Original Research Article Biochemistry

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

PHYTOCHEMICAL COMPOUND IDENTIFICATION AND ANTI-INFERTILITY PROPERTY OF N-MIRACLE (POLYHERBAL FORMULATION) BY UV-VISIBLE SPECTROSCOPY, FTIR, TLC, HPLC AND GC-MS STUDY

MEENATCHI SUNDARAM ANGAPPAN*[1] AND JEYAPRAKASH KARUPPAIAH[2]

[1]Ph.D Research Scholar, R&D Centre, Bharathiar University, Coimbatore –641 045.
[2]Head, PG and Research Department of Biochemistry, Rajah Sarfoji Govt. College, Thanjavur – 613 005.

ABSTRACT

Male infertility is a world-wide medical and social problem. In Ayurveda, single or multiple herbs (polyherbal) are used for the treatment. The concept of polyherbalism is to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity. N-Miracle, a polyherbal formulation, which was prepared by the combination of five medicinal plants and selenium having diversified pharmacological effect, however, the anti-infertility effect of the combination of the above plants are not yet carried out. Hence, the present study has been designed with the purpose of identifying the active principles of methanolic extract of N-Miracle (Polyherbal formulation) by UV-Visible spectrophotometer, FTIR, TLC, HPLC, and GC-MS analysis. UV-Visible spectrum revealed the presence of phenolic compounds and flavonoid compounds in the range of 220 nm to 1100 nm. FTIR spectrum unveiled the presence of phenol, alkane, carboxylic acid, aromatic compound, aliphatic amines and halogen compounds. TLC of methanolic extract of N-Miracle (Polyherbal formulation) revealed the presence flavonoid compound namely isoquercetin. HPLC analysis of the methanolic extract of N-Miracle (Polyherbal formulation) showed the presence of polyphenols and flavonoid compound at RT 5.541, RT 13.060, and RT 32.028. GC-MS screening revealed the presence of alcohol, fatty alcohol, alkane, phytol, and fatty acid methyl esters groups.

KEYWORDS: *Infertility, Polyherbalism, Phytochemical, N-Miracle, Flavonoids.*

MEENATCHI SUNDARAM ANGAPPAN*
Ph.D Research Scholar, R&D Centre, Bharathiar University, Coimbatore –641 045.

Received on: 27-11-2016
Revised and Accepted on: 17-01-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.1.b567-576

INTRODUCTION

Infertility is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse.[1] Aging and stress exposure are the inevitable phenomena. A large body of evidence has demonstrated that male sexual behaviours gradually decline with age.[2] Numerous factors including both physical and mental factors are regarded as the important aetiology for sexual dysfunction in elderly. Stress is regarded as one important factor to induce sexual dysfunction. It was reported that chronic exposure to a variety of mild stressors significantly decreases male sexual behavior.[3-4] Plants have a great potential for producing new drugs for human benefit. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases. According to a report of world health organization, more than 80% of world population depends on traditional medicine for their primary healthcare needs. The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential for systematic evaluation of plants used in traditional medicine for various ailments. Hence, there is need to screen medicinal plants for promising biological activity.[5] Herbal medicines are used to cure diseases since a great many year back in light of its simple accessibility, social worthiness and less side effect.[6]In Ayurveda, single or multiple herbs (polyherbal) are used for the treatment. The concept of polyherbalism is to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity. Polyherbalism confers some benefits not available in single herbal formulation. It is evident that better therapeutic effect can be reached with a single multi-constituent formulation. For this, a lower dose of the herbal preparation would be needed to achieve desirable pharmacological action, thus reducing the risk of deleterious side-effects. Besides, polyherbal formulations bring to improved convenience for patients by eliminating the need of taking more than one different single herbal formulation at a time, which indirectly leads to better compliance and therapeutic effect. All these benefits have resulted in the popularity of polyherbal formulation in the market when compared to single herbal formulation.[7]N-Miracle, a polyherbal formulation, was prepared by the combination of the following medicinal plants. Each 100 g contains the following composition:

A. *Conium maculatum* L (20%).
B. *Lycopodium Clavatum* (20%).
C. *Selenium* (0.005%).
D. Vitex agnus castus L (20%).
E. *Pausinystalia yohimbe* (20%).
F. *Caladium seguinum* (20%).

AIM AND OBJECTIVE OF THE STUDY

The aim and objective of the present study is to identify phyto-active compounds from the polyherbal formulation (N-Miracle) using analytical tools such as chromatographic and spectroscopic method.

MATERIALS AND METHODS

Drugs and Chemicals

N-Miracle (Polyherbal formulation) was provided by Dr. Ramesh Shankar, Sai Brindavan Homeo Clinic, Omalur Main Road, Salem, Tamil Nadu, India, 636 455, as a gift sample and it was used to carry out the research work. All other chemicals and reagents used in the present study were obtained commercially and were of analytical grade.

Preparation of Extract

5g of the powder of N-Miracle (Polyherbal formulation) were transferred into three different conical flask (250mL). The conical flask containing 100 mL of three different solvents viz. Ethanol, Methanol and Water. The conical flask containing plant powder and solvent was mixed well for 48 hours by free hand. After three days, the extracts were filtered using Whatmann filter paper No.1. and was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45^{o}C. The obtained extracts were stored at 4°C in air tight bottle until further use.

