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Phytochemical studies and GC/MS analysis on the isolated essential oil from the leaves of *Citrus Aurantium* Linn

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ABSTRACT

Preliminary phytochemical studies of the leaves of C.aurantium and Gas chromatography –Mass spectrometry (GC – MS) analysis of its essential oil for the determination of the constituents. In the present study to perform the preliminary phytochemical studies and organoleptic characters by standard procedures. Chemical composition of leaf essential oil was determined by GC-MS analysis. Preliminary phytochemical screening on the leaves revealed the presence of flavanoids, carbohydrates, phytosterols, volatile oil, saponins, tannins, proteins terpenoids and absence of glycosides, alkaloids and fixed oil. GC-MS profile of the essential oil obtained by hydro distillation from the fresh leaves showed the presence of 35 compounds. The major compounds were Eucalyptol (1, 8 Cineole - 43.05%), Sabinene (16.65%), β-Linalool (15.25%), α-Terpineol (8.025), α-Pinene (1.34%), β-Myrcene (1.20%), 4-Terpineol (1.11%), β- Pinene (1.01%), D-Limonene (0.97%), O-Cymene (0.88%). Some variations were observed in the composition and percentage of constituents in the essential oil when compared to previous studies. This is may be due to climate change, soil, altitude and other conditions. GC-MS study revealed essential oil extracted from the fresh leaves possesses poly phenol compounds. Traditionally leaves were used as an anti –microbial and from that are assumed that poly-phenols present in the essential oil may be the reason for the activity. The essential oil may be a good candidate of potential therapeutic effects like antimicrobial activity against bacteria, virus, particularly to MRSA with resistance modifying property and stimulation of innate immunity.

Keywords: *Citrus aurantium* leaves, phytochemical studies, Essential oil, GC-MS analysis.

INTRODUCTION

Citrus aurantium commonly known as bitter orange is widely and easily available plant belonging to the family Rutaceae. The leaves, fruit, barks are used traditionally for the treatment of wide panel of diseases [1].

The attention of many researchers has recently been attracted to study the biological activities of essential oils (EO) from plants. Essential oils are valuable natural substances used in many areas like perfumery, cosmetics, spices, herbal therapy, aromatherapy etc., [2]. Essential oils are complex mixtures mixtures of hydrocarbons and oxygenated compounds derived from these hydrocarbons [3]. Detailed knowledge of the constituents of the EO will lead to a better and specifically diverted application and also to know its purity only can obtain by means of carefully performed GC/MS experiments [4]. Worldwide demand of EO especially citrus oils has been in increasing phase during past few decades. Carvacrol, Cinnamaldehyde, Citral, Thymol and Limonene are some major bioactive compounds of some essential oils which are recommended as food additives by European commission with no harm

to human health [5]. The chemistry of EO of citrus species like *Citrus aurantifolia*, *Citrus aurantium*,*Citrus limon*, etc have been reviewed in detail [6]. *Citrus aurantium* (Khatta: Hindi, Narangam, Narattai': Tamil) is a tree with greenish white, glabrous shoots which cultivated in India for its fruit and used for various medicinal purposes [7]. Its ethno-medicinal application has been well known for a long time. Leaf is traditionally used for emmemagogue[8], blood pressure, cough, cold, bronchitis[9], ear ache, dysentery, diarrhea[10], UI ailments, dysmenorrheal, influenza, insomnia[11],anti-inflammatory, headache, nervousness, weakness [12], hypoglycaemic, carminative, fever, sedative, digestive[13].The leaves used as cytotoxic[14], antiyeast, antifungal and antibacterial[15]. Essential oil of the leaves used as antibacterial and antifungal [16], anxiolytic [17]. *Citrus aurantium* leaves from different regions vary in smell and taste. The oil content of different Indian types varies from 0.3 to 0.4%. Mostly quantitative analysis has been published for the EO of fruit peel. In our study to report on phytochemical studies on the dried powdered leaves was carried out which can help for the identification of pharmacological activities based on the results for secondary metabolites and also to find out the adulterants.The constituents of essential oils are identified using GC in combination with mass spectrometry. GC-MS is the most powerful technique used to identify the components present in the oils. In the present study, *C.aurantium* leaf oil was studied for their organoleptic and physical properties, and analysed by GC-MS method to know their chemical composition.

MATERIALS AND METHODS

Plant materials:
Fresh leaves were collected in the month of January 2011 from Chinthamani village, Villupuram District. The climate was normal. The plant was authenticated by Dr.P. Jayaraman, Director of Plant Anatomy Research Institute, Tambaram, Chennai, and Tamilnadu. Voucher herbarium specimen (PCG-1/300) was prepared and preserved at the herbarium of College of Pharmacy, Madurai Medical College, and Madurai.

