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Research Article

DETERMINATION OF VOLATILE BIOACTIVE COMPOUNDS FROM EXTRACTS OF BAEL *(AEGLE MARMELOS)* PLANT PARTS AND THEIR COMPARATIVE ANALYSIS

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ABSTRACT

Objective: The main objective of this study is to determine the bioactive compounds from the extracts of wildly growing *Aegle marmelos* plant parts.

Methods: *A. marmelos* root, stem, leaves, bark, fruit peel, and pulp were screened for the presence/absence of phytochemicals. Bioactive compounds in all the plant parts were analyzed by gas chromatography–mass spectrometry (GC/MS) analysis. For evaluation of bioactive compounds first, the column chromatography was done using various solvents and found that the methanolic extracts gave better elution and separation of compounds and hence used further for GC/MS analysis.

Result: GC/MS analysis revealed chromatograms of methanol extract of *A. marmelos* plant parts, and all the plant parts were found to have a number of phytochemicals. Some compounds, namely, benzene, nitro-, benzenepropanoic acid, 3, 5-bis (1, 1-dimethylethyl)-4-hydroxy-, methyl ester, and tetradecene were found in all parts with a varying percentage. Phenol only found in the fruit of the plant with more percentage in fruit peel (4.38%) than in fruit pulp (0.58%). Dibutyl phthalate is the major compound found in *Aegle* root (10.43%), fruit peel (34.56%), and pulp (13.18%). Other important compounds such as coumarin (2H-1-Benzopyran-2-one, 7-[(3,7-dimethyl-2,6-octadienyl)oxy]-, (E)-), skimmianine (Furo[2,3-b] quinoline, 4,7,8-trimethoxy-), and cyclobarbital were found in plant root.

Conclusion: After the GC/MS analysis, it was concluded that all the parts of this wildly growing plant contain a significant amount of pharmaceutically important compounds.

Keywords: *Aegle marmelos*, Rutaceae, Gas chromatography–mass spectrometry analysis, Bioactive compounds, Biomedicine.

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INTRODUCTION

Plants synthesize a variety of secondary metabolites against the infectious agents [1]. Bioactive compounds are defined as secondary metabolites that elicit pharmacological or toxicological effects in man and animals. Frequent use of synthetic drugs has made the pathogens resistant to multiple drugs. This impels the need to screen medicinal plants for novel bioactive compounds due to their biodegradability, safety, and fewer side effects [2].

Aegle marmelos is one such medicinal plant having a plethora of bioactive compounds in every plant part. Mentions of this plant have also been found in the pre-historic writings dating back to 800 B.C. It was not only known in the ancient era for its medicinal properties but also being studied nowadays extensively using advanced scientific techniques. *A. marmelos,* plant of family Rutaceae, is commonly known as wood apple plant and other vernacular names are Bael Fruit, Indian Bael, Holy Fruit, Golden Apple, Elephant Apple, Indian Quince, and Stone Apple [3]. It subsists well in a wide range of climatic conditions and can be cultivated worldwide. It is a subtropical plant which can grow up to an altitude of 1200 m from the sea level and also in the dry forests of hilly and plain areas. It is native to India and grown throughout India, mainly near the temples due to its mythological importance [4]. It has its origin from the Eastern Ghats and Central India. It has been used in medicines due to its significant phytochemical constitution making it potent as a remedy for diseases such as diabetes, peptic ulcer, inflammation, diarrhea and dysentery, constipation, respiratory infection, and cancer. It also has cardioprotective, antimicrobial, radioprotective, antipyretic, analgesic, antioxidant, hepatoprotective, and wound healing properties [5].

Various other researchers throughout the world have done the gas chromatography/mass spectrometry (GC/MS) analysis of *A. marmelos* plant parts, but till now no researcher has reported GC/MS analysis of the *A. marmelos* fruit peel. In this study, GC/MS analysis of fruit peel shows that it possesses a significant amount of the bioactive compounds which are potent as antioxidants. Wildly growing *A. marmelos* plant from the semiarid area of the Indian state of Rajasthan was studied for its bioactive compound composition through GC/MS analysis. Bioactive compounds from different plant parts such as root, stem, leaf, bark, fruit peel, and pulp were compared and analyzed.

