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Identification of Novel Phytocomponents from the leaf of *Morinda Tinctoria* a potential Indian Medicinal herb by GCMS analysis.

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ABSTRACT

Herbs become the integral part of the mankind since several centuries. India is considered to be one of the most significant zones for cultivation and export of the medicinal plant. According to the literature nearly 60–80% of the world's population still relies on traditional medicine. Indian system of traditional medicine such Ayurveda, siddha and unani formulation comprises greater of herbs or herbomineral combinations. There is always a constant demand for alternate herbal therapy because of known potential sided effect caused by conventional allopathic drugs. Still now there is no proper documentary evidence prevails on nature of phytocomponents present in most of the Indian medicinal herbs. An attempt of bringing some most significant herbs to the lime light this study aimed at evaluating the systematic phytocomponent profiling of the herb *Morinda tinctoria* by GCMS analysis. From the results of the present investigation it was evident that GCMS analysis of ethanolic extract of *Morinda tinctoria*(MT) reveals the presence of most significant phytocomponents like lysine, ascorbic acid, linoleic acid, oleic acid, stigmasterol, lycopene etc. Lysine present in MT used for the treatment of Herpes simplex virus, Osteoporosis, whereas ascorbic acid possess anti-oxidant, anti-ulcer, anti-microbial and anti-cancer properties. linoleic acid helps to maintain the health of cell membranes, improve nutrient absorption. Oleic acid associated with decreased low-density lipoprotein (LDL) cholesterol, and possibly increased high-density lipoprotein (HDL) cholesterol level. Lycopene involved in preventing heart disease similarly stigmasterol reduced Aβ generation by decreasing the enzymes such as β and γ-secretase and also reducing presenilin distribution in lipid rafts implicated in amyloidogenic amyloid protein precursor (APP) cleavage.

Keywords: Morinda tinctoria , GCMS , Phytocomponents, Lysine, Ascorbic acid, Linoleic acid, Oleic acid, Stigmasterol, Lycopene.

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INTRODUCTION

In India, nearly 70% of modern drug are derived from natural resources and number of other synthetic analogues have been prepared from prototype compounds isolated from plants [1-3]. It was reported that more than 60% of cancer drug available in market are based on natural products. Currently, about 80% of antimicrobial, immunosuppressive, cardiovascular, and anticancer drugs are derived from plant sources. More than 70% entities among 177 anticancer drugs approved are based on natural products or mimetic. About 25% prescription drug found globally are derived from plant sources, and nearly 121 such drugs entity are in use. Thirteen drugs of natural origin are approved in United States between 2005 and 2007, and clinical trials are going on more than 100 natural product-based drugs. It was also estimated that 11% of the total 252 drugs found in essential medicine list of WHO are exclusively of plant origin [4, 5]. In Indian traditional medicine a large number of plants are used. It was estimated that Ayurveda uses 1200–1800 plants, Siddha medicine includes 500–900 plants, Unani utilize 400–700 medicinal plants and Amchi medicine uses nearly 300 plants while folk healers of India use more than 7500 medicinal plants in different medicine. Three classical Ayurvedic literatures Charaka Samhita, Sushruta Samhita and Astanga Hridaya mentioned about 526,573 and 902 numbers of plants [6-8].

Morinda tinctoria belongs to the family Rubiaceae grows wildly and distributed throughout Southeast Asia, commercially known as Nunaa, is indigenous to tropical countries and is considered as an important folklore medicine. In the traditional system of medicine, leaves and roots of MTR are used as astringent, deobstruent, emmenagogue and to relive pain in the gout [9] .It has been reported to have a broad range of therapeutic and nutritional values [10] .There is a greater demand for fruit extract of morinda species in treatment for different kinds of illness such as arthritis, cancer, gastric ulcer and other heart disease [11]. The major components have been identified in the Nunaa plant which includes octoanicacid, potassium, vitamin C, terpenoids, scopoletin, flavones glycosides, lineoleic acid, anthraquinones, morindone, rubiadin and alizarin [12-14].

The main aim of the present investigation is to extract the leaf of *Morinda Tinctoria* by soxhlet extraction using ethanol as solvent and to subject the extract for GCMS analysis to explore the presence of biologically significant phytocomponents present within it.

