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Qualitative and Quantitative Analysis of plants Extracts: *Mucana prurita, Mesua ferrea, Punica granatum*

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Abstract

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 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 like 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.

Key-words: Metnstrum, Extraction, Biosynthetic, Quantitative, Characterization

Introduction

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 (Ghosh and Bhattacharya, 2004). Family planning has been promoted through several methods of contraception, including oral contraceptives. Therefore, there is a need of drug which is effective but with lesser side effects (Thakare *et.* al.,2009). Global search on anti-fertility agents is going on, to tackle the problem of population explosion. Many hormonal drugs are available for the purpose but they are not free from side effects. Hence, the search for a suitable product from indigenous medicinal plants is proposed which could be effectively used in the place of oral Pills (Singh, 1990).

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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 (Shahu,2014). The practice of traditional medicine for the control of fertility in most parts of Africa is based on the use of plant medicines for many years. The fact that the herbal medicines have been employed for such a long time there are no reports on both ethno botanical and pharmacological profile of these plants **(**Ramya *et al*.,2011 **)**.In fertility regulation; the ancient literature has mentioned the use of a number of plants/preparations as abortifaciant and local contraceptives. WHO and ICMR provide systemic guidelines for the evaluation

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of anti-fertility plants to generate reproducible results, i.e., proper authentication and systemic screening methods. Many plant preparations are reported to possess antifertility properties in ancient Indian literature. Many plants have been tested for their anti-fertility activity in laboratory animals. Only one plant through vascicine from *Adhatoda vasika* have been claimed to have abortifaciant properties but it could not be used clinically; the centchroman, non hormonal oral contraceptives also has not been proved very successful in phase IV clinical trials. Hence, the search needs to be continued **(**Kachroo and Agrawal, 2011**).** This may reduce the population burden of our country.

Aphrodisiacs are the substances which are used to increase sexual activity and help in fertility. Sexual feelings are an inevitable part of life. The basic and fundamental purpose of sex and sexuality is the "continuation of progeny" and the survival of human race (Kothari P,2001**).** The sex is the most intimate, indispensable and an integral part of every individual and can be a source of pleasure and fulfillment. The sexual myths and misconception also leads to sexual dysfunctions (Kar and Chandra, 2005**).**

Infertility is a worldwide medical and social problem. It affects above 10-15% of married couples. WHO estimates that there is 60-80 million infertile couples worldwide (Badami et al,2000). Infertility in itself may not only threaten physical health but it can certainly have a serious impact on the mental and social wellbeing of infertile couple, especially in our country. In many countries the stigma of infertility often leads to marital disharmony, divorce or ostracism (Khanna, 1992). Research during the past two decades has an unfolded focus on impotence (erectile failure), premature ejaculation and male infertility. There are a number of prescription drugs which may act as sex stimulant and enhancing the sexual desire and activity in both men and women, although their use have shown significant improvement in treating sexual disorders, but at the same time they are not devoid of large number of side effects. These include arrhythmia, suicidal tendencies, mental disorders, tremors etc. The use of synthetic aphrodisiacs results in the dilatation of blood vessels in other parts of the body also, causing headache and even fainting. Other side effects include facial flushing, blurred vision and sensitivity to light which usually occur at higher doses (Kulkarni and Reddy, 1998)**.**

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.

Plant Profile

1. Mucuna prurita

- **Common name:** Kaunch Beej
- **Family:** Leguminosae
- **Habitat:** It is found almost all over the country
- **Parts used:** Seeds, roots and legumes

Pharmacological action

Astringent, anthelimentic, nervine tonic, aphrodisiac, diuretic, vermifuge and stimulant, anodyne, antidotal, psychedelic, leucorrhea, spermatorrhea, dyspepsia, colic, hemiplegic, and facial paralysis and powerfully aphrodisiac **(**Kumar and Saha,2013**).**

Chemical Constituents

L-dopa, sulphur, manganese, 4-dihydroxy phenylalanine, glutathayon, lacithine, galic acid and glucoside. These seeds contain saturated fatty acid stearic and palmitic acid, oliac acid and linolinic acid (http://www.singleherbs.org/products/kaunch.htm Retrieved on date 10-2-14 at 2.40 pm).