METHODS

Plant material and extraction

Bilva plant samples were collected from fields of Chaumun area of Jaipur district in Rajasthan. Identification of the plant was confirmed by Rajasthan Agricultural Research Institute, Jaipur. *A. marmelos* plant parts, leaves, root, stem, bark, fruit peel, and pulp were taken and shade dried and then crushed to make a fine powder. For evaluation of bioactive compounds first, the column chromatography was done using various solvents and it was found that the methanolic extracts gave better elution and separation of compounds and hence used further for GC/MS analysis.

GC/MS

GC/MS technique is used in this study to identify the bioactive components present in the extract. This method involves a very little amount of the test sample and gives the molecular weights of even fraction of compounds. GC/MS analysis of this extract can be performed using GC SHIMADZU QP2010 system and GC interfaced to a MS (GC/MS) equipped with Elite-1 fused silica capillary column (Length: 30.0m, Diameter: 0.25 mm, film thickness: 0.25 µm, Composed

of 100% dimethyl polysiloxane). The components can be identified by comparing their retention times with those of authentic samples as well as by comparing their mass spectra with those of Wiley 275 library [6].

RESULT

The bioactive fraction on GC/MS analysis revealed chromatograms of methanol extract of *A. marmelos* plant parts. Some compounds, namely, benzene, nitro-, benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester, and tetradecene were found in all the plant parts with a varying percentage. Phenol only found in the fruit of the plant with more percentage in fruit peel (4.38%) than in fruit pulp (0.58%). Phenol is supposed to be responsible for the antioxidant activity of the sample. Dibutyl phthalate is the major compound found in *Aegle* root (10.43%), fruit peel (34.56%), and pulp (13.18%), having the highest

Table 1: Compounds identified in the *Aegle marmelos* fruit peel

Peak no.[a]	Retention time[b]	Area[c]	Area%[d]	Name
1	5.319	28558	4.38	Phenol
2	6.698	47781	7.33	Benzene, Nitro-
4	9.722	31092	4.77	5-Tetradecene, (E)-
5	11.426	49454	7.59	5-Tetradecene, (E)-
6	12.949	45627	7.00	5-Eicosene, (E)-
7	13.455	225194	34.56	Dibutyl phthalate
8	13.940	120586	18.50	Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydroxy-, methyl ester
9	14.326	34662	5.32	5-Eicosene, (E)-
10	15.581	25527	3.92	Phosphonic acid, dioctadecyl ester
		651663	100.00	

[a]Peak No: Number of peak occurring subsequently in the chromatogram of sample. [b]Retention time: Measurement of time (in min.) spent by solute in the column (the time from injection into the column is made to when elution occurs). [c]Area: Area covered by a peak in chromatogram which is proportional to the amount of compound present. [d]Area %: It is calculated by dividing the area of each peak by total area and multiply it by 100
Area %=Area of Peak÷Total Area×100