UV-Visible Spectroscopic Analysis

Phenolic and flavonoid compounds of methanolic extract of N-Miracle (Polyherbal formulation) was determined by UV-Visible Spectroscopic analysis.[7] The methanolic extract was examined under UV-Visible spectral analysis. The extract was centrifuged at 3000 rpm for 10 minutes and filtered through Whatmann No.1 filter paper by using high pressure vacuum pump. The sample was diluted to 1:10 with the methanol solvent. The extract was scanned in the wavelength ranging from 260-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected.

Fourier Transform Infrared Spectrophotometer (FTIR)

Functional groups of methanolic extract of N-Miracle were screened by Fourier transform infrared spectrophotometer as per the method of Neha et al.[8] Fourier transform infrared spectrophotometer was performed to assess the functional groups of the extract. The transmittance was recorded between 400-4000 cm^{-1} on FTIR spectrophotometer. The functional groups present in the leaves were identified from the spectra.

Thin Layer Chromatography (TLC)

Active principles of methanolic extract of N-Miracle (Polyherbal formulation) was identified by the method of thin layer chromatography.[9] A thin layered plate was prepared by spreading aqueous slurry of Silica gel G on the clean surface of a glass or rigid plastic. Calcium carbonate or starch was also added to the adsorbent to increase adhesion. The plate was then heated in an oven for about 30 minutes at 105°C to activate the plate. It was then cooled inside the oven itself. The extracts were drawn with capillary tubes and applied as spots on a stationary phase (silica-gel coated plate) about 1 cm from the base. The plate was then dipped into a suitable

solvent system (n-Butanol: Acetic acid: Water (4:1:5). The plate was then placed in a container with enough solvent in a well-covered tank. The solvent migrates up the plate. As the solvent rising through thin layer separates different components of the mixture at different rates which appear as spots in the thin layer. After the solvent has reached almost the top edge of the plate, nearly 3/4th of the plate, the plate was removed from the tank and dried briefly at moderate temperatures 60-120°C. The presence of secondary metabolites in the extract was detected by TLC using suitable spraying reagents. The presence of flavonoid was detected by the formation of yellow color in the plate a positive reaction by exposure of ammonia to TLC plate.

R_f Value

It was a ratio of distance travelled by the sample and distance travelled by the solvent.

$$R_f = \frac{\text{Distance travelled by the sample (solute) from the origin}}{\text{Distance travelled by the solvent from origin}}$$

High Performance Liquid Chromatographic (HPLC)

Flavonoid and phenolic contents of methanolic extract of N-Miracle (Polyherbal formulation) were screened by the method of HPLC as described by Biswas et.al. [10] The HPLC analysis of N-Miracle (Polyherbal formulation) was carried out with Chromatographic system (Shimadzu Class-VPV6.14SP2, Japan) consist of autosampler with 20µl fixed loop and an UV-Visible detector. The gradient elution of solvent A [water-acetic acid (25:1 v/v)] and solvent B (methanol) had a significant effect on the resolution of compounds. As a result, solvent gradients were formed, using dual pumping system, by varying the proportion of solvent A [water-acetic acid (25:1, v/v)] to solvent B (methanol). Solvent B was increased to 50% in 4 min and subsequently increased to 80% in 10 min at a flow rate of 1.0 mL/min. The samples were run for 25min. and detection was done at 280 nm by UV detector (Lamp-D2). All chromatographic data were recorded and processed using auto chro-software.

GC-MS (Gas Chromatography-Mass spectrometry)

Molecular structure and molecular mass of the phytochemical compounds were screened by GC-MS analysis as per the method Srinivasan et al.[11] GC-MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter was 0..32mm, column length was 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 mL /min and an injection volume of 0.5 µl was employed (split ratio of 10:1) injector temperature 270 ºC; ion-source temperature 200 ºC. The oven temperature was programmed from 40ºC (isothermal for 2 min), with an increase of 8 ºC/min, to 150ºC, then 8ºC/min to 250ºC, ending with a 20min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time was 51.25min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0. Interpretation on GCMS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.[12]

RESULTS

1. UV-Visible Spectroscopy

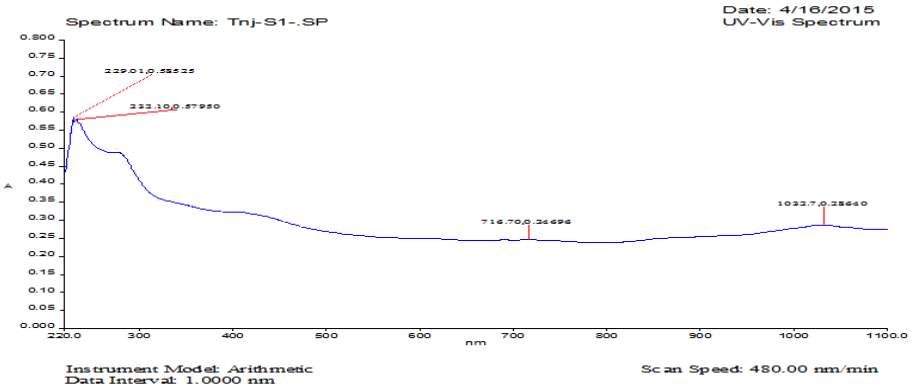

Figure 1
UV-Visible Spectrum of methanolic extract of N-Miracle (Polyherbal formulation)

Table 1
UV-Visible Peak Values of Methanolic Extract of N-Miracle (Polyherbal formulation)

S. No	Nanometer	Absorption Value
1.	229	0.58
2.	232	0.57
3.	716	0.24
4.	1032	0.28

The qualitative UV-Visible spectrum profile of methanolic extract of N-Miracle (Polyherbal formulation) was selected from 220 nm to 1100 nm due to sharpness of peaks and proper baseline. UV-Visible spectrum profile of methanolic extract of N-Miracle (Polyherbal formulation) was given in (Fig.1) and its absorption values were given in (Tab.1). The profile showed that the peaks from 220 nm to 1100 nm and the profile showed the peaks at 229, 232, 716, and 1032 nm with the absorption value of 0.58, 0.57, 0.24, and 0.28 respectively. The spectra for phenolic compounds and flavonoids typically lie in the range of 230-290 nm.[7] The result of UV-Visible spectroscopic analysis confirmed the presence of tannins and flavonoids in N-Miracle (Polyherbal formulation).

