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GAS CHROMATOGRAPHY-MASS SPECTROSCOPY ANALYSIS OF *ORMOCARPUM COCHINCHINENSE* LEAF EXTRACT- TRADITIONAL BONE HEALING PLANTS

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Abstract: The present study was to investigate the *Ormocarpum cochinchinense* leaf for phytochemical compounds and GC-MS analysis. Phytochemical screening of the leaf extract was carried out according to the qualitative method. GC-MS analysis was performed on the ethanolic extract of *Ormocarpum cochinchinense* to find out the chemical constituents. The results showed the presence of flavonoids, alkaloids, steroids, terpenoids, glycosides, tannins, saponins, acidic compounds, coumarins, and not detected fat and oils. GC-MS results revealed the presence of eleven different phytochemical constituents like, 1-Butano, 3-methyl-, acetate 1), 4-Vinylphenol 2), Acetic acid, trichloro 3), Mome Inositol 4), 2,6,10-Trimethyl,14-ethylene-14-pentadecne 5), Hexadecanoic acid 6), Phytol 7), 1,14-Tetradecanediol 8), Hexadecanoic acid, 1-(Hydroxymethyl)-1,2-Ethanediy ester 9), 2-Hydroxy-3-[(9E)-9-Octadecenoyloxy]propyl (9E)-9-octadecenoate 10), Eicosanoic acid, 2-ethyl-2-methyl-, methyl ester 11). The components were isolate and identified by comparing their relation indices and mass spectra fragmentation patterns with those stored on the MS-Computer library and also form the published literatures. However, isolation of individual phytochemical constituents may proceed a novel drug.

Keywords: Phytochemical, Ethanolic leaf extract, GC-MS analysis, *Ormocarpum cochinchinense*.



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INTRODUCTION

Medicines from plant origin are effective weapon to cure diseases. Herbal plants are natural sources of phytochemical constituents that can be used against many human diseases in present day^[1]. Novel therapeutic agents were developed by using pharmacological properties of plants and may be used as leads^[2]. The therapeutic values of these plants lie in bioactive compounds that produce definite physiological actions on the human body. These phytochemical constituents in medicinal plant include flavonoids, alkaloids, steroids, terpenoids, glycosides, tannins, saponins, acidic compounds, coumarins, fat and oils^[3]. The world is now looking to India due to its rich biodiversity of medicinal plants and abundance of conventional medicine systems^[4]. In India, many herbal plants are commonly used by all section of people either directly as folk remedies or indirectly in pharmaceuticals preparations of modern medicine. There is a required that the medicinal plants be evaluated for phytochemistry so as to determine the potentials of these indigenous sources of medicines. Therefore in current study one medicinal plant was selected for phytochemical analysis.

Plants have fundamental nutritional importance by their content of protein, carbohydrate, fats and oils, minerals, vitamins and water responsible for growth and development in man and animals. Plant based medicines are considerable interest because of their resistance to many antibiotic, people perceive that green medicine is safe than synthetic drugs which are costly and have adverse effects^[5]. Many researchers have come up with the fact that some plant compound which have been regarded as nutritional or anti-nutrients have Potentials in helping to reduce the risk of several deadly diseases in man^[6,7,8]. Medicinal plants provide health care and disease free life. The active principles contained in it are exploited in traditional medicine for various ailments^[9].

Ormocarpum cochinchinense is a small herb found in Tamil Nadu, commonly known as Elumbotti belong to leguminaceae family. This herb was conformed as medicine by humans from earlier days to till now. Most medicines in practice were formulated by plant based metabolites^[10]. The traditional knowledge about medicinal herbs and their effect against diseases was known by ancient Indians^[11]. But scientific evidence was unknown as it has been kept secreted by village vaidyas^[12]. The present investigation, focus on this hidden treasure, to exploit treatment for bone healing activities.

Materials and Methods

Collection of plant materials

The leaves of *O. cochinchinense* were collected from local area of Villupuram District, Tamil Nadu, India during October- December-2012.

Chemicals

All chemicals were purchased from SD fine chemical company Mumbai and all chemicals were of analytical grade.

Preparation of powder from leaves

In the laboratory, bone healing leaves were dried and then powdered with a mechanical grinder and stored in a jib lock cover. The dried powder (1.00Kg) was refluxed with ethanol (EtOH), for seven days. Removal of whole dry plants was done by filtration through whatman No.1 filter paper. The extracts were placed in glass petridishes 90mmX15mm; pyrex, germany). The total filtrate was concentrated to dryness, in hot air oven at 32°C to render ethanol solvent extract. The dried crude extract (blackish-green yield 5.5% w/w) was found which was then re-dissolved in ethanol to obtain a solution containing 2.0mg/ml of extracts to be used for further assays.

