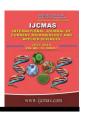


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Hydro-distillation of Essential Oil of *Mangifera indica L*. Flowers and its GC-MS Analysis

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ABSTRACT

Medicinal and aromatic plants (MAPs), such as those from Anacardiaceae family and their metabolites, namely Essential Oils (EOs) are of great pharmaceutical interest and are also a source of biologically relevant compounds having broad spectrum anti-microbial activity as well as extremely high degree of druggability. These EOs are obtained through the hydro-distillation of different parts of MAPs. Therefore, the aim of this work is to extract the essential oil from flowers of wild *Mangifera indica L*. plant through hydro-distillation method using Clevenger's apparatus and to analyze the various constituents present in that Wild mango flower essential oil (WMFEO) by gas chromatography coupled with mass spectrometry (GC-MS).

Introduction

The medicines derived from herbs have gained special interest in the subject of commercial as well as scientific exploration. The scientific exploration of the ethnobotanicals is an important step to introduce the drug of interest into the pharma industries. Medicinal plants have always attracted man-kind because of their higher degree of therapeutic index along with its properties of nontoxicity. Among these, there is sub group of medicinal and aromatic plants (MAPs) which are always thought for its extremely high degree of druggability. The MAPs are renowned as powerhouse of thousands of botanicals (phytochemicals) synthesized through the secondary metabolic pathways. The MAPs possess those active

principles or compounds which are having broad spectrum anti-microbial activity (Singh et al., 2021). The MAPs contain aromatic volatile substances which are also known as hydro-distillates, because the volatile principles of the said plants are hydro-distilled in the form of essential oils with sweet aroma. Information on the chemistry and properties of hydro-distillates (essential oils) of MAPs species make it a sure source of phyto-drugs as well as excipients in Pharma industry. Furthermore, essential oils have gained widespread popularity worldwide due to their relatively low risk of adverse effects, easy accessibility, affordability, and high effectiveness (Yang et al., 2021; Nasri, 2013). This broad utilization has generated considerable enthusiasm for exploring the potential of essential oils in various fields,

particularly in natural healthcare (Bhalla et al., 2013). Gas chromatography-mass spectrometry (GC-MS) is the best technique to identify the bioactive constituents of long-chain hydrocarbons, alcohols, acids, esters, amino acids, alkaloids, steroids and nitro compounds (Geetha et al., 2013). Mangifera indica L. is a prominent member of the cluster of MAPs (Shivran et al., 2023) which is affluent in Indian Climate. Besides source of edible fruit, is also an essential oil-bearing plant, inferring about its secondary metabolite resource. Therefore, the Traditional medicine have enlisted its name and application in curing diabetes. cough. diarrhea and more over immunomodulation (Lauricella et al., 2017). Essential oil (EO) from the leaves, peel, seed and barks of Mangifera indica L. is reported to contain active ingredients that are utilized in the food and pharmaceutical industry (Maharaj et al., 2022). Also, it has been documented that the fruit of Mangifera indica contains substantial quantities of protein, fat, dietary fiber, ash, carbohydrates, and energy. The present study is aimed to investigate the phytoconstituents present in the essential oil from flowers of Mangifera indica for the first time. More precisely, this work is devoted towards selection of plant in the near locality, hydro-distillation of essential oil (hydro-distillate) using Clevenger's apparatus (Clevenger, 1928) and detection of its Phytoconstituents using GC-MS.

Materials and Methods

Sample preparation

The mature inflorescence of *Mangifera indica L*. (wild mango flowers) were collected from Chaunrpur village from the interior of Sambalpur district of Western Odisha, India and were authenticated and deposited in the laboratory of Medical Microbiology, Department of Biotechnology and Bioinformatics, Sambalpur University, Burla, Sambalpur, Odisha. From the inflorescence, the flowers were separated manually, washed with sterile water and shade dried. After that, the flowers were packed into the round bottom flask and subjected to hydro distillation in the Clevenger's apparatus.

Extraction of Essential oil

The hydro-distillation of flowers of *Mangifera indica L.* gives essential oil (Wild mango flower essential oil, WMFEO) via Clevenger apparatus (Ullah *et al.*, 2022;

Sampath et al., 2011). The mango flowers were washed with tap water followed by washing in distilled water. The flowers were subjected to shade drying for 4 hrs. The bench top Clevenger's apparatus was taken as the distillation unit. The heating mantle was set initially at a temperature of 100° C till the boiling of the water inside (1 hour). Then, the temperature was decreased to 70° C for a period of 12 hours. The oil droplets distilled inside was found to be condensed inside the condenser after a prolonged period of hydro-distillation. A layer of EO was observed on the surface of water layer, then the heating mantle was switched off and the over layer EO was collected by discarding the water in a sterile container. Then, the oil was allowed to be free from moisture content inside a desiccator filled in with silica. The neat oil was taken out form the desiccator and was kept in refrigerator at +40 C until GC-MS profiling and biological screening.