2. Fourier Transform Infrared Spectroscopy (FTIR) Spectroscopic

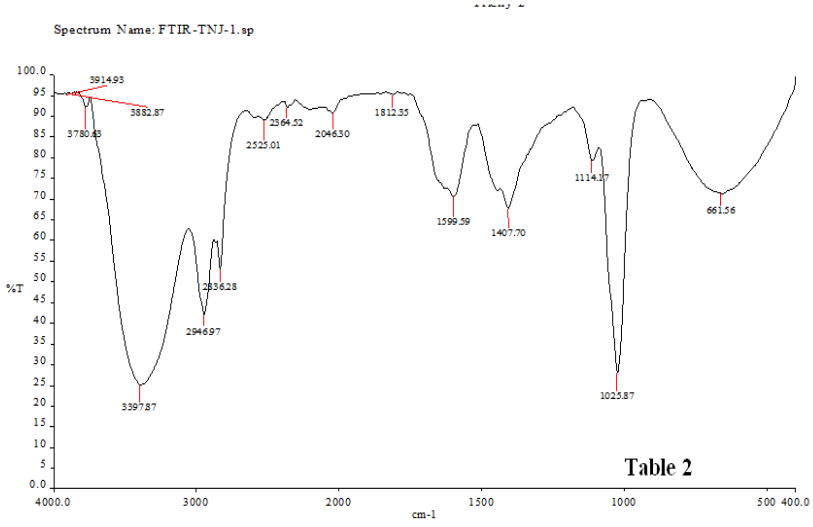

Figure 2
Fourier Transform Infrared Spectrum of methanolic extract of N-Miracle (Polyherbal formulation)

Table 2
Functional Group Analysis of Methanolic Extract of N-Miracle (Polyherbal formulation) by Using FTIR Spectroscopy

S. No	Peak Values	Bond	Functional Groups
1.	3397.87	O-H	Phenol
2.	2924.94	C-H	Alkanes
3.	2836.28	C=O	Carboxylic Acid
4.	1599.59	C=C	Aromatics
5.	1407.70	C=C	Aromatics
6.	1114.17	N-C	Aliphatic amines
7.	1025.87	C=O	Carboxylic acid
8.	661.56	C-X	Halogen Compound

The FTIR spectrum was used to identify the functional groups of the active components based on the peak value in the region of infrared radiation. The results of FTIR spectrum and its peak values with functional groups were represented in (Fig.2) and (Tab.2). When the N-Miracle (Polyherbal formulation) extract was passed into the FTIR, the functional groups of the active components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of phenol, alkane, carboxylic acid, aromatic compound, aliphatic amines, halogen compounds which showed major peaks at 3397.87, 2924.94, 2836.28, 1599.59, 1407.70 respectively.

3. Thin layer chromatography (TLC)

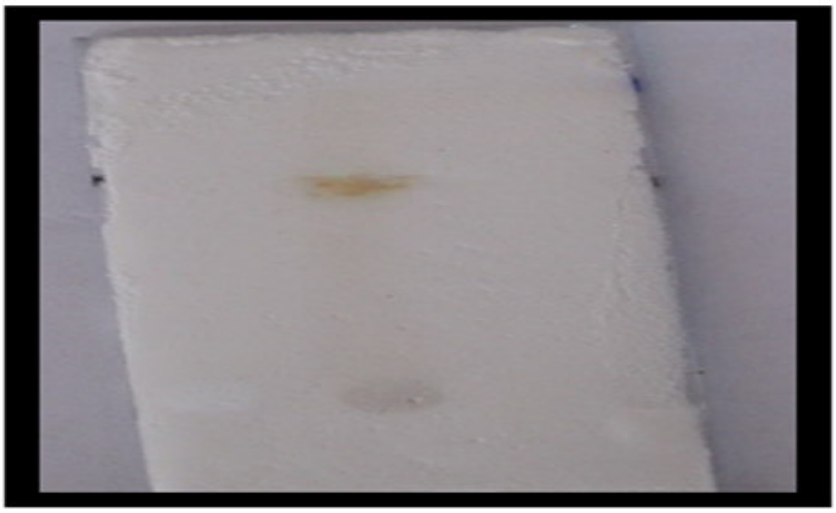

Figure 3
TLC of methanolic extract of N-Miracle (Polyherbal formulation)

Table 3
Analysis of Flavonoids by TLC

Phytochemical	R_f Value	Result	Literature
Flavonoid	3.8/5	0.76	Isoquercetin

All determinations were performed in triplicates
Thin layer chromatogram of methanolic extract of N-Miracle (Polyherbal formulation) was given in **(**Fig 3**)** and its R_f value was given in (Tab 3). TLC of methanolic extract of N-Miracle (Polyherbal formulation) revealed the presence of a spot having R_f value of 0.76 when a solvent phase of n-Butanol: Acetic acid: Water (4:1:5) solvent system was used. This R_f value 0.76 was compared with standard R_f value of flavonoid and it was identified as flavonoid compound as isoquercetin[13] in the methanolic extract of N-Miracle (Polyherbal formulation).