Qualitative phytochemical group test

Extract was subjected to qualitative screening for the detection of phytochemical groups by standard method^[13,14]. In each test 10% (w/v) solution of the extract was taken unless otherwise mentioned in the individual test.

Gas chromatography-Mass spectrometry (GC-MS) analysis

The analysis of the leaves powder were performed using a Hewlett Packard 5890 II GC, equipped with a HP-5 MS capillary column (30m.25, film thickness 0.251m) and a HP5972 mass selective detector. For GC-MS detection an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1m/min. Injector and MS transfer line temperature were set at 220 and 290°C respectively. The diluted samples (1/100 in acetone, v/v) of 1µl were injected manually and in the split less mode. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, NBS75K library data of the GC-MS system and literature data. The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phases reported in the literature^[15].

RESULT AND DISCUSSION

Qualitative phytochemical screening of *O. cochichinense* leaves extract

Ethanol extract of *O. cochichinense* leaves showed the presence of alkaloids, steroids, flavanoids, glycosides, saponins, tannins, acidic compounds, resins and coumerines but not detected fats and oils. Quantitatively, alkaloids were more whereas resins were least among

the detected secondary metabolites (Table 1). The extract was found to have alkaloid, flavonoids and the results are consistent with partial studies conducted earlier^[16].

GC-MS Analysis of *O. cochinchinense* leaf extract The eight compounds from *O. cochinchinense* leaves were identified by gas chromatography-mass spectrometry (GC-MS). The main constituents identified in the leaves of *Ormocarpum cochinchinense* were 1), 4-Vinylphenol 2), Acetic acid, trichloro 3), Mome Inositol 4), 2,6,10-Trimethyl,14-ethylene-14-pentadecne 5), Hexadecanoic acid 6), Phytol 7), 1,14-Tetradecanediol 8), Hexadecanoic acid, 1-(Hydroxymethyl)-1,2-Ethanediy ester 9), 2-Hydroxy-3-[(9E)-9-Octadecenoyloxy]propyl (9E)-9-octadecenoate 10), Eicosanoic acid, 2-ethyl-2-methyl-, methyl ester 11). In the bone healing leaf extract showed the 11 compounds (Table-2, 3). This study has revealed the presence of many secondary metabolites and bioactive phytocomponents in the leaf of *O. cochinchinense* which might be of a very important medicinal value and further study includes isolation and purification of bioactive components^[17]. To the best of my knowledge, compounds 1-11 were isolated for the first time from *Ormocarpum cochinchinense*. Herbal plants in all fact of life have served a important starting material for drug design and development^[18]. Antimicrobial substances like saponins, glycosides, flavonoids and alkaloids etc are found to be distributed in plants, yet these compounds were not well established due to the lack of knowledge and techniques^[19]. From this phytoconstituents, saponins have been reported to exhibit hemolytic and foaming activity^[20].

Generally, herbal plants are valuable plant materials having a wide range of local therapeutic applications, the leaves, roots, barks and seeds are found to be antipyretic, laxative, analgesic, antioxidant, antifungal, antibacterial and non inflammatory^[21]. To the best of my knowledge, compounds 1-11 were isolated for the first time from *Ormocarpum cochinchinense*.

1. Phytochemical screening of *Ormocarpum cochinchinense* leaves using ethanol extract

S.No.	Phytoconstituents	Name of the test	Observed changes	Result
1.	Flavonoids	Ammonium test	Yellow color	+
2.	Alkaloids	Dragendorff's test	Red precipitate	+
		Wagner's test	Redish-brown precipitate	+
		Hagers test	Yellow precipitate	+
3.	Steroids	General test	Redish brown	+
4.	Terpenoids	General test	Grey color	+
5.	Glycosides	Fehling's test	More dense red	+
6.	Tannins	Lead acetate test	Cream gelatinous precipitate	+
		Ferric chloride test	Greenish to black color	+
7.	Resins	Precipitate test	Precipitate occure	+
		Color test	Pink color changed	+
8.	Saponins	Frothing test	Stable froth	+
9.	Acidic compounds	General test	Turn blue litmus paper red	+
10.	Coumarins	General test	Intense fluorescence under UV light	+
11.	Fat and oils	General test	No change	ND