MATERIALS AND METHODS

Plant material

The fresh leaves of *Morinda tinctoria*(MT) were collected from (Peranakkavur Village is a Village in Uttiramerur Taluk in Kanchipuram District, Tamil Nadu, India). The plant materials were identified and authenticated by botanist one Dr. Sasikala Ethirajulu. Captain Srinivasa Murthy research Foundation, Chennai, Tamil Nadu, India. The specimen voucher was deposited in the Department of Pharmacology and toxicology, Sathyabama University, Chennai, Tamil Nadu, India.

Preparation of the Plant Extract

The fresh leaf of MT was collected and washed with running water. It was shade dried at room temperature and 1kg of the dried leaf and fruit was made in to coarse powder. The powder was passed through a 60 No mesh sieve. Air dried Powdered drug was extracted with the solvent ethanol by using soxhlet extraction. Then the extracts obtained such as ethanolic extract of *Morinda tinctoria* (EEMT)was filtered, concentrated by rotary vacuum pump to get the solid mass.

GCMS Specification

Agilent 7890B GC connected to5977A MSD, NIST Ver.2.1 MS data library

Column Name

- HP_5MS 5% Phenyl Methyl Silox -60°C-325°C (325°C) 30m×250µm×0.25µm
- Split less mode injection

- 1µL injection volume

Oven program

- 50°C for 2min then ramp 5°C per minute till 270°C, then 270°C maintained for 2min,total run time 42 min
- Detector temperature 275°C
- Injector temperature 250°C
- Solvent delay 2min
- m/z Scan range 50-600amu

Start Time(min)	End Time(min)	Start m/z	End m/z Scan	Speed
2.50	18.00	50.00	650.00	2000

GC-MS Plays a key role in the analysis of unknown components of plant origin. GC-MS ionizes compound and measures their mass numbers. Ionization method includes EI (Electron Ionization). The EI method produces ions by colliding thermal electrons emitted from a filament with sample gas molecules. This method provides high stability in ionization and obtained mass spectra show good reproducibility. The EI method provides good result for quantitative analysis as well. Quantitative analysis with GC-MS, in which only ions specific to the compounds are measured, is highly selective method without interfering components. Gas chromatography Technique involves the separation of volatile components in a test sample using suitable capillary column coated with polar or non-polar or intermediate polar chemicals. Elite-1 column (100% Dimethyl polysiloxane) is a non-polar column used for analysis of phyto-components. Elite -5 column (5% phenyl and 95% methyl polysiloxane) is an intermediate column and also used for the estimation of Phytochemical. An inert gas such as hydrogen or nitrogen or helium is used as a carrier gas .The compounds of test sample is evaporated in the injection port of the GC equipment and segregated in the column by absorption and adsorption technique with suitable GC programme.

RESULTS

GC-MS analysis of EEMT reveals the presence of 11 compounds. The GC-MS analysis was done using the instrument Agilent 7890B GC connected to 5977A MSD, NIST Ver.2.1 MS data library. The sample volume was 1 to 5.0 µL. The sample of EEMT was run for 18 minutes.

The Chromatogram (Figure.1) shows 11 prominent peaks in the retention time range 12.4 – 46.7. The first peak at 12.4 retention time with Mol wt of 94 corresponds to Dimethyl sulfone. The Second prominent peak at 12.5 retention time with Mol wt 157 is due to the presence of Proline. The third peak at 13 having retention time with the Mol wt 174 denotes the presence of Lysine.