4. High performance liquid chromatography (HPLC):

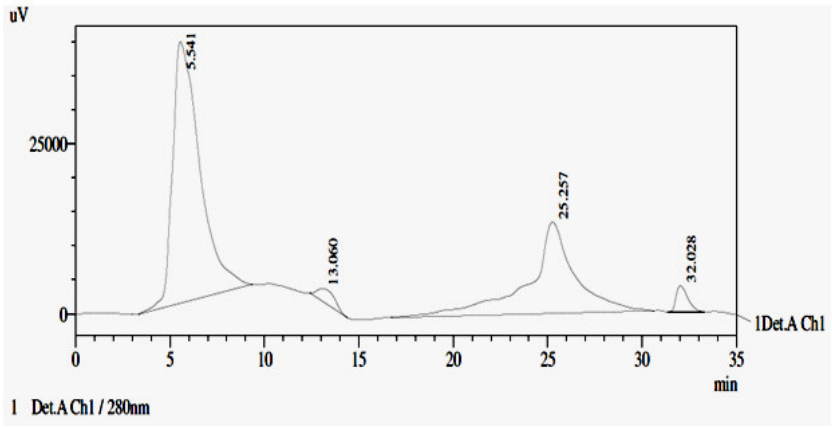

Figure 4
HPLC Chromatogram of Methanolic Extract of N-Miracle (Polyherbal formulation)

Table 4
Retention times and peak areas (%) of methanolic extract of N-Miracle (Polyherbal formulation)

Peak	RT	Area	Height	Area%	Height%
1	5.541	3866732	38356	59.760	66.833
2	13.060	132508	1819	2.048	3.169
4	32.028	164120	3822	2.536	6.660

Table 5
Analysis of *phenolic* and flavonoid compound by HPLC with RT

S. No	RT	Phytoconstituents	Compound	literature
1	5.541	Tannic acid	Polyphenol	Mradu et al., 2012
2	13.060	Procyanidin	Flavonoid	Vagiri et al., 2012
3	32.028	quercetin-3-6-malonyl-glucoside	Flavonoid	Biswas et al., 2013

HPLC profile of methanolic extract of N-Miracle (Polyherbal formulation) was given in (Fig 4**)** and their peak values were given in (Tab 4) and (Tab 5)**.** HPLC analysis of the methanolic extract of N-Miracle (Polyherbal formulation) showed the presence of various constituents as evidenced by the chromatogram obtained at various retention times (5.541, 13.060, and 32.028). The separation of the peaks such as 5.541, 13.060 and 32.028 were compared with the retention times of isolated compounds with literature data. The phenolic acid like tannic acid was identified at RT 5.541[14], the flavonoids were identified like Procyanidin at RT of 13.060[15] and quercetin-3-6-malonyl-glucoside at RT of 32.028 [9] at λ_{max} 280 nm.

5. Gas Chromatography-Mass Spectrometry (GC-MS):

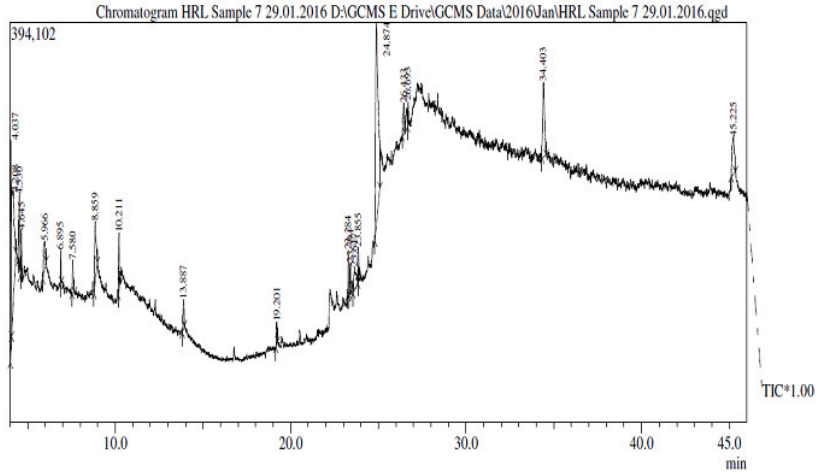

Figure 5
GC-MS Spectrum of Methanolic extract of N-Miracle (Polyherbal formulation)

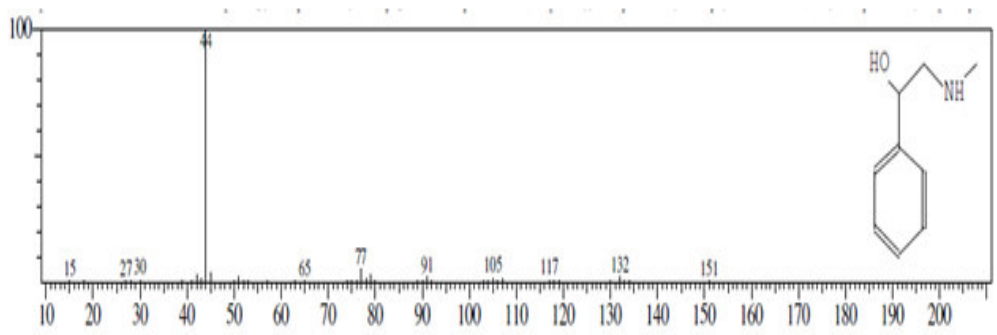

Figure 5(a)
Mass Spectrum of Benzene methanol, alpha-(1-aminoethyl)