Phytochemical studies:
Various chemical tests were employed in the preliminary screening for various secondary metabolites like alkaloids, carbohydrates, glycosides, sterols, saponins, tannins, proteins and free amino acids, mucilage, terpenoids, essential oil, flavanoids and fixed oil on the shade dried powdered leaves of *C.aurantium* [18, 19].

Extraction of essential oil:
The fresh leaves of *C.aurantium* were hydro distilled using Clevenger apparatus for 3 hours with an average yield of 0.4% (w/v). The oil obtained was dried over anhydrous sodium sulphate and stored in closed glass vials at 4ºC until analysis.

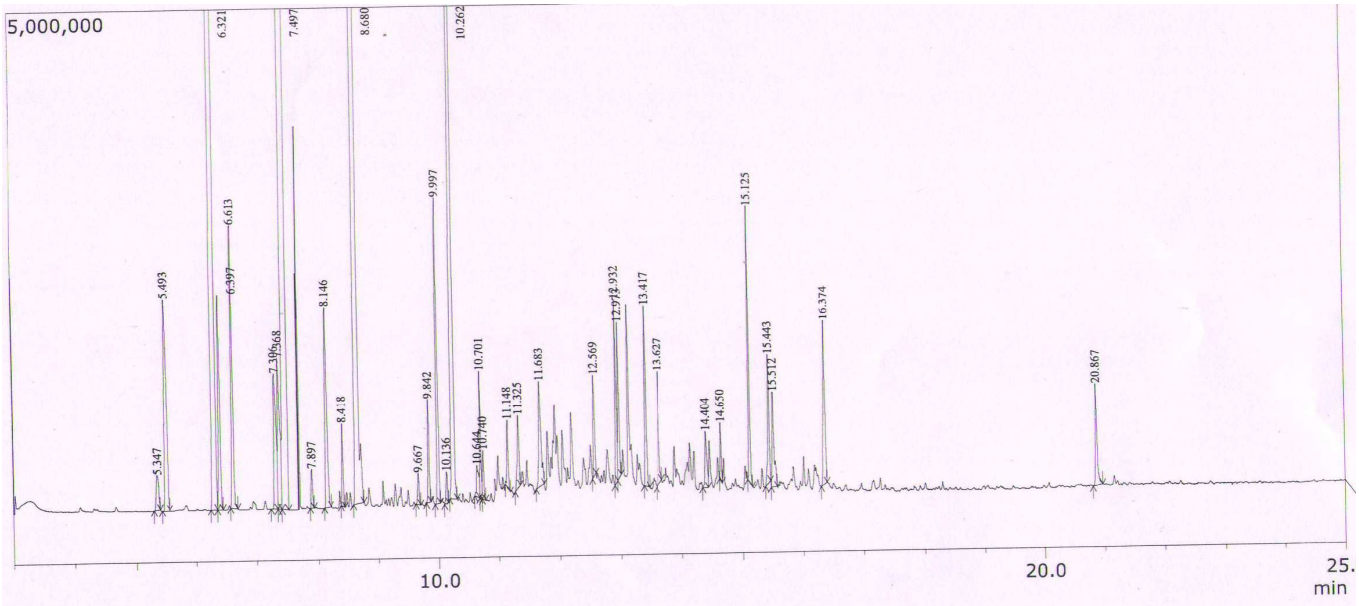

Figure 1: GC-MS chromatogram of *Citrus aurantium* leaf oil

Gas chromatography – Mass spectrometry analysis (GC-MS):
GC-MS analysis were performed on a GC – 2010 Shimadzu capillary gas chromatography directly coupled to the mass spectrometer system (GC-MS – model QP 2010; S/N column (70464300019 SA; Shimadzu) DB – 5ms non

polar fused silica capillary column (30m X 0.25mm, 0.25µm film thickness) was used under following conditions: oven temperature program isotherm 2 min at 70ºC, 3ºC/min gradient to 200ºC and final temperature kept for 35 min; injection temperature 200ºC carrier gas is helium with flow rate 1.51ml/min; linear velocity 45.1 cm/sec. The effluent of the GC column was introduced directly into the source of MS and spectra obtained in the EI mode with ionization energy 70eV, in the electronic ionization mode and ion source temperature is 200ºC. The solvent cut time 3 min. The sector mass analyzer was set to scan from 40 to 1000m/z with interface temperature of 240ºC.

Identification of components:
The components of the essential oil were identified on the basis of comparison of their relative indices and mass spectra by computer matching with WILEY8 and National Institute of Standards and Technology (NIST08) libraries provided with the computer controlling GC-MS system [20].

RESULTS AND DISCUSSION

Preliminary phytochemical screening on the leaves of *Citrus aurantium* showed the presence of different chemical compounds like carbohydrate, phytosterol, saponins, tannins, proteins,essential oil, terpenoids and flavanoids. Other phyto constituents like alkaloids, glycosides and fixed oil, mucilage were absent.