Medicinal Uses

- **Digestive system:** it is used in intestinal worms and colic
- **Central nervous system:** It is used in paralysis, hemiplegic and other nervine Disorders and spasms associated with Parkinson's or Bell's palsy.
- **Genito- urinary system:** It is used in leucorrhea and profuse menstruation
- **Reproductive system:** It is used as an aphrodisiac in seminal weakness and spermatorrhea.

2. *Mesua ferre*

- **Common name:** Nagkeshar
- **Family:** Guttiferae
- **Habitat:** Eastern Himalayas, Assam, West Bengal, Western Ghats, Travancore, and Andaman
- **Parts used:** Flower (stamen), oil, bark, leaf, bud, seed.

Chemical constituents: Glutathayon, lacithine, galic acid and glucoside. These seeds contain saturated fatty acid stearic and palmitic acid.

Medicinal Uses

- It has astringent, digestant, and ant poisonous, antimicrobial, anti-inflammatory, antipyretic and anthelminthic.
- It is used in fever, itching, nausea, leprosy, skin disorders, erysipelas, bleeding piles, metrorrhagea, menorrhagea, excessive thirst, and sweating.
- In bleeding piles, it should be used in the dose of 3 gm along with mishri.
- Its oil should be use externally to cure skin disorders.

3. *Punica granatum*

- **Common Name:** Anar
- **Family Name:** Lythraceae
- **Part Used:** Seed, Roots, Leaves
- **Habitat:** Southern Europe, Northen Africa, tropical Afrika, Central asia, India, America, california

Constituents: Vitamin C, vitamin K , polyphenols, such as ellagitannins and flavonoids, Pomegranate seed oil contains punicic acid , palmitic acid , stearic acid, oleic acid and linoleic acid .Juice, seeds and peel apparently contain steroid hormones, including estrone (Kho *et al*,2010).

Uses

- It is used in diarrhoea, dysentery and intestinal parasites.
- The seeds and juice are considered a tonic for the heart and throat, especially when sweet; pomegranate fruit is nourishing for (*pitta* or fire) systems and is known as a blood builder. The astringent qualities of the flower juice, rind and tree bark are considered valuable for a variety of purposes, such as stopping nose bleeds and gum bleeds, toning skin, firming-up sagging breasts, and treating haemorrhoids.
- Pomegranate juice (of specific fruit strains) is also used as an eye drop, as it is believed to slow the development of cataracts. It is being used as a contraceptive by means of consuming the seeds, or rind, as well as by using the rind as a vaginal suppository. This practice is recorded in ancient Indian literature, in medieval sources, and in modern folk medicine**(**John Riddl, 1992**).**

Material and Methods

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 (60-800C)
(d) Ethanol

Procedure

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 desiccators. Weighed the obtained extract and calculated its percentage yield in terms of air dried powdered crude material.

TLC analysis of plant *Mucuna prurita, Mesua ferrea, Punica granatum*

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.

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 ***Mucuna prurita, Mesua ferrea, Punica granatum*** 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.

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

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 in result and discussion.

HPTLC Analysis

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 F254 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

Nuclear Magnetic Resonance Spectroscopy

NMR for structural analysis relies on the few basic principles. Certain nuclei (^{1}H, ^{13C}) have spin states, thus behave like tiny bar magnets with magnetic dipole moments in the presence of magnetic field the nuclei can align either towards or against the magnetic field. Nuclei aligned with a field are in a lower state than those aligned against the induced field. If electromagnetic radiation of frequency is applied to the sample lower energy nuclei aligned along the direction of the field will absorb the energy and flip its orientation. The excited nuclei will than return to its original state emitting radio frequency energy as it returns. These changes in absorption/emission of energy due to resonating nuclei can be detected& plotted to give NMR spectrum.

NMR spectrophotometers have a specific operating frequency, which is depended on the strength of the applied magnetic field. A 500 MHz spectrophotometer has a much stronger magnetic field than 60 MHz spectrophotometer. The higher the operating frequency, the better the resolution of the spectrum. Additionally, different nuclei have different magnetogyric ratios or "Spin Properties". Therefore each require different radio frequency for resonance at any given magnetic field strength.