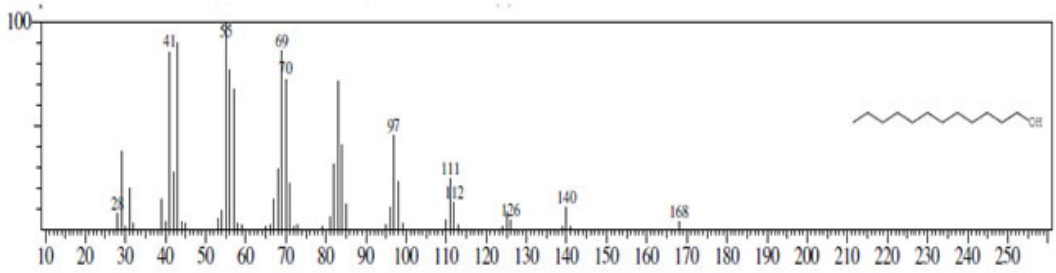

Figure 5(b)

Mass Spectrum of1-Dodecanol

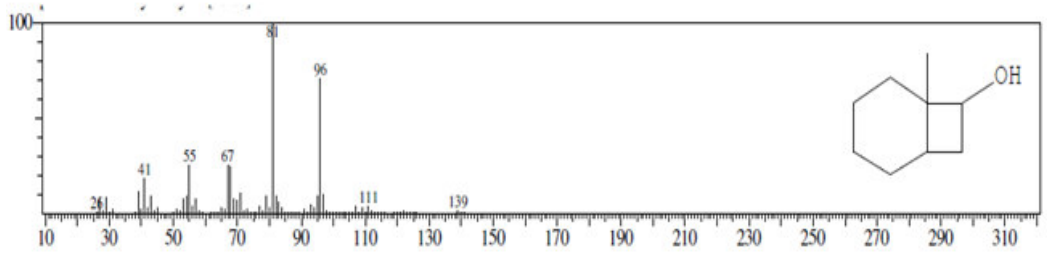

Figure 5(c)
Mass Spectrum of 6-Methyl-bicyclo [4.2.0] octan-7-ol

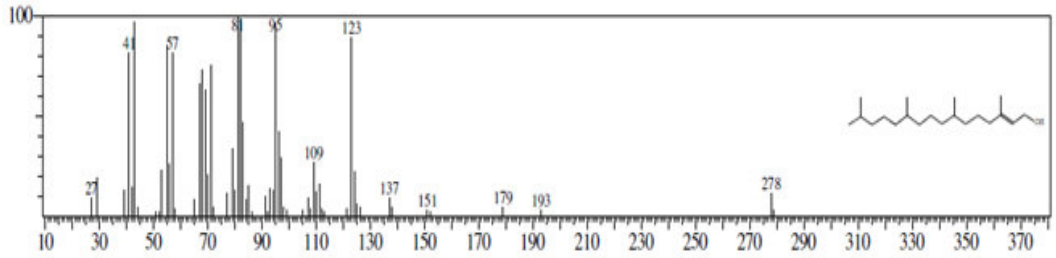

Figure 5(d)
Mass Spectrum of 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol

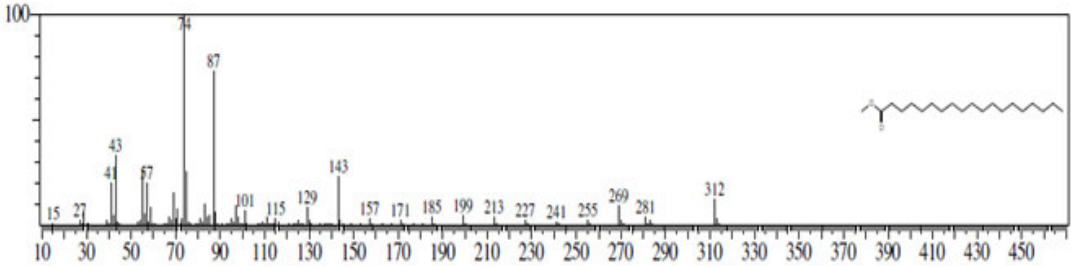

Figure 5(e)
Mass Spectrum of Nonadecanoic acid, methyl ester

Table 6
GC-MS Analysis of Methanolic Extract of N-Miracle (Polyherbal formulation)

S. No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	4.208	Benzenemethanol alpha-(1-aminoethyl)	$C_9H_{13}NO$	151	17.56
2	10.211	1-DODECANOL	$C_{12}H_{26}O$	186	2.17
3	23.391	6-Methyl-bicyclo [4.2.0] octan-7-ol	$C_9H_{16}O$	140	1.61
4	23.855	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.48
5	26.693	Nonadecanoic acid, methyl ester	$C_{20}H_{40}O_2$	312	0.74

Table 7
GC-MS Analysis -Phytochemical Compounds, Nature and Biological Activities of Methanolic Extract of N-Miracle (Polyherbal formulation)

