

A SPECTROSCOPIC STUDY OF MUCANA PRURITA, MESUA FERREA, PUNICA GRANATUM WITH THEIR MEDICINAL IMPORTANCE

SONU SHARMA^{a1} AND SHAILESH SHARMA^b

^aDepartment of Pharmacognosy, Faculty of Pharmacy, School of Pharmaceutical Sciences, Jaipur National University, Jagatpura Jaipur, India

^bDepartment of Pharmaceutics, Principal and HOD of Dr. K.N. Modi University, Newai, Rajasthan, India

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. 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, glycosides, alkaloids, tannins, phytosterol etc. All these three plants have pharmacological activity like Astringent, anthelmintic, nervine tonic, aphrodisiac, diuretic, vermifuge and stimulant, anodyne, antidotal, psychedelic, leucorrhoea, spermatorrhea, facial paralysis and powerfully aphrodisiac, leprosy, scabies, skin diseases, pruritus, haemorrhoids, ulcers, dyspepsia, impotency, leucorrhoea, haemoptysis, cephalalgia, fever and cardiac debility.

KEYWORDS: Haemorrhoids, Impotency, Leucorrhoea, Haemoptysis, Cephalalgia

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).

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 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 vasicine from *Adhatodavasika* have been claimed to have abortifaciant properties but it could

¹Corresponding author

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 *Mucunaprurita*, *Mesuaferrea*, *punicagranatum* 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

Mucuna prurita

Common Name: KaunchBeej

Family: Leguminosae

Habitat: It is found almost all over the country

Parts Used: Seeds, roots and legumes

Pharmacological Action

Astringent, anthelmentic, nervine tonic, aphrodisiac, diuretic, vermifuge and stimulant, anodyne, antidotal, psychedelic, leucorrhoea, 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 leucorrhoea and profuse menstruation

Reproductive system: It is used as an aphrodisiac in seminal weakness and spermatorrhea.

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 anthelmintic.
- 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.

Punicagranatum

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 puniceic acid , palmitic acid , stearic acid, oleic acid and linoleic acid .Juice, seeds and peel apparently contain steroid hormones, including estrone(Khoet *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).

MATERIALS 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 (60-80°C)
- (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 upto 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.

Nuclear Magnetic Resonance Spectroscopy

NMR for structural analysis relies on the few basic principles. Certain nuclei (^1H , ^{13}C) 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 then 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

Fraction 1 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

Fraction 1 and 2 isolated were undertaken for Mass Spectrum (LC- MS) by electron impact ionization on various MAT 1020. Direct inlet probe at 270°C and electron energy 70eV.

RESULTS AND DISCUSSION

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

NMR & Mass Spectra Interpretation of Fraction 1

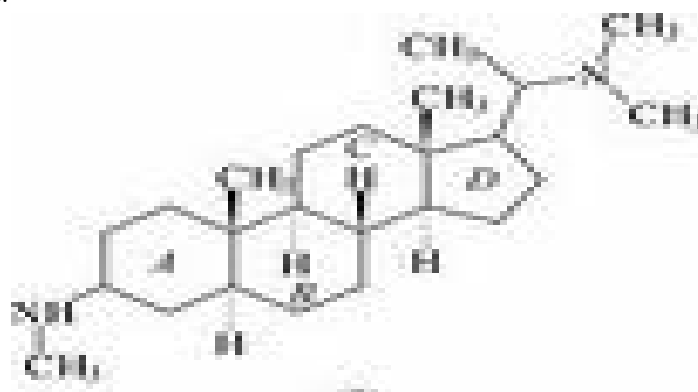
There are four chemical shifts (δ PPM) seen in NMR spectra of fraction 1, which are as follows:-

- First chemical shift is at 2.35 δ PPM, this peak indicates Triplet multiplicities (2.3695 ; 2.3456 ; 2.3202) of signal with one proton.
- Second chemical shift is at 1.65 δ PPM, this peak indicates Quadruplet multiplicities (1.6557; 1.6326; 1.6213; 1.6118) of signal with two protons.
- Third chemical shift is at 1.25 δ PPM, this peak indicates Singlet multiplicities (1.2539) of signal with maximum no of protons i.e 40 protons.