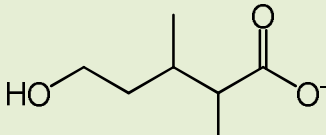
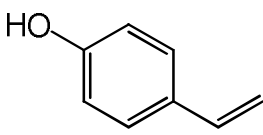
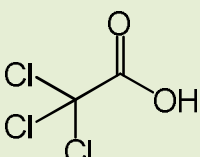
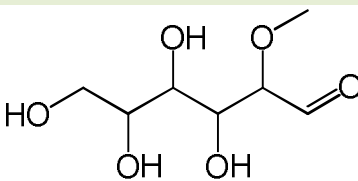
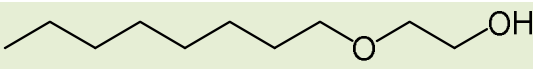
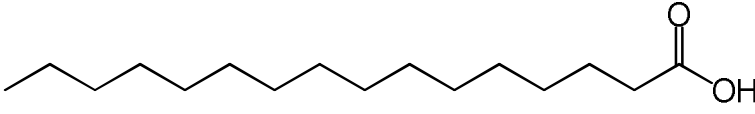
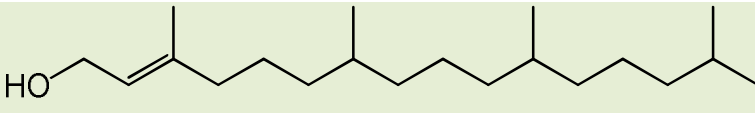
"+" Presence, ND Not detected

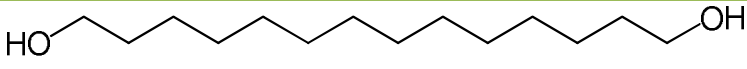
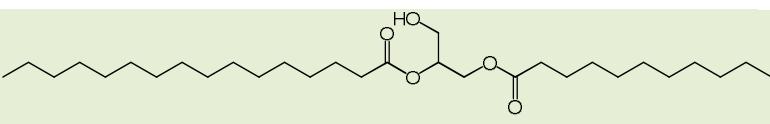
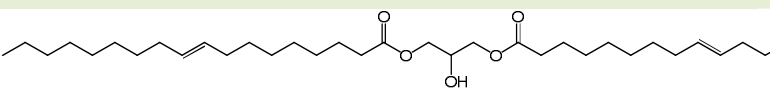
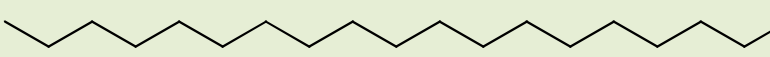
Table 2. Identified phytochemicals from the ethanolic leaf extract of *Ormocarpum cochinchinense*

Retention time	Name of the compounds	Molecular formula	Molecular weight	Area(%)
6.926	1-Butano, 3-methyl-, acetate	C7H14O2	130	21.93
8.544	4-Vinylphenol	C8H8O	120	4.35
9.502	Acetic acid, trichloro	C7H11Cl3O2	232	1.21
14.792	Mome Inositol	C7H14O6	194	49.73
15.755	2,6,10-Trimethyl,14-ethylene-14-pentadecne	C20H38	278	1.76
17.148	Hexadecanoic acid	C16H32O2	256	2.58
18.487	Phytol	C20H40O	296	4.29
19.984	1,14-Tetradecanediol	C14H30O2	230	1.72
20.149	Hexadecanoic acid, 1-(Hydroxymethyl)-1,2-Ethanediy ester	C35H68O5	568	6.45
21.617	2-Hydroxy-3-[(9E)-9-Octadecenoyloxy]propyl (9E)-9-octadecenoate	C39H72O5	620	5.25

21.812	Eicosanoic acid, methyl-, methyl ester	2-ethyl-2-	C ₂₄ H ₄₈ O ₂	368	0.71
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Table.3. Extracted ions of the compounds examined, and the relative retention times during the GC-MS analysis for Retention time (RT), extracted ions (m/z).

Compound Name	Formula	RT	m/z
1-Butano, 3-methyl-, acetate		6.926	88
4-Vinylphenol		8.544	120
Acetic trichloro acid,		9.502	70
Mome Inositol		14.792	116
2,6,10-Trimethyl,14-ethylene-14-pentadecne		15.755	123
Hexadecanoic acid		17.148	129
Phytol		18.487	123

1,14-Tetradecanediol		19.98 4	12 9
Hexadecanoic acid, 1-(Hydroxymethyl)-1,2-Ethanediy ester		20.14 9	18 5
2-Hydroxy-3-[(9E)-9-Octadecenoyloxy]propyl (9E)-9-octadecenoate		21.61 7	12 9
Eicosanoic acid, 2-ethyl-2-methyl-, methyl ester		21.81 2	12 9

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