The yield of essential oil from these leaves was 0.4% w/v through hydro distillation. The oil was greenish yellow with aromatic odour and has pungent taste with refractive index of 1.4570 [21]. The constituents of leaf oil were listed in order of their elution on the DB – 5ms column (Fig.1).

In total 35 volatile components were identified. In which sesquiterpene hydrocarbon were found to be the major group of compounds. These were Eucalyptol (1, 8 Cineole - 43.05%), Sabinene (16.65%), β-Linalool (15.25%),α-Terpineol (8.025),α-Pinene (1.34%), β-Myrcene (1.20%), 4-Terpineol (1.11%), β- Pinene (1.01%), D-Limonene (0.97%), O-Cymene (0.88%) and other minor compounds [Table -1].

Table 1: Components of *Citrus aurantium* oil identified by GC-MS analysis

Components identified	Retention time	Peak area (%)
Eucalyptol (1,8-cineole)	7.49	43.05
Sabinene	6.32	16.65
Beta linalool	8.68	15.25
Alpha-Terpineol	10.26	8.02
Alpha-Pinene	5.49	1.34
Beta-Myrcene	6.61	1.20
4-Terpineol	9.99	1.11
Beta.-Pinene	6.39	1.01
D-Limonene	7.36	0.97
Trans-Nerolidol	15.12	0.93
5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	8.14	0.93
o-Cymene	7.30	0.88
Beta -Eudesmol	16.37	0.77
Caryophyllene	13.41	0.65
Cyclohexene, 3-acetoxy-4-(1-hydroxy-1-methylethyl)-1-methyl-	11.68	0.64
1-Methyl-4-(1-methylethyl)-3-cyclohexen-1-ol	12.93	0.63
Beta-Elemene	12.97	0.56
Caryophyllene oxide	15.51	0.52
Spathulenol	15.44	0.51
Phytol	20.86	0.47
s-(+)-5-(1-Hydroxy-1-methylethyl)-2-methyl-2-cyclohexen-1-one	13.62	0.44
3-Methoxy-p-cymene	10.70	0.40
(+)Alpha-terpineol	9.84	0.38
Ho-trienol	12.56	0.35
4-Methyl-2,3-hexadien-1-ol	11.32	0.33
Germacrene B	14.40	0.31
Trans-linalool oxide	8.41	0.28
(E)-2-Decenyl acetate	11.14	0.24
Trans-Geraniol	10.64	0.23
Alpha.-Thujene	5.34	0.23
Tau.-Cadinol	14.65	0.18
2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-	10.74	0.16
(3Z)-3-Hexenyl 2-methylpropanoate	10.13	0.13
Gamma.-Terpinene	7.89	0.13
Sabina ketone	9.66	0.11

A comparative chemical investigation on leaf and peel oil showed linalyl acetate in leaf oil and Limonene in peel oil as major component. Limonene was also reported to be a major component in commercial sample and peel oil obtained at different storage condition. Previous study determined the variation in chemical composition of a number of cold pressed bitter orange oils. The monoterpene hydrocarbon (Limonene) in Cuban bitter orange oil was also reported as major components [22]. Comparison of the chemical composition of Andalucian (Spanish) with Sicilian (Italian) bitter orange oil were carried out previously and reported Limonene as major component on both oil [23]. Linalool as major component was reported in the leaf oil (Mauritius origin) by Capillary GC and GC-MS [24]. Very recently one study examined cold pressed daidai peel oil by GC and GC-MS and found limonene as main component. These results showed linalool / Linalyl acetate as major component in leaf oil. However, limonene was found in the peel oil of this plant [25]. Some variations were observed in the composition and percentage of constituents in the essential oil when compared to previous studies. This is may be due to climate change, soil, altitude and other conditions.

The essential oil was effective against wide spectrum of microbes. These may be due to the presence of multiple components in the volatile oil. It can be optimistically concluded that the essential oil may be used in variety of effective pharmaceutical formulations to treat various chronic diseases.

CONCLUSION

The present study was undertaken to isolate and analyze the essential oil of the leaves of *C.aurantium* by GC-MS as it is widely available and menacingly wasted part of it and also to identify the various chemical compounds present in the leaves by preliminary phytochemical screening.

The essential oil of *C.aurantium* proved to be effective against microbes like bacteria, virus, particularly to MRSA with resistance modifying property and stimulation of innate immunity. Its medicinal application are still to be explored well so as to minimize the menacing wastage and to maximize the revenue generated by this crop to boost up our national economy as well as the proper exploitation of the plant for the therapeutic purposes. By investigating its bioactivity of its essential oil we can meet the situation of unsettling facts of modern pharmaceutical industry which facing lately it pipeline of new drug discovery seems to be almost empty.

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