- Last chemical shift is at 0.85 δ PPM, this peak indicates Triplet multiplicities (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 as 299.7 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

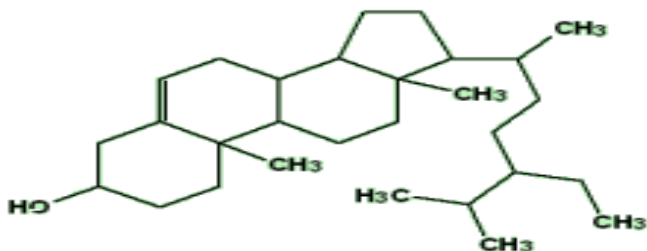
There are seven chemical shifts (δ PPM) seen in NMR spectra of fraction 2, which are as follows:-

- First chemical shift is at 3.95 δ PPM, this peak indicates doublet multiplicities (3.8961; 3.9571) of signal with maximum no of protons i.e. 7 protons.
- Second chemical shift is at 6.36 δ PPM, this peak also indicates doublet multiplicities (6.3561; 6.3644) of signal with one proton.
- Third chemical shift is also a doublet at 6.55 δ PPM, this peak indicates doublet multiplicities (6.55497; 6.5571) of signal with one proton.
- Fourth chemical shift is at 6.72 δ PPM, this peak indicates doublet multiplicities (6.7364; 6.7066) of signal with one proton.

- Fifth chemical shift is also a doublet at 7.25 δ PPM, this peak indicates doublet multiplicities (7.2223; 7.2601) of signal with three protons.
- Sixth chemical shift is at 11.39 δ PPM, this peak indicates singlet multiplicities (11.3993) of signal with one proton
- Last seventh chemical shift is at 11.98 δ PPM, this peak indicates singlet multiplicities (11.9884) of signal with one proton.

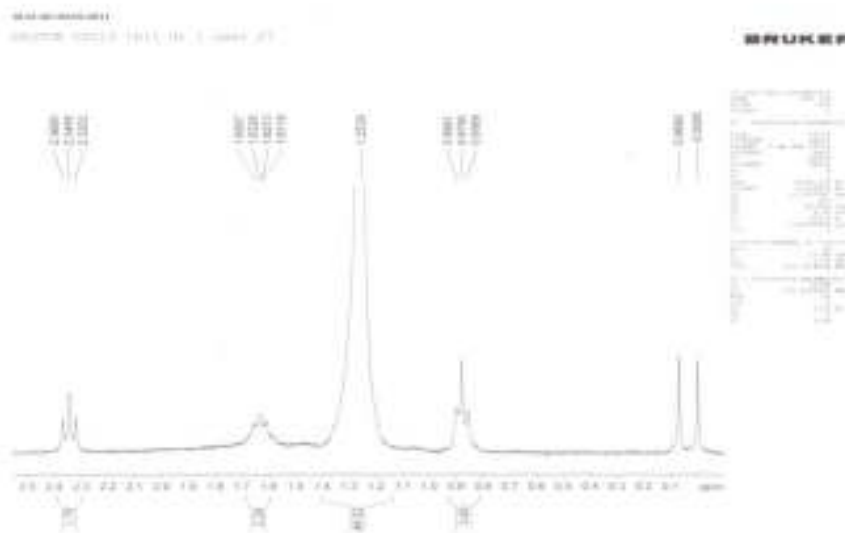
No shift of TMS (Tetra Methyl Saline) is prominent in the spectra.