Detection of Components in *Mangifera* flower Extract/Hydro-distillates through GC-MS analysis

The essential oil of *Mangifera indica L*. flowers (WMFEO) was examined for the occurrence of various compounds by GC-MS technique. The GC-MS analysis of the hydro-distillate was carried out in the Laboratory of Chemical Engineering department, National Institute of Technology, Rourkela, Odisha, India using Agilent GC: 7980B Agilent MS: 5977A with auto-sampler Column: DB5MS and DB1MS. The capillary column MS detector was attached in the interface with auto injector. Further, the eluted chromatograph was referred with NIST laboratory and eluted compound were identified with respective M/Z ratio.

Identification of test components

Further, the eluted chromatograph was referred with NIST laboratory and eluted compound were identified with respective M/Z ratio, with Mass Spectra steak peaks of molecular peaks, and fragmentation peaks.

Results and Discussion

Physico- chemical characterization of oil

The yield of essential oil obtained by hydro-distillation of *Mangifera indica L*. flowers had a bright dark yellow colour with sweet aroma.

GC-MS Analysis of WMFEO

The result obtained from the GC-MS analysis, the eluted peak spectra are depicted in the Supplementary Page No. 1 (SP-1). While, Supplementary Pages 2-24 (SP 2-24) contains the peak data of M/Z ratio with its abundance of each of the components detected. It was noted that, the first peak was at 16.740 while 4.304 was the last peak, which was eluted in the chromatograph. From the MS-MS data, GC result at page no 2, with M/Z - 71.0 was considered as 100% abundance implying that, this was the molecular peak while M/Z - 88.0 was defined as base peak with abundance of 60.88%. Hence, from the library search report using Chem Station Integrator, it was observed that, the compound is Butanoic acid, ethyl ester while it was further observed that, there was presence of the said compound with different m/z ratio a) 88.0, 60.88% b) 73.00, 20.44% c) 60.00, 20.06% d) 89.00, 16.9% indicated by black, blue, red and green stick peak set respectively. There was identification of β-Phellandrene based upon elution of molecular peak 93.1 (100%) while base peak 91.10 (47.52%). Another m/z peak was also eluted with m/z 136.50 (24.38%). Likewise, in addition to these two components, a third component, β- Pinene, MS data was analyzed which has molecular peak at 93.0 and m/z base peak with 91.00 (26.09%) and also m/z 79.00 base peak (22.4%). Further, with the molecular peak of 119.0 (100%), the base peak m/z 134.00 (26.79%) and also m/z 91.00 (21.18%) were agreeable with the structure of o-Cymene and p-Cymene respectively. In addition to these above mention components molecular peak m/z 68.10 (100%), the base peak m/z 93.00 (71.49%); base peak m/z 67.10 (68.46%), m/z 45.10 (38.51%) were depicted with D-Limonene structure. Thus, it is inferred about presence of D-Limonene. Besides, a molecular peak m/z 93.10 (100%), a base peak at 45.10 (50.90%), base peak 91.00 (41.49%), base peak m/z 92.00 (38.28%) has inferred about structure of transβ-Ocimene. Further, the molecular peak 121.20 (100%) with base peak m/z 93.10 (96.65%) inferred about structure of 2-Carene, base peak m/z 136.20 (91.00%) has inferred about (+)-4-Carene respectively. Bicyclobutylidene has molecular peak m/z 79.00 (100%) while base peak m/z 95.00 (32.18%).

Thymol was identified from the MS having molecular peak m/z 135.00 (100%) with base peak m/z 150.00 (17.72%). In addition to this, Citronellol, MS was found to be having molecular peak m/z 69.10 (100%) and base peak m/z 67.00 (57.16%). Besides, some unknown compounds, there was elution of Resorcinol with

molecular peak m/z 137.00 (100%) and with base peak m/z 109.0 (24.26%). Furanone was also observed to be one constituent which was eluted in the mass spectrum with molecular peak m/z 85.00 (100%), base peak m/z 56.10 (8.52%), base peak m/z 55.00 (8.21%) base peak135.00 (7.54%). Vanillin was inferred from the mass spectra where molecular peak m/z was 51.00 (100%), base peak m/z 152.00 (92.52%), base peak m/z 81.00 (21.13%) and base peak m/z 109.00 (17.24%). Further, Coumarin was also eluted having molecular peak m/z 118.00 (100%), base peak m/z 146.00 (91.26%), base peak m/z 90.00 (39.24%), base peak 89.00 (37.80%). And ethyl, Vanillin, was also found to be in the mass spectra with molecular peak m/z 166.00 (48.33%). Further, Caryophyllene oxide was also eluted in the spectra with molecular peak m/z 121.00 (100%), base peak m/z 55.10 (87.41%). The identified compound from the Mass spectrum, of eluted peaks of Gas Chromatography is given in Table 1. The results obtained from Gas chromatography analysis made for mango flower hydro-distillate is given in Table no 2 and the results showing peaks is attached.