S. No	RT	Peak area %	Name of the compound	Compound Nature	Activity
1	4.208	17.56	Benzenemethanol. alpha-(1-aminoethyl)	Alcohol	Vasoconstriction, bronchodilator, CNS stimulant, weight loss
2	10.211	2.17	1-DODECANOL	Fatty alcohol	Anti-bacterial and Anti-inflammatory activity
3	23.391	1.61	6-Methyl-bicyclo [4.2.0] octan-7-ol	Alkane	antiproliferative, antivascular, antiviral, and antiparasitic
4	23.855	1.48	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phytol	Antioxidant activity
5	26.693	0.74	Nonadecanoic acid, methyl ester	Fatty acid methyl esters (FAME)	Antihyhpercholesterolemic activity

The GC-MS analysis of N-Miracle (Polyherbal formulation) revealed the presence of phytochemical constituents that could contribute the medicinal quality of the plant. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their retention time (RT), molecular formula, molecular weight and peak area in percentage were presented in (Tab.6, 7) and (Fig 5, 5 a-e). Phytochemical constituents were detected in the methanolic extract of N-Miracle (Polyherbal formulation) revealed that Benzene methanol, alpha.-(1-aminoethyl) (17.56%) was found to be the major component followed by 1-Dodecanol (2.15%), 6-Methyl-bicyclo[4.2.0]octan-7-ol (1.61%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (1.48%) and Nonadecanoic acid, methyl ester (0.74%) were found as the major compound in methanolic extract of N-Miracle (Polyherbal formulation). The phytochemicals identified through GC-MS analysis showed many biological activities relevant to this study were listed in (Tab 7).

DISCUSSION

UV-Visible Spectroscopic methods have become a powerful tool for secondary metabolite profiling as well as for qualitative and quantitative analysis of the pharmaceutical and biological materials.[16] This study was supported by Yushchyshena and Tsurkan[17] and the findings of the study revealed that *Vitex agnus castus* (one of the herb present in our polyherbal formulation N-Miracle) contains 2.7% of flavonoids in leaves and no more than 1.5% in other parts of plant. The results of the study were compared with the work of Males[18] revealed 3 times more flavonoid. The findings of the previous study also revealed that *Vitex agnus castus* (one of herb present in our polyherbal formulation N-Miracle) possesses fertility efficacy and was a well-known remedy for female reproductive system disease[19]. In general, flavonoids are products of plant metabolism and have different phenolic structures. Flavonoids are potent antioxidants because of their free radical scavenging properties and may protect testicular cells against free oxygen radicals and lipid peroxidation. There by it may increase spermatogenesis. Flavonoids are well known chelators and it chelates toxic heavy metals and protect testicular tissues against toxic metals[20]. The present study has demonstrated that N-Miracle (Polyherbal formulation) was rich in flavonoids and may have a beneficial effect on spermatogenesis. The results of FTIR spectrum indicates that the various functional constituents present in N-Miracle (Polyherbal formulation) and suggested the various medicinal properties of this polyherbal formulation. The study was also supported by Baranska[21] who revealed Yohimbine (one of the herb present in our polyherbal formulation N-Miracle) was an aphrodisiac and the active principle of Yohimbine was hydrochloride and the alkaloids containing C-H orientation. The results of the present study were also confirmed by Sahiba et al[22], which revealed that *Vitex agnus castus* L. (one of the herb present in our polyherbal formulation N-Miracle) had phenols and hydroxyl as a functional tested by FTIR with different absorbance. The results of the Sahiba et al[22] also showed that *Vitex agnus castus* L. exhibited anti-angiogenic activity through the polyphenols and these chemical groups proven to inhibit angiogenesis, proliferation of tumor cells and endothelial cells. At present, a number of analytical tools (chromatographic and spectroscopic) have been used to analyze flavonoids in plant samples or crude drugs. TLC was one of the most popular and widely used separation techniques because of its ease of use, cost effectiveness, high sensitivity, speed of separation as well as its capacity to analysis multiple samples simultaneously.[23] The technique could be utilized for separation, isolation, identification and quantification of components in a mixture. It could also be utilized on a preparative scale to isolate a particular component. The present study was also correlated with Yuan et al[24] who isolated flavonoid from *Vitex agnus castus* L. (one of the herb present in our polyherbal formulation N-Miracle) using TLC, which has cytotoxic effect on leukemic cell lines and also have reported that *Vitex agnus castus* L. possess a selective cytotoxic activity against tumor cells. Das et al[25] separated bioactive flavonoid using TLC in *Lycopodium clavatum* (herb present in N-Miracle), which protect the skin keratinocytes from ROS and DNA damage. Arash Khaki et al[26] stated that quercetin is a strong antioxidant and has been shown to reduce oxidative stress and improves testicular and sperm functions by reducing ROS production and enhances epididymal sperm quality and numbers. Quercetin is one of the major flavonoid present in our polyherbal formulation N-Miracle which might be responsible for the beneficial effect on spermatogenesis. High performance liquid chromatography (HPLC) has recently emerged as a preferred analytical tool for fingerprints and quantification of marker compounds in plant extracts and herbal drugs because of its simplicity, sensitivity, accuracy, suitability for high throughput screening. Over the past decade, HPLC has been successfully used in the analysis of pharmaceuticals, plant constituents and biomacromolecules[27]. A major advantage of HPLC was that it has the ability to easily separate a wide variety of chemical mixtures. The results of the present study were also supported by the finding of Shah et al[28] who have indicated the presence of glycosidein in *Vitex agnus castus* L. (one of the herb present in our polyherbal formulation N-Miracle) and suggested hepatoprotective activity of *Vitex* species. The report of this study also supported by Socaciu et al[29] who have revealed that *Lycopodium clavatum* (one of the herb present in our polyherbal formulation N-Miracle) contained phenolic acid derivative in HPLC-UV analysis. Because of its simplicity, sensitivity and effectiveness in separating components of mixtures, GC-MS was one of the most important tools in biochemistry. The GC-MS analysis of N-Miracle (Polyherbal formulation) revealed the presence of phytochemical compounds. The identified compounds possess many biological active properties. This results suggested that (i) Benzenemethanol, alpha.-(1-aminoethyl) (RT 4.208) possesses vasoconstriction, bronchodilation, CNS stimulant, and weight loss property[30], (ii) 1-Dodecanol (RT 10.211) possesses antibacterial and anti-inflammatory property[31], (iii) 6-Methyl-bicyclo[4.2.0]octan-7-ol (RT 23.391) possesses antiproliferative, antivascular, antiviral, and antiparasitic property[32], (iv) 3,7,11,15-Tetramethyl-2-hexadecen-1-ol

