene- acetone- acetic acid (9 : 0.3 : 0.7)	Iodine vapor	0.60	

Result- The given sample may contain ursolic acid as per TLC profile.

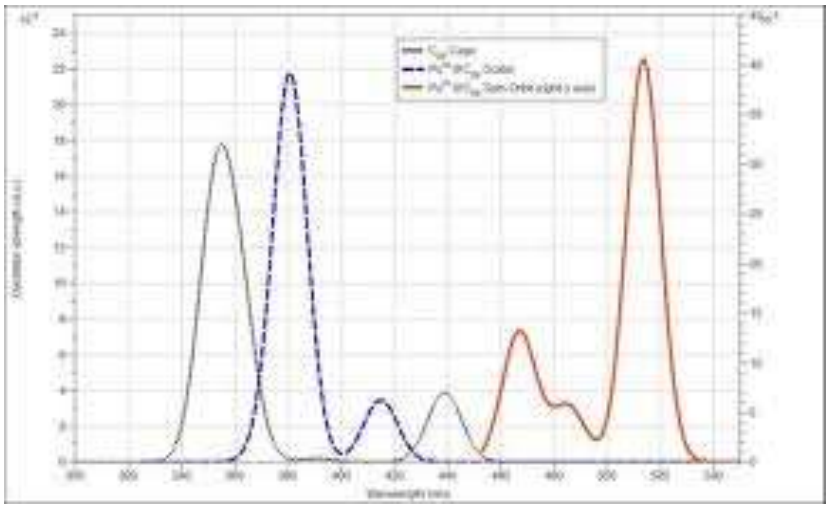
TLC FOR FLAVONES ACID:

Solvent System	Detector	Rf (detected)	Picture
Chloroform- acetic acid-water (5 : 4.5 : 0.5)	Iodine vapor	0.71	
toluene- chloroform-acetone (4 : 2.5 : 3.5)	Iodine vapor	0.67	

Result- The given sample may contain flavones as per TLC profile.

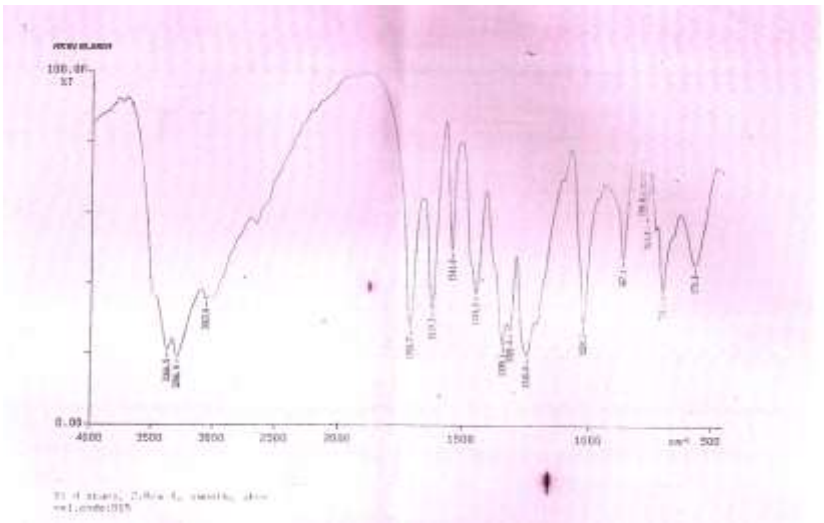


UV Spectra of Fraction 1



UV Spectra of Fraction 2

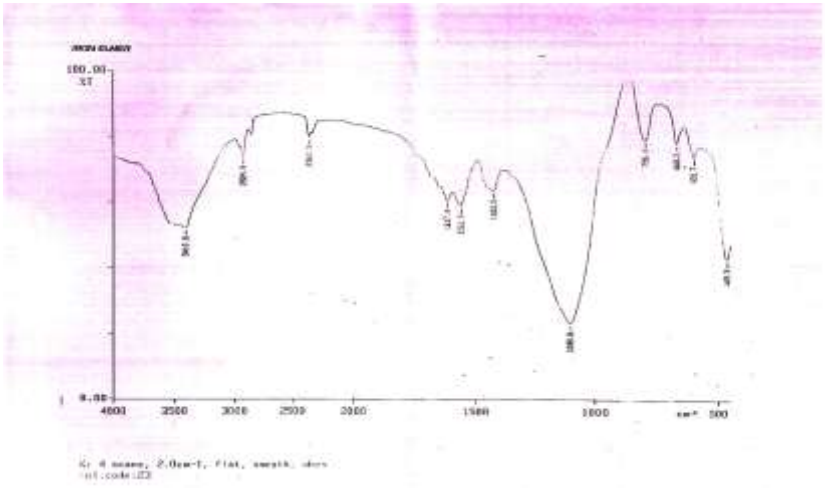
FTIR SPECTRA OF FRACTION 1



➢ **FTIR data for fraction 1**

- It Shows absorption peak in the region of 1450-1660 cm^{-1} at 1541 and 1619 which indicates presence of aromatic group.
- The absorbance band found in the region of 1700-2000 cm-1 indicates presence of carbonyl group.
- The band extends in the region of 2000-3100cm-^{1}at 3063 indicates the presence of C-H (Alkenes).
- The peak at 3366.5 cm^{-1} indicates the presence of Amine group.

FTIR SPECTRA OF FRACTION 2



➢ **FTIR data for fraction 2**

- It Shows absorption peak in the region of 1300-1500 cm^{-1} at 1423 which indicates presence of conjugation.
- The absorbance band found in the region of 1617.4 is due to aromatic group.
- The band extends in the region of 2000-2500cm-^{1}at 2362 indicates the presence of Sulphur group.
- The absorbance band found in the region of 2500-3000cm^{-1} at 2925.9 indicates the presence of alkyl group.
- The absorbance band found in the region of 3000-3500cm^{-1} at 3410.6 indicates the presence of Alchol group.

1) ETHANOLIC EXTRACT

From Etanolic extract of powder of whole plant of ***Mucuna prurita, Mesua ferrea, punica granatum.*** Beta sitosterol was isolated and quantitative estimation was carried out by HPTLC

HPTLC DATA

a) Rf of Sterol = 0.41

b) AUC of Sterol = 19007.8

c) Conc of Sterol = 5ug

d) Conc of extract = 100ug

e) Corresponding AUC of Extract = 1607.1

Thus 19007.8 AUC = The 5ug of sterol

Than 1670.1 AUC of extract = X ug of sterol

X= 1670.1*5/19007.8

X=0.4393ug of Sterol

RESULT: Sterol in petroleum ether extract = 0.4393%w/w of extract.

2) PET ETHER EXTRACT

From Pet Ether extract of powder of whole plant of ***Mucuna prurita, Mesua ferrea, punica granatum.*** Eugeneol was isolated and quantitative estimation was carried out by HPTLC.

HPTLC DATA

a) Rf of Eugeneol = 0.85

b) AUC of Eugeneol = 807.00

c) Conc of Eugeneol = 5ug

d) Conc of extract = 100ug

e) Corresponding AUC of Extract = 591.10

Thus 807.00 AUC = The 5ug of Eugeneol

Than 591.10 AUC of extract = X ug of Eugeneol

X= 591.10*5/807

X=3.66ug of Eugeneol

RESULT: Eugeneol in etanolic extract = 3.66%w/w of extract.

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