Identification of components in WMFEO

The identified compounds were (1) butanoic acid, (2) Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-, (3) (1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (4) β -Phellandrene, (5) β-Pinene, (6) o-Cymene, (7) D-Limonene, (8) β- Ocimene, (9) 2-Propanol, 1,1'-oxybis, (10) Methane, diethoxy, (11) 2-Carene, (12) 2-Butanol, 3,3'-oxybis-(13)Phenylethyl Alcohol, Methylenebicyclo[2.1.1]hexane, (15) Thymol, (13) Benzenemethanol, (14) Benzenemethanol, alpha., alpha, (15) Thymol, (16) Citronellol, 1(7) Resorcinol, (18) 2,4-Hexadiene, 3,4-dimethyl-, (E,Z),, (19) 2(3H)-Furanone, dihydro-5- (20) Vanillin, 42 (21) Coumarin, (22) Ethyl Vanillin, (23) Caryophyllene oxide.

In Conclusion, GC-MS analysis is the first step towards understanding the nature of active principles in medicinal plants and this type of study will be helpful for further detailed study. The presence of various biologically active compounds in the essential oil obtained from the flowers of *Mangifera indica* (WMFEO) greatly contributed to its pharmacological activities. This plant can serve as a new natural source for obtaining many therapeutically valued metabolites against various diseases. However, the isolation of individual phytochemical constituents and subjecting it to biological activity will give effective and beneficial results.

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Table.1 Physico- chemical characterization of oil

Color	Odour/ Aroma	Consistency	Degree of evaporation	Solubility
Bright dark yellow Color	Strong sweet smell	Oily	Medium	Alcohol, Acetone, Benzene and DMSO

Table.2 The identified compounds from Mass Spectra of GC eluted peaks

Sl. No	Peak No	Identified Compounds	Molecular Formula	Structure	Peak Area (%)	M/Z ratio	Nature of compound
1	1	Butanoic acid, ethyl ester	C ₆ H ₁₂ O ₂		0.25	88.00	Ester
2	2	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	C ₆ H ₁₄ O ₂		0.14	91.00	Alcohol/ Ether
3	3	((1R)-2,6,6- Trimethylbicyclo[3.1.1]hept -2-ene	C4H10O		0.15	91.00	Ether
4	4	betaPhellandrene	C8H18O2		6.91	91.00	Alcohol/ Ether
5	5	betaPinene	C ₈ H ₁₀ O		1.65	91.00	Alcohol
6	6	o-Cymene	C ₈ H ₈ O ₃		0.42	134.0	Phenol derivative
7	7	D-Limonene	C4H6O2		1.24	93.00	Lactone
8	8	transbetaOcimene	C ₈ H ₈ O ₃		3.51	45.10	Aromatic Aldehyde

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9	9	2-Propanol, 1,1'-oxybis	C9H10O3		18.3 7	89.00	Aromatic Aldehyde
10	10	Methane, diethoxy	C9H6O2	****	28.0	103.1	Lactone
11	11	2-Carene	C10H16		24.0	93.10	Monoterpene
12	12	2-Butanol, 3,3'-oxybis-	C10H16		4.47	103.0	Monoterpene
13	13	Phenylethyl Alcohol	C10H16		2.07	92.00	Monoterpene
14	14	Methylenebicyclo[2.1.1]hex ane	C10H16		1.11	110.0	Monoterpene
15	15	Thymol	C10H14		1.17	65.00	Aromatic Hydrocarbon
16	16	Citronellol	C10H16		0.42	67.00	Monoterpene
17	17	Resorcinol, 2-acetyl-	C10H16		0.23	119.0 0	Monoterpene
18	18	2,4-Hexadiene, 3,4- dimethyl-, (E,Z)	C10H16		0.33	59.00	Monoterpene
19	19	2(3H)-Furanone, dihydro-5-	C7H10	D-D	0.86	56.10	Hydrocarbon

20	20	Vanillin	C10H14O	2.24	152.0 0	Phenol
21	21	Coumarin	C10H20O	0.84	146.0 0	Alcohol (Monoterpenoid)
22	22	Ethyl Vanillin	C ₆ H ₁₂ O ₂	1.27	166.0	Sesquiterpene oxide
23	23	Caryophyllene oxide	C ₆ H ₁₄ O ₂	0.33	55.10	Ester

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Author Contributions

Dr Snigdharani Dash: Conceived the original idea, gather the resources, designed the model, use the software and wrote the manuscript.; Dr Smaranika Pattnaik: Designed the model and the computational framework and analysed the data.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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