Procedure used

Fraction1 and 2 isolated were undertaken NMR in $CDCL^{3}$ and reference used was TMS (Tetra Methyl Saline) in 10mm sample tube. The spectrum was recorded over 4000Hz range on FT 80, A-80 MHz. Spectra were interpreted as below:-

Mass Spectroscopy

Mass spectrometry is an analytical technique which can provide information concerning the molecular mass of organic and inorganic compounds. The tools of mass spectrometry - the mass spectrometers - are characterized by the technique used to separate ions according to their m/z values and/or by the way ions are formed (Ionization techniques).

Mass spectrophotometers can be used in combination with separation technique such as gas chromatography and liquid chromatography (GC or LC). These combination instruments that accomplish separations prior to mass spectrometry are sometimes referred as "Hyphenated Instruments" e.g., Gas Chromatograph-Mass spectrophotometer (GC-MS) or Liquid chromatography-mass spectrophotometer (LC-MS).The techniques of Mass spectrophotometer, (GC-MS) or Liquid chromatography-mass spectrophotometer (LC-MS) have dominated much of the use of spectrometry in organic analysis.

Procedure used

Fraction1 and 2 isolated were undertaken for Mass Spectrum (LC- MS) by electron impact ionization on various MAT 1020. Direct inlet probe at 270^{0}C and electron energy 70eV.

Results and Discussion

Column Chromatography Of Ethanolic Extract of Powder Of Whole Plant Of *Mucuna Prurita, Mesua Ferrea, Punica Granatum*

S. No	Solvent Fraction	No. of Spots	Rf Value of Spots	Color 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	1	0.61	Light Blue

	(50:50)			
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	-	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	_	_

Column Chromatography of Plant Extracts *Mucuna prurita, Mesua ferrea, Punica granatum* gives 4 Fractions Which Are Given Below

S. No	Solvent Fraction	No. of Spots	Rf Value of Spots	Colour of Spots
1	Benzene:Chloroform (75:25)	**1**	0.62	Blue
2	Benzene:Chloroform (50:50)	1	0.61	Light Blue
3	Benzene:Chloroform (25:75)	1	0.64	Dark Blue
4	Chloroform (100)	1	0.61	Light Blue

Thin Layer Chromatography of plants *Mucuna prurita, Mesua ferrea, Punica granatum* Gives Two Spots Which Are Given Below

S. No	Solvent Fraction	No. of Spots	Rf Value of Spots	Colour of Spots
1	**BETA SITOSTEROL** *(Toluene- ethyl acetate)* ***(9.5 :0.5)***	**1**	0.82	Bluish green
2	**EUGENOL** *(Toluene- ethyl acetate)* ***(9.3 : 0.7)***	1	0.52	Bluish green

Fluorescence Study of Various Extracts of Powder of Aerial Part of *Mucuna prurita, Mesua ferrea, Punica granatum*

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

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	-	-	+
Carbohydrates	-	-	+
Tannins and phenolic compounds	-	-	+

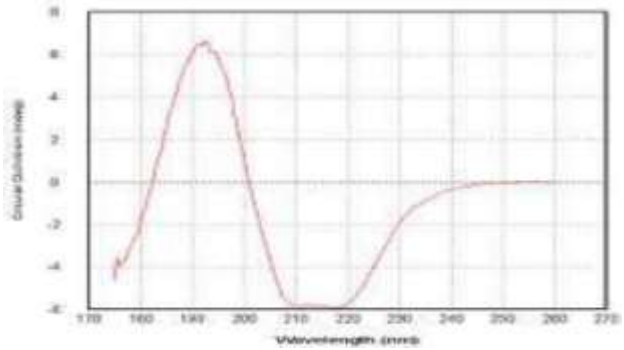
Flavanoids	+	+	+
Phytosterol	+	-	-
Proteins & amino acids	-	-	-
Triterpenoids	-	-	-
Fixed oil & fats	+	-	-
Gums & Mucilage	+	+	+