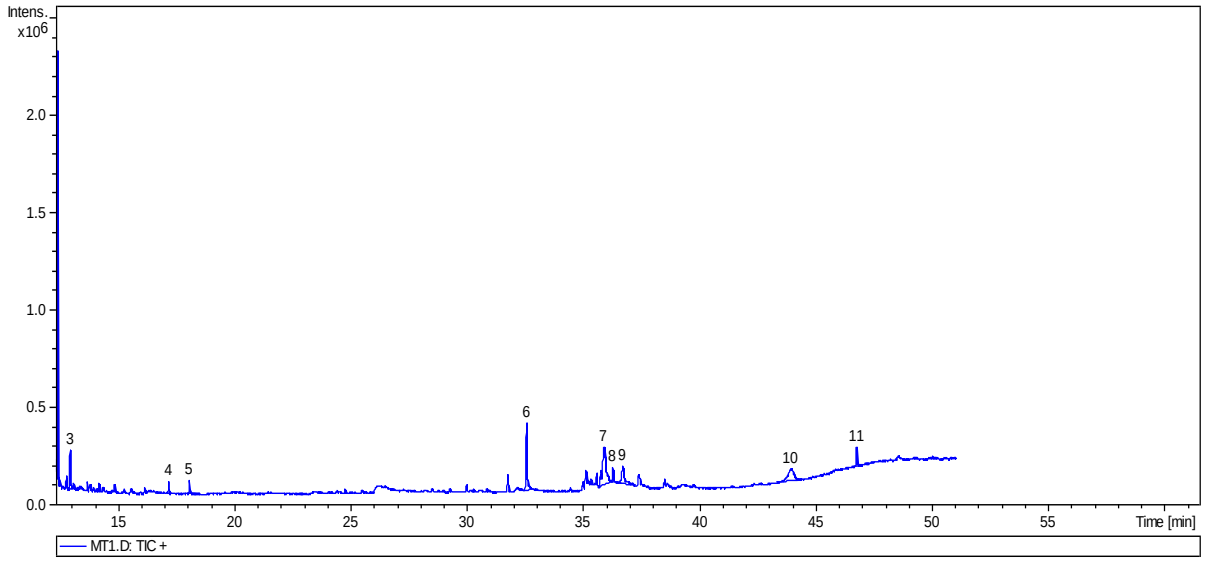

Fig1: GCMS Chromatogram of EEMT

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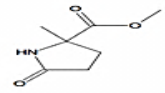
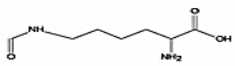
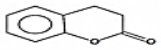
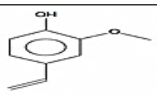
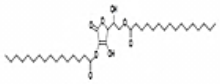
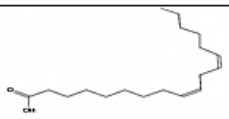
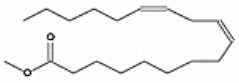
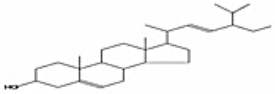
Table 1: Peak Table and component analysis of EEMT

Peak No	Retention Time	Peak Area	Mol. wt	Structure	Mol. Formula	Name
1	12.4	2021843	94		C2H6O2S	Dimethyl sulfone
2	12.5	2618524	157		C7H11NO3	Proline
3	13	448202	174		C7H14N2O3	Lysine
4	17.2	117053	148		C9H8O2	2H-1-Benzopyran-2-one, 3,4-dihydro-
5	18.1	181416	150		C9H10O2	2-Methoxy-4-vinylphenol

The fourth less prominent peak at 17.2 retention time with the Mol wt 148 denotes the presence of 2H-1-Benzopyran-2-one, 3,4-dihydro-.The fifth significant peak at 18.1 retention time with the Mol wt 150 is characteristic of 2-Methoxy-4-vinylphenol.The sixth significant peak at 32.6 retention time with the Mol wt 652 denotes the presence of Ascorbic acid. The seventh less prominent peak at 35.8 retention time with the Mol wt 280 is due to the presence of Linoleic acid. The eighth prominent peak at 36.3 retention time with the Mol wt 282 is due to the presence of Oleic Acid. The ninth less prominent peak at 36.7 retention time with the Mol wt 294 is due to the presence of Methyl linoleate.

Table 2: Peak Table and component analysis of EEMT

Peak No	Retention Time	Peak Area	Mol.wt	Structure	Mol.Formula	Name
6	32.6	1340134	652		C38H68O8	Ascorbic acid
7	35.9	2105251	280		C18H32O2	Linoleic acid
8	36.3	246873	282		C18H34O2	Oleic Acid
9	36.7	817940	294		C19H34O2	Methyl linoleate
10	43.9	989035	412		C29H48O	Stigmasterol
11	46.7	331311	554		C40H58O	Lycopene

The tenth peak at 43.9 retention time with the Mol wt 412 is due to the presence of Stigmasterol. The eleventh less prominent peak at 46.7 retention time with the Mol wt 554 is due to the presence of Lycopene 1,2-dihydro-1-hydroxy- compound.