Table 2: Compounds identified in the *Aegle marmelos* root

Peak no.[a]	Retention time[b]	Area[c]	Area%[d]	Name
1	5.324	8332	0.58	Phenol
2	6.119	12691	0.88	Benzene, 1-bromo-2-methyl-
3	6.700	41400	2.87	Benzene, Nitro-
4	7.151	37564	2.60	Acetic acid, Propyl ester
5	7.305	19301	1.34	2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-One
6	8.062	69957	4.85	2,3-Dihydro-Benzofuran
7	8.276	21823	1.51	Acetic Acid, Propyl Ester
8	9.547	83181	5.77	Ethyl 2-hydroxybenzyl sulfone
9	9.723	38165	2.65	5-Tetradecene, (E)-
10	10.057	93607	6.49	Cytidine
11	10.600	73062	5.07	D-Allose
12	11.265	66830	4.63	3-Deoxy-d-mannoic lactone
13	11.427	53526	3.71	7-Hexadecene, (Z)-
14	11.602	28066	1.95	1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic Acid
15	12.950	39882	2.77	7-Hexadecene, (Z)-
16	13.456	190108	13.18	Dibutyl phthalate
17	13.862	12165	0.84	Dodecanoic Acid, Methyl Ester
18	13.941	92917	6.44	Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydroxy-, methyl ester
19	14.327	20413	1.42	3-Octadecene, (E)-
20	15.012	18778	1.30	3,6-Octadecadienoic Acid, Methyl Ester
21	15.328	7365	0.51	trans-2-Dodecen-1-ol, pentafluoropropionate
24	16.996	124611	8.64	Tetracosamethyl-cyclododecasiloxane
25	17.224	104388	7.24	9-Tetradecenal, (Z)-
		1442145	100.00	

Table 3: Compounds identified in the *Aegle marmelos* stem

Peak no.[a]	Retention time[b]	Area[c]	Area%[d]	Name
1	6.122	10638	1.57	Benzene, 1-bromo-2-methyl-
2	6.703	39789	5.87	Benzene, nitro-
3	7.820	28507	4.21	1-Propene, 3-(ethenyloxy)-
5	9.723	44302	6.54	5-Tetradecene, (E)-
6	11.427	50107	7.40	5-Tetradecene, (E)-
7	12.344	16941	2.50	Hexestrol, O-trifluoroacetyl-
8	12.410	17360	2.56	Spiro-1-(cyclohex-2-ene)-2'-(5'-oxabicyclo[2.1.0]pentane), 1',4',2,6,6-pentamethyl-
9	12.700	13987	2.06	Hexestrol
10	12.951	44074	6.50	9-Octadecene, (E)-
11	13.456	217627	32.12	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester
12	13.942	116224	17.15	Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydroxy-, methyl ester
13	14.328	33689	4.97	9-Eicosene, (E)-
14	15.584	27374	4.04	Z-5-Nonadecene
		677574	100.00	

amount in the fruit peel. Whereas, some other important compounds such as coumarins (2H-1-benzopyran-2-one, 7-[(3,7-dimethyl-2,6-octadienyl)oxy]-, (E)-), skimmianine (Furo[2,3-b] quinoline, 4,7,8-trimethoxy-), and cyclobarbital were found in plant root only. Hence, after a comparative analysis of compounds identified, it was found that there are compounds which are found only in particular plant parts (Tables 1-7).

DISCUSSION

Jorge *et al.* from Cuba in 2005 did the GC/MS analysis of *A. marmelos* leaf and identified 65 compounds comprising more than 85% leaf oil. Major components identified were β-caryophyllene (10.0 %) and δ-cadinene (12.1%) [7]. Satyal *et al.* from Nepal in 2012 also identified 82 compounds by GC/MS analysis of *Aegle* leaves [8]. In 2014, Mujeeb *et al.* from Lucknow and Rajeshkannan and Lakshmanan from Tamil Nadu also studied the *A. marmelos* leaves and fruit, respectively, and identified various compounds by GC/MS analysis [9,10]. Bajaniya *et al.* in 2015 studied the fatty acid profile of *Aegle* seed oils and characterized the phytochemicals present in it through GC/MS analysis [11]. Nadhiya and Vadivazhagi in 2015 studied the *A. marmelos* and *Mentha piperita* leaves. In this study, GC/MS were used to identify phytochemicals present in the *A. marmelos* with *M. piperita* leaves extract. Phytochemical identified from *A. marmelos* and *M. piperita* combined extract was used to investigate the various antioxidant activities [12]. In 2016, Ritu Jha and Rajinder Gupta developed energy drink from the combination of *A. marmelos, Rubia cordifolia, Phyllanthus emblica,* and *Beta vulgaris*