(+) Present (-) Absent

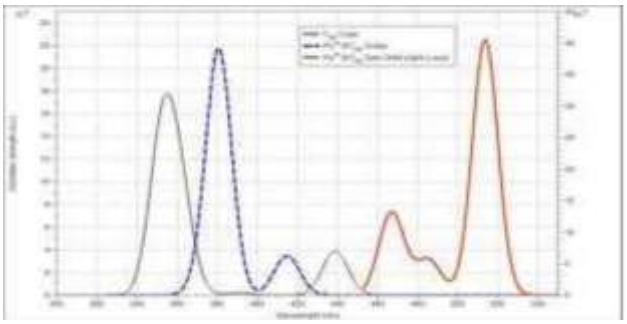
UV Spectra

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



UV Spectra of Fraction



UV Spectra of Fraction 2

FTIR data for fraction 1

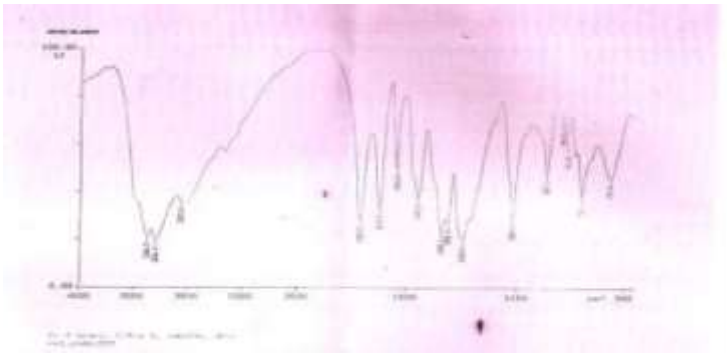
It Shows absorption peak in the region of 1450-1660 cm-1 at 1541 and 1619 which indicates presence of aromatic group.

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-1at 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 1



FTIR data for fraction 2

It Shows absorption peak in the region of 1300-1500 cm-1 at 1423 which indicates presence of conjugation.

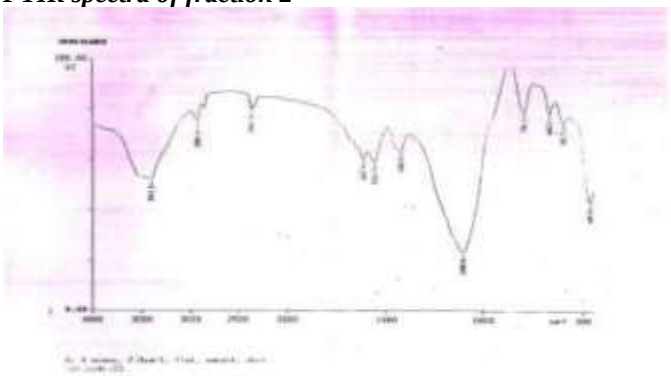
The absorbance band found in the region of 1617.4 is due to aromatic group.

The band extends in the region of 2000-2500cm-1at 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.

FTIR spectra of fraction 2



HPTLC Data

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.

NMR & Mass Spectra Interpretation of Fraction 1

There are four chemical shifts (δPPM) seen in NMR spectra of fraction 1, which are as follows:-

a) First chemical shift is at 2.35 δPPM , this peak indicates Triplate multiplaicities (2.3695 ;2.3456 ; 2.3202) of signal with one proton.

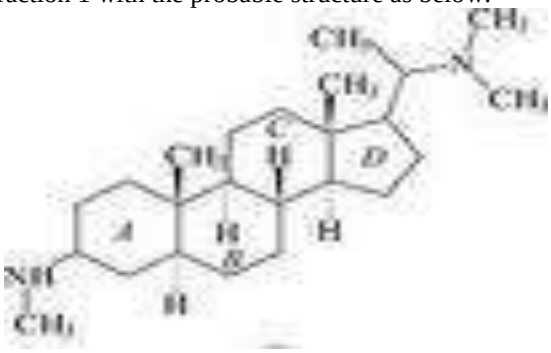
b) Second chemical shift is at 1.65 δPPM , this peak indicates Quadrate multiplaicities (1.6557;1.6326;1.6213;1.6118)of signal with two protons.

c) Third chemical shift is at 1.25 δPPM , this peak indicates Singlate multiplaicities (1.2539) of signal with maximum no of protons i.e 40 protons.

d) Last chemical shift is at 0.85 δPPM , this peak indicates Triplate multiplaicities 0.8991;0.8795 ;0.8568)of signal . This shift is probably of TMS (Tetra Methyl Saline)

A mass spectrum of fraction 1 implies the base peak at 73.9 m/e and the total mass of the compound as299.7 m/z.