(RT 23.855) possesses antioxidant property[33], and (v) Nonadecanoic acid, methyl ester (RT 26.693) possesses anti-hyhpercholesterolemic activity which was supported by Miettinen et al.[34] Benzenemethanol, alpha.-(1-aminoethyl) is a major compound present in N-Miracle (polyherbal formulation) is a catecholamine which has stimulatory property and it exerts its effect via alpha-1 adrenergic receptors which stimulates the release of nitric oxide. The nitric oxide actives cGMP production which activates the inflow of blood into the penis and maintains normal erection.[35]

CONCLUSION

Fertility medication, better known as fertility drugs were drugs which enhance reproductive fertility. Management of male infertility was always a difficult task with current allopathic medical treatment option. The treatment of men with unexplained idiopathic infertility remained a challenge in allopathic medicine. Side effects of allopathic aphrodisiac drugs include libido changes, acne, nausea, headache, weight gain, visual field changes, dizziness, gynecomastia and allergic dermatitis and gastrointestinal side effects. A phytotherapeutic approach to modern drug development can provide many valuable drugs from traditional medicinal plants. Search for pure phytochemical as drugs were time consuming and expensive. Numerous plants and polyherbal formulations were used for the treatment of male infertility. The evaluation of plant products on the basis of medicinal and therapeutic properties forms a platform for the discovery of newer drug molecules from different plant sources. The present study showed N-Miracle (Polyherbal formulation) contains phytoconstituents which are potent antioxidants. These antioxidants prevent testicular tissue damage from free radicals and thereby increases spermatogenesis. The catecholamine isolated from GC-MS study may also have beneficiary role during erection. Further study is required to demonstrate the ameliorative effect of N-Miracle (Polyherbal formulation) in patients with infertility.

ACKNOWLEDGEMENT

The authors are very thankful to Dr. Ramesh Shankar, Sai Brindavan Homeo Clinic, Omalur Main Road, Salem, Tamil Nadu, India, 636 455 for providing kind support to carry out the research work.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, Sullivan E, Van der Poel S. The international committee for monitoring assisted reproductive technology (ICMART) and the world health organization (WHO) revised glossary on ART terminology, 2009. Human Reproduction. 2009 Oct 4:dep343.
2. Phanijo AL. Sexual dysfunction in old age. Advances in Psychiatric Treatment 2000; 6:270-277.
3. Brotto LA, Gorzalka BB, Hanson LA. Effects of housing conditions and 5-HT 2A activation on male rat sexual behavior. Physiology & behavior. 1998 Feb 15;63(4):475-9.
4. Paolo SD, Brain P, Willner P. Effects of chronic mild stress on performance in behavioural tests relevant to anxiety and depression. Physiology & behavior. 1994 Nov 1;56(5):861-7.
5. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC complementary and alternative medicine. 2006 Oct 17;6(1):1.
6. Mahish PK, Mahobia R, and Yadav J. Use and awareness of herbal medicines among literate population. Int J Pharm Bio Sci (2016): 7(4):174 – 178.
7. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of ayurveda. Pharmacognosy reviews. 2014 Jul 1;8(16):73.
8. Neha SL, DhiNgRa R. Plastinated knee specimens: A novel educational tool. Journal of clinical and diagnostic research: JCDR. 2013 Jan;7(1):1.
9. Harborne JB. Methods of plant analysis. InPhytochemical methods 1984 (pp. 1-36). Springer Netherlands.
10. Biswas N, Balac P, Narlakanti SK, Haque ME, Hassan MM. Identification of phenolic compounds in processed cranberries by HPLC method. Journal of Nutrition & Food Sciences. 2013 Feb 14;2013.
11. Srinivasan K, Sivasubramanian S, Kumaravel S. Phytochemical profiling and GC-MS Study of Adhatoda vasica leaves. Int J Pharm Bio Sci. 2013;5(1):714-20.
12. Edewor TI, Olabisi KN. Gas chromatography-mass spectrometric analysis of methanolic leaf extracts of *Lannea kerstingii* and *Nauclea diderrichii*, two medicinal plants used for the treatment of gastrointestinal tract infections. Asian Journal of Pharmaceutical and Clinical Research. 2016 Jun 28:179-82.
13. Kaya B, Menemen Y, Saltan FZ. Flavonoid compounds identified in Alchemilla L. species collected in the north-eastern Black Sea region of Turkey. African Journal of Traditional, Complementary and Alternative Medicines. 2012 Jan 1;9(3):418-25.
14. Mradu G, Saumyakanti S, Sohini M, Arup M. HPLC profiles of standard phenolic compounds present in medicinal plants. International Journal of Pharmacognosy and Phytochemical Research. 2012;4(3):162-7.
15. Vagiri M, Ekholm A, Andersson SC, Johansson E, Rumpunen K. An optimized method for analysis of phenolic compounds in buds, leaves, and fruits of black currant (Ribes nigrum L.). Journal of agricultural and food chemistry. 2012 Oct 15;60(42):10501-10.