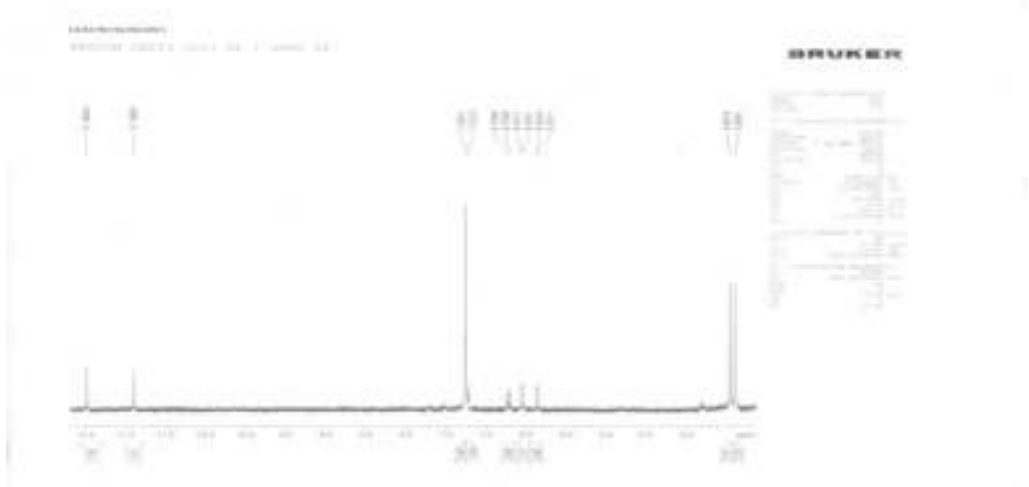
Mass spectra of fraction 2 imply the base peak at 321.5 m/e and the total mass of the compound as 4854. M/z Thus the overall instrumental analysis results in the probability of any Beta Sito-steroidal nucleus ring in the fraction 2 with the probable structure as below:-