Thus the overall instrumental analysis results in the probability of any ketoxy steroidal nucleus ring in the fraction 1 with the probable structure as below:-



NMR & Mass Spectra Interpretation of Fraction 2

There are seven chemical shifts (δPPM) seen in NMR spectra of fraction 2, which are as follows:-

a) First chemical shift is at 3.95 δPPM, this peak indicates doublet multiplicities (3.8961; 3.9571) of signal with maximum no of protons i.e. 7 protons.

b) Second chemical shift is at 6.36 δPPM, this peak also indicates doublet multiplicities (6.3561 ; 6.3644)of signal with one proton.

c) Third chemical shift is also a doublet at 6.55 δPPM, this peak indicates doublet multiplicities (6.55497 ; 6.5571) of signal with one proton.

d) Fourth chemical shift is at 6.72 δPPM , this peak indicates doublet multiplicities (6.7364 ; 6.7066)of signal with one proton .

e) Fifth chemical shift is also a doublet at 7.25 δPPM , this peak indicates doublet multiplicities (7.2223 ; 7.2601) of signal with three protons.

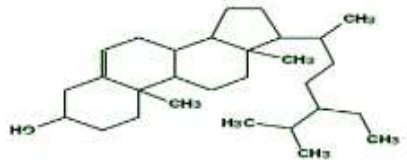
f) Sixth chemical shift is at 11.39 δPPM, this peak indicates singlet multiplicities (11.3993) of signal with one proton

g) Last seventh chemical shift is at 11.98 δPPM , this peak indicates singlet multiplicities (11.9884) of signal with one proton.

No shift of TMS (Tetra Methyl Saline) is prominent in the spectra.

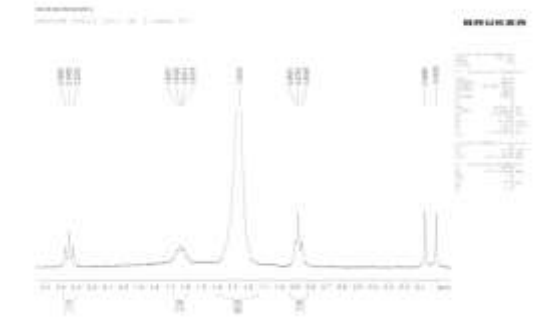
Mass spectra of fraction 2 imply the base peak at 321.5 m/e and the total mass of the compound as 4854. M/z Thus the overall instrumental analysis results in the probability of any Beta Sito-steroidal nucleus ring in the fraction 2 with the probable