16. Owen AE. Fundamentals of UV-visible spectroscopy.
17. Yushchyshena O, Tsurkan O. Phenolic compounds content in Vitex agnus-castus L. and V. cannabifolia Sieb. growing in Ukraine. J. of Medicinal Plants Studies. 2014;2(5):36-40.
18. Maleš Ž. Determination of the content of the polyphenols of Vitex agnus-castus Lf rosea. Acta pharmaceutica. 1998 Jan 1;48(3):215-8.
19. Ye Q, Zhang QY, Zheng CJ, Wang Y, Qin LP. Casticin, a flavonoid isolated from Vitex rotundifolia, inhibits prolactin release in vivo and in vitro. Acta Pharmacologica Sinica. 2010 Dec 1;31(12):1564-8.
20. Khaki A, Ouladsahebmadarek E, Javadi L, Farzadi L, Fathiazad F, Nouri M. Anti-oxidative effects of citro flavonoids on spermatogenesis in rat. African Journal of Pharmacy and Pharmacology. 2011 Jun 30;5(6):721-5.
21. Baranska M, Schulz H. Determination of alkaloids through infrared and Raman spectroscopy. The alkaloids: Chemistry and biology. 2009 Dec 31;67:217-55.
22. Sahib HB, AlZubaidy AA, Jasim GA. Anti-Angiogenic Activity of Vitex agnus castus Methanol Extract in vivo Study. Iranian Journal of Pharmaceutical Sciences. 2016 Jan 1;12(1):59-68.
23. Stahl E. Thin-layer chromatography. A laboratory handbook. Thin-layer chromatography. A laboratory handbook.. 1967(2nd edition).
24. Kikuchi H, Yuan B, Nishimura Y, Imai M, Furutani R, Kamoi S, Seno M, Fukushima S, Hazama S, Hirobe C, Ohyama K. Cytotoxicity of Vitex agnus-castus fruit extract and its major component, casticin, correlates with differentiation status in leukemia cell lines. International journal of oncology. 2013 Dec 1;43(6):1976-84.
25. Das S, Das J, Paul A, Samadder A, Khuda-Bukhsh AR. Apigenin, a bioactive flavonoid from Lycopodium clavatum, stimulates nucleotide excision repair genes to protect skin keratinocytes from ultraviolet B-induced reactive oxygen species and DNA damage. Journal of acupuncture and meridian studies. 2013 Oct 31;6(5):252-62.
26. Khaki A, Fathiazad F, Nouri M, Khaki A, Maleki NA, Khamnei HJ, Ahmadi P. Beneficial effects of quercetin on sperm parameters in streptozotocin-induced diabetic male rats. Phytotherapy Research. 2010 Sep 1;24(9):1285-91.
27. Kadam AB, Havele SS, Dhaneshwar SR. Validated HPTLC method for simultaneous estimation of lafutidine and domperidone in bulk drug and formulation. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(4):221-5.
28. Sundararajan V. Evaluation of SMA-RL71, a curcumin analogue nanomicelle as a drug in xenograft models of triple negative breast cancer (Doctoral dissertation, University of Otago).
29. Pop RM, Csernatoni F, Ranga F, Fetea F, Socaciu C. HPLC-UV analysis coupled with chemometry to identify phenolic biomarkers from medicinal plants, used as ingredients in two food supplement formulas. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology. 2013 Nov 13;70(2):99-107.
30. Jain A, Choubey S, Singour PK, Rajak H, Pawar RS. Sida cordifolia (Linn)–An overview.
31. Babu NP, Pandikumar P, Ignacimuthu S. Anti-inflammatory activity of Albizia lebbeck Benth., an ethnomedicinal plant, in acute and chronic animal models of inflammation. Journal of ethnopharmacology. 2009 Sep 7;125(2):356-60.
32. Manpadi M, Kireev AS, Magedov IV, Altig J, Tongwa P, Antipin MY, Evidente A, van Otterlo WA, Kornienko A. Synthesis of Structurally Simplified Analogues of Pancratistatin: Truncation of the Cyclitol Ring. The Journal of organic chemistry. 2009 Aug 18;74(18):7122-31.
33. Sannigrahi S, Mazumder UK, Pal D, Mishra SL. Terpenoids of methanol extract of Clerodendrum infortunatum exhibit anticancer activity against Ehrlich's ascites carcinoma (EAC) in mice. Pharmaceutical biology. 2012 Mar 1;50(3):304-9.
34. Miettinen T, Vanhanen H, Wester I, inventors; Raision Tehtaat Oy Ab, assignee. Use of a stanol fatty acid ester for reducing serum cholesterol level. United States patent US 5,502,045. 1996 Mar 26.
35. Seilicovich A, Lasaga M, Befumo M, DuvILANSKI BH, del Carmen Diaz M, Rettori V, McCann SM. Nitric oxide inhibits the release of norepinephrine and dopamine from the medial basal hypothalamus of the rat. Proceedings of the National Academy of Sciences. 1995 Nov 21;92(24):11299-302.