Table 4: Compounds identified in the *Aegle marmelos* leaves

Peak no.[a]	Retention time[b]	Area[c]	Area%[d]	Name
1	6.006	18779	2.18	Cyclobutane, 1,2-bis (1-methylethenyl)-, trans-
2	6.700	35919	4.18	Benzene, nitro-
3	9.723	35955	4.18	4-Tetradecene, (E)-
4	10.573	14078	1.64	Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)-, (R)-
5	11.425	51774	6.02	7-Tetradecene, (E)-
6	12.949	45396	5.28	7-Hexadecene, (Z)-
7	13.262	13497	1.57	3-Tridecene
8	13.454	295847	34.40	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester
9	13.940	106510	12.38	Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydroxy-, methyl ester
10	14.325	28226	3.28	9-Eicosene, (E)-
11	14.922	54060	6.29	Dimethyl {bis[(4,8,8-trimethyldecahydro-1,4-methanoazulen-9-yl) methoxy]}silane
12	15.582	30770	3.58	1,7-Dimethyl-4-(1-methylethyl) cyclodecane
13	18.206	83193	9.67	Carbamic acid, methylnitroso-, 1-naphthalenyl
		860116	100	

Table 5: Compounds identified in the *Aegle marmelos* bark

Peak no.[a]	Retention time[b]	Area[c]	Area%[d]	Name
1	6.701	33134	4.26	Benzene, nitro-
2	9.724	26666	3.43	6-Dodecene, (E)-
3	11.214	15171	1.95	2,5-Dihydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one
4	11.425	47739	6.13	7-Tetradecene, (E)-
5	12.697	13118	1.69	Hexestrol, O-trifluoroacetyl-
6	12.949	48494	6.23	5-Octadecene, (E)-
7	13.456	205078	26.35	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester
8	13.941	96234	12.36	Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydroxy-, methyl ester
9	14.094	28914	3.71	Isoquinolin-6-ol, 7-methoxy-1-methyl-
10	14.326	32293	4.15	1-Hexadecanol
11	16.485	197906	25.42	2-(1-Hydroxy-1-methylethyl)-2,3-dihydrofuro[3,2-g] chromen-7-one
12	16.733	33654	4.32	(S)-7-Hydroxy-8,8-dimethyl-7,8-dihydropyrano (3,2-g) chromen-2 (6H)-one
		778401	100.00	

Table 6: Compounds identified in the *Aegle marmelos* fruit pulp

Peak no.[a]	Retention time[b]	Area[c]	Area%[d]	Name
1	6.121	12707	0.63	Benzene, 1-bromo-2-methyl-
2	6.701	41879	2.08	Benzene, nitro-
4	7.782	39209	1.95	7-Tetradecene, (Z)-
5	7.898	95566	4.75	Tricyclo[3.3.1.1 (3,7)]decan-6-one, 2-(4-allyloxyphenyl)-5,7-dipropyl-1,3-diaza-
6	9.723	30074	1.49	7-Tetradecene, (E)-
7	11.428	52424	2.60	5-Tetradecene, (E)-
8	12.950	41437	2.06	5-Octadecene, (E)-
9	13.456	209997	10.43	Dibutyl phthalate
10	13.941	111967	5.56	Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydroxy-, methyl ester
11	14.326	41176	2.05	5-Eicosene, (E)-
12	15.581	24992	1.24	n-Heptadecanol-1
13	16.665	117565	5.84	Furo[2,3-b] quinoline, 4,7,8-trimethoxy-
15	18.387	73670	3.66	2H-1-Benzopyran-2-one, 7-[(3,7-dimethyl-2,6-octadienyl) oxy]-, (E)-
17	19.083	40266	2.00	Cyclobarbital
18	19.508	889357	44.19	2,6-Dimethyl-3,5,7-octatriene-2-ol, E, E-
		2012616	100.00	