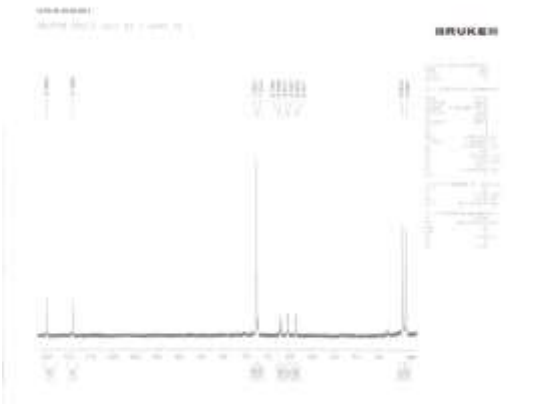
structure as below:-



Beta sito-sterol

Mass Spectra of Fraction 1





Mass Spectra of Fraction 2

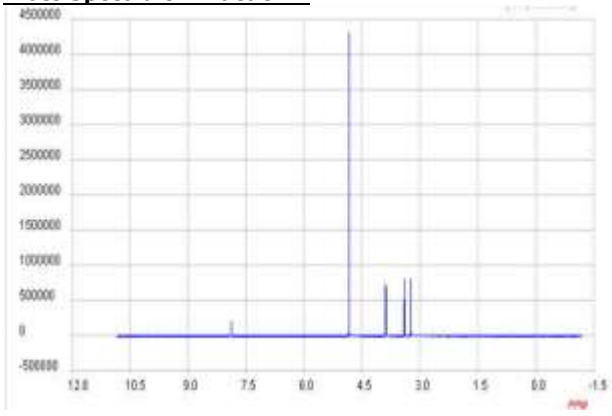
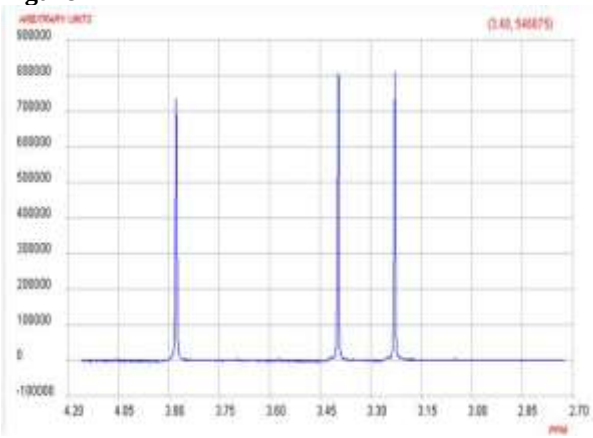


Figure 1



Figures 2- In this figure, three singlets are observed. The first peak between 3.15-3.30 represents the hydrogen's on the methyl group that is connected to the single bonded nitrogen. The peak between 3.30-3.45 represents the hydrogen's proton of the methyl group next to the double bonded oxygen and double bonded carbon. The third peak between 3.75-3.90 represents the hydrogen protons present on the methyl group between the two carbons with the double bonded oxygen. The deshielding due to the nitrogen's near all the hydrogen's causes the shift in the spectra. This is most evident in the third peak because the hydrogen's are located near a carbon that is attached to nitrogen near two carbons with double bonded oxygen

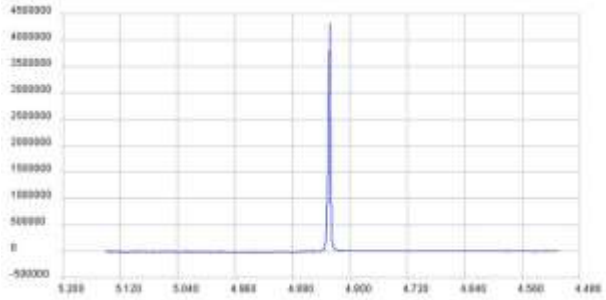


Figure 3: In this figure, one peak is observed due to the solvent used to conduct the H NMR. A D2O was used and caused this large peak between 4.800-4.880

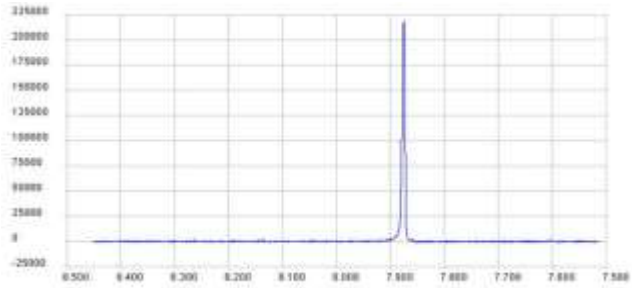
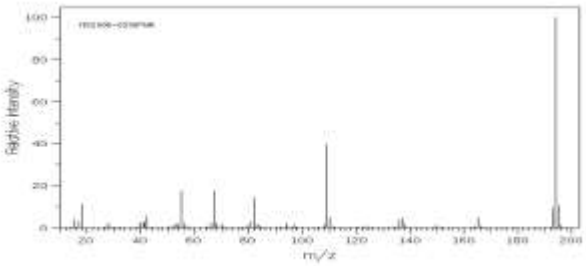


Figure 4- In this figure, one peak is observed due to the hydrogen proton located on the carbon between the two nitrogen groups. The DE shielding due to the carbons are located next to nitrogen that cause a great shift in the spectra. After checking the intergration data, it is apparent that one hydrogen is represented by this peak.