Beta sito-sterol

Mass Spectra of Fraction 1





Mass Spectra of Fraction 2

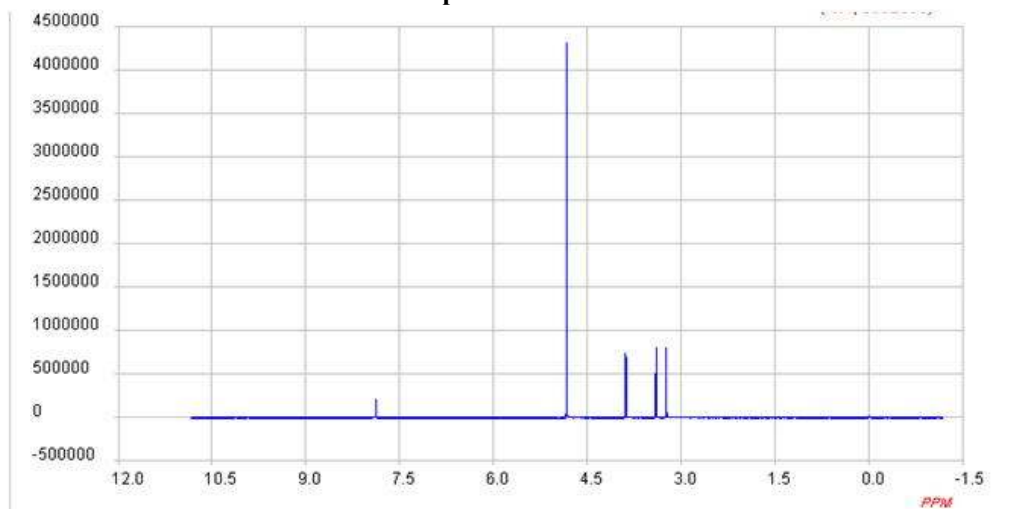


Figure 1

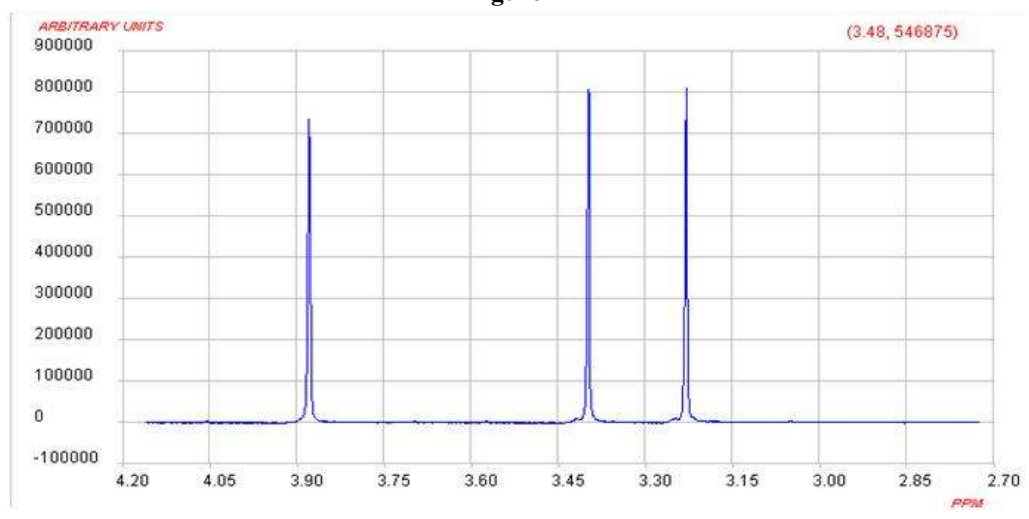


Figure 2

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

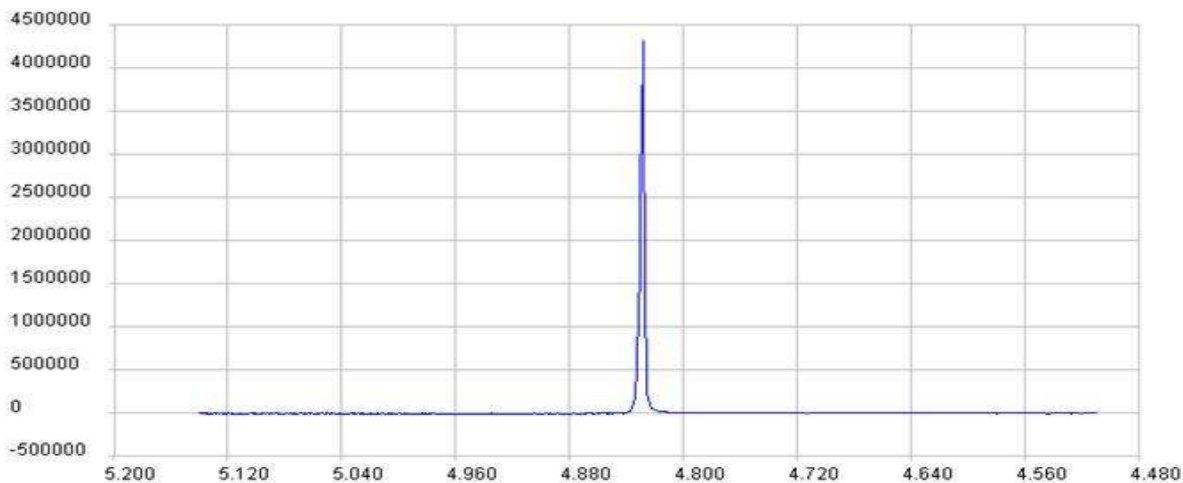


Figure 3

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

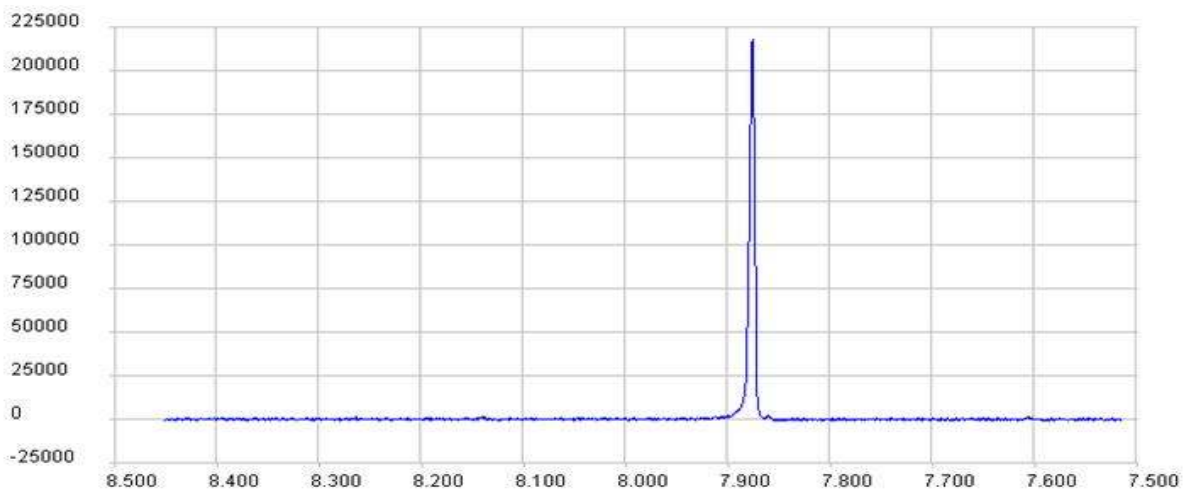


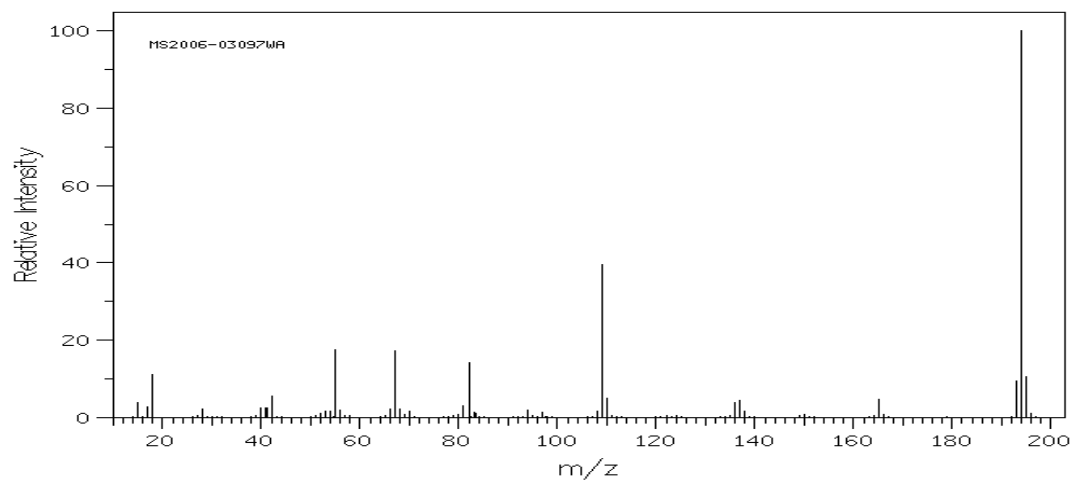