DISCUSSION

In recent time most of the traditional medical practitioners often recommend herbs, herbal products, or complementary and alternative medicine (CAM) therapy to their patients for the effective treatment of certain diseases [15,16]. As per the survey in 2007 indicated that about 40% of adults and 11% of children used CAM therapy (CAMT), and among the adult users, white and black adults constituted 43.1% and 25.5%, respectively [17].In developing countries, botanical dietary supplements and traditional medicines are often the primary sources of health care for disease prevention and treatment [18, 19].

L-lysine a vital amino with rich nutritive value. Increased concentration of lysine was found in muscles. In recent times lysine supplementation has been adopted for the treatment of herpes simplex infection [20] and osteoporosis [21]. It is an essential amino acid and has very good oral bioavailability [22] and brain penetration [23, 24].Presence of lysine Further Lysine play a crucial role in production of carnitine, a micro nutrient responsible for lipid lowering process. Lysine also possesses certain extensive role in absorption of calcium, formation of collagen in tendon, bone, skin and cartilages. It further strengthens the scope of clinical usage of the plant *morindatinctoria* for such indications.

Vitamin C commonly known as ascorbic acid known for its betterment in treating scurvy .Human biological systems are unable to synthesis the ascorbic acid hence they rely on other dietry supplementation for their daily needs .In addition to this ascorbic acid is an important micro phytocomponents aids in management of H-pylorin induced ulcers [25,26].In the present investigation it was found that the sixth significant peak at 32.6 retention time with the Mol wt 652 denotes the presence of Ascorbic acid in EEMT.Ascorbic acid is a potential anti-oxidant which offers protection from lipid hydroperoxyl (LHP) radicals there by it rescues the cells from oxidative stress induced damages [27, 28].

Oleic acid is the most abundant and significant monounsaturated fatty acid which prevails in most of the Indian medicinal herbs. According to literatures oleic acid along with prolactin synergistically stimulates the growth and development of islet cells in pancreas [29]. This finding strengthens the supplementation of the herb *morindatinctoria* for such ailments.

Lycopene is a carotenoid hydrocarbon it is also reported to be a potential anti-oxidant in scavenging oxygen free radicals [30].Many research finding has suggested that lycopene halts the progression of cancer in mammary gland, liver, skin and lung in mouse ref [31].Further high plasma level of lycopene was associated with a decreased risk of CVD in women [32].

Phytosterols are structural constituents of plant cell membranes and function similarly to cholesterol in animal cells. The most abundant phytosterols are sitosterol, campesterol, and stigmasterol, with the structural differences occurring in the side chain attached to the steroid ring. Plant stanols are saturated sterols and are much less abundant in nature compared to their corresponding sterols. In the human diet, phytostanols comprise about 5 to 10% of the total sterol [33-35]. Due to their low abundance in the diet, phytostanols are often reported as a part of the total “phytosterol” content of a particular food or dietary intake. Western societies consume about 200 to 300 mg phytosterols per day [36- 40], whereas Asian and vegetarian diets provide higher amount of phytosterols in the diet [41, 42]. In the present result the tenth peak at 43.9 retention time with the Mol wt 412 is due to the presence of Stigmasterol.

Scientific investigation of the plant derived stigmosterol e reveals that stigmasterol reduced Aβ generation by decreasing the enzymes such as β and γ-secretase and also reducing presenilin distribution in lipid rafts implicated in amyloidogenic amyloid protein precursor (APP) cleavage [43].

CONCLUSION

From the results of the present investigation it was concluded that the herb *Morindatincotria* has potential phyto components such as lysine, ascorbic acid, linoleic acid, oleic acid, stigmasterol, lycopene etc. Regular intake of the leaf and fruits of the plant morinda may compensate the dietary requirement of lysine,

lycophene and ascorbic acid and offers good protection against oxidative stress induced diseases. Further the presence of plant sterols offers better neuro protection and monounsaturated fatty acid promotes growth and development of islet cells in pancreas. By considering all these medical benefits *morindatinctoria* may considered to be one of the valuable lead for the clinical management of various disorders.

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CONFLICT OF INTEREST: Author approved the manuscript and they declare no conflict of interest

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