Table 7: Some important compounds identified

Compound	Description [16]	Plant part
Dibutyl phthalate (DBP)	Additive to adhesives, printing inks and makes nail polish flexible. It acts as ectoparasiticide and also as a plasticizer	Found in root, fruit peel, and pulp
n-Heptadecanol-1-	A chemical compound from the group of fatty alcohols used as an antifoaming agent and in the synthesis of complex organic compounds	Found in the root
Cyclobarbital (cyclobarbitone)	It is a barbiturate derivative drug which is used to treat insomnia in Russia	Found in the root
Coumarin (2H-1-Benzopyran-2-one, 7-[(3,7-dimethyl-2,6-octadienyl) oxy]-, (E)-)	It is a fragrant compound in the benzopyrone chemical class and used in perfumes and fabric conditioners, aroma enhancer in pipe tobaccos and alcoholic drinks. It is also used as a precursor in the synthesis of anticoagulant. It is banned as a flavoring food additive, due to its hepatotoxicity. "Coumarins" are a type of Vitamin K antagonists	Found in the root
Isoquinolin-6-ol, 7-methoxy-1-methyl-	It is a quinoline alkaloid with painkilling properties	Found in the bark
5-Octadecene, (E)-	An alkene with the molecular formula C18H36 and used in the synthesis of colloidal quantum dots. It acts as an antimicrobial agent	Root, stem, bark, and fruit pulp
Hexestrol, O-trifluoroacetyl-	It is also known as hexanestrol, hexoestrol, and dihydrodiethylstilbestrol. It was used to treat estrogen deficiency but is now no longer employed medically	Found in the stem
Marmesin (2-(1-Hydroxy-1-methylethyl)-2,3-dihydrofuro[3,2-g] chromen-7-one)	It is a compound precursor in psoralen and linear furanocoumarins biosynthesis	Found in the bark
Skimmianine (Furo[2,3-b] quinoline, 4,7,8-trimethoxy)	This group of alkaloids is essentially limited to plant family Rutaceae. Simplest member is dictamnine, whereas skimmianine is the most widespread. It has *in vitro* pharmacological properties such as antimicrobial, antiviral, mutagenic, and cytotoxic	Found in the root

and its phytochemical, nutritive, and antimicrobial analysis. GC/MS screening of sample revealed the presence of hexadecanoic acid and octadecanoic acid. The ingredients used in the energy drink had very good nutrition as well as pharmacological use [13]. Kasireddy *et al.* studied the neuroprotective potential and efficacy of *A. marmelos* fruit extract against neurodegenerative disorders, and it was concluded that dry fruit extract improves antihypoxic effects induced by sodium nitrite. This effect was supposed to be mediated by the antioxidant properties caused by the bioactive compounds present in the plant [14]. Antibacterial activity of *A. marmelos* (L.) *Correa* was studied by Yadav *et al.*, and it was found that methanolic extracts of the plant showed better inhibition activity which may be due to the efficiency of methanol to extract more bioactive compounds than other solvents [15].

After reviewing the research work on *A. marmelos* plant parts from different countries and comparing it with the present study, it was observed that compounds identified also varied with the geographical and climatic conditions.

CONCLUSION

In this study, the plant parts were found to have a significant amount of bioactive compounds responsible for its medicinal and phytochemical importance. This study has been done on the wild variety of *A. marmelos* plant grown in Rajasthan state of India, which is a semiarid area. Rajasthan state covers almost 60% area of the Thar Desert of India. This plant can be seen almost everywhere and mostly near the temples due to its mythological importance. Charak, the "father of medicine" from ancient Indian history, also mentioned the importance of *A. marmelos* in medicine in his treatise. As this plant can thrive well in a wide range of climatic conditions, it would be of dire importance to grow this plant in the desert area of Rajasthan under afforestation program for reducing the growth of desert as well as serving as a potential source of medicine. The results of this GC/MS analysis show that various parts of the plant contain sufficient amount of plethora of bioactive compounds which play a major role in providing the plant its medicinal property.

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AUTHORS CONTRIBUTION

NS reviewed the literature of same nature research work, carried out the experiments and prepared the manuscript. WD helped to carry out Study, manuscript preparation and critical revision of the manuscript. Both the authors agree with the content of the manuscript.

CONFLICT OF INTEREST

There is no conflict of interests.

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