Figure 4

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

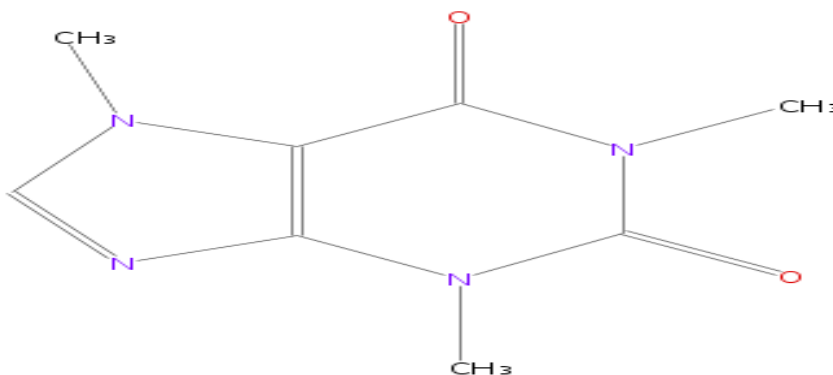
C₈H₁₀N₄O₂ (Mass of molecular ion: 194)

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

Mass of molecular ion: 194

Reasonable guess of the assignments but we can't be completely sure till we compare

With similar compounds [C₈H₁₀N₄O₂]



Caffeine

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