Mass Spectra

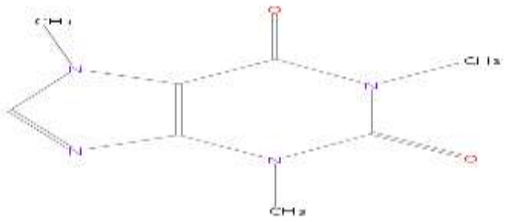
MS2006-03097WA SDBS NO. 1898
C8H10N4O2 (Mass of molecular ion: 194)
Reasonable guess of the assignments but we can't be completely sure till we compare With similar compounds JCB]



Peak data

15.0	3.8
17.0	2.6
18.0	11.2
28.0	2.1
40.0	2.4
41.0	2.5
42.0	5.4
52.0	1.1
53.0	1.6
54.0	1.7
55.0	17.5
56.0	2.0
66.0	2.2
67.0	17.3
68.0	2.1
70.0	1.6
81.0	3.1
82.0	14.2
83.0	1.4
83.5	1.1
94.0	1.9
97.0	1.4
108.0	1.7
109.0	39.7
110.0	4.9
136.0	3.8
137.0	4.4
138.0	1.7
165.0	4.8
193.0	9.3
194.0	100.0
195.0	10.6
196.0	1.0

Mass of molecular ion: 194



Caffeine

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References

1. Badami S, Desai V B, Suresh B. Drugs play a major role in male fertility. Express Pharma Pulse 2000; 2: 18
2. Ghosh K and Bhattacharya T K. Preliminary study on the anti implantation activity of compound from the extracts of seeds of thespesiapopulnea. India Journal of Pharmacology. 2004; 36: 288-291
3. John Riddle. Contraception and Abortion from the Ancient World to the Renaissance. Harvard University Press, Cambridge, Massachusetts; London, England.1992, 25.
4. Heftmann, Shui-TzeKo and Raymond D. Bennett. Identification of estrone in pomegranate seeds. Erich Phytochemistry. 1966;5,(6):1337–1339
5. Kachroo Monica and Agrawal S S. Anti-implantation activity of different extract of the peels of Citrus medica Linn. International Journal of PharmTech Research. 2011;3 (1): 535-539,
6. Kothari P. Common Sexual Problems. 3rd edition, UBS publishers, New Delhi, 2001; 1(6): 155, 203, 204, 221.
7. Kar N, and Chandra G. Comprehensive Textbook of Sexual Medicine. 1st edition, Jaypee Brothers Medical Publishers. New Delhi, 2005; 280-285, 309-313.

8. Khanna J. Prevention of infertility. Office of Publication, W.H.O., Geneva, 1992; 32: 161-166.
9. Kulkarni S K and Reddy D S. Pharmacotherapy of Male Erectile Dysfunction with Sildenafil. Indian Journal of Pharmacology. 1998; 30 : 367-378
10. Kumar Pradeep and SahaSudipta. An updated review on Taxonomy. Phytochemistry, Pharmacology and Toxicology of Macunapruriens. 2013; 2 (I) 1:306-314
11. Ramya R, Sivasakthi R, Senthilkumar C, Anudeepa J, Santhi N, Venkata Narayanan N: Preliminary phyto chemical and anti fertility Studies on Dodonea viscose Linn.Asian Journal Res Pharm Sci, 2011; 1 : 77-79
12. Singh Shiv Pal. Fertility Control of Female through SesbaniSesbian Seeds. The Journal of Research and Education in Indian Medicine. 1990; 9 (4): 27- 32.
13. ShahuAlakh N. Phyto-pharmacological review of mesuaferrealinn. International Journal OfPhytopharmacology. 2014; 5(1): 6-14.
14. Thakare V N, Kothavade P S, Dhote V V, Deshpande A D. Antifertility Activity of Ethanolic Extract of Allium cepaLinn in Rats. International Journal of PharmTech Research. 2009;1 : 73-78
15. Young Lim Kho, Woong Jung, Dohyeong Kwon and Jung HoanKim.identification of estrone in pomegranate (Punicagranatum) extracts by liquid chromatography-tandem mass spectrometry. Food Science and Biotechnology,2010;19, (3):809-813,
16. http://www.singleherbs.org/products/kaunch .htm Retrieved on date 10-2-14 at 2.40 pm

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