DETERMINATION OF VOLATILE BIOACTIVE COMPOUNDS FROM EXTRACTS OF BAELE (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 (3.56%), and pulp (13.18%). Other important compounds such as coumarin (2H-1-Benzopyran-2-one, 7-[(3,7-dimethyl-2,6-octadienyloxy)-, (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 Bad, Holý 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 Aegle marmelos from the semi-arid 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
Bilha 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).
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. | Retention time | Area | Area% | Name                                           |
|----------|----------------|------|-------|------------------------------------------------|
| 1        | 5.319          | 28558| 4.38  | Phenol                                         |
| 2        | 6.698          | 47781| 7.33  | Benzene, Nitro-                                |
| 3        | 9.722          | 31092| 4.77  | 5-Tetradecene, (E)-                            |
| 4        | 11.426         | 49454| 7.59  | 5-Tetradecene, (E)-                            |
| 5        | 12.949         | 45627| 7.00  | 5-Eicosene, (E)-                               |
| 6        | 13.455         | 22519| 34.56 | Dibutyl phthalate                              |
| 7        | 13.940         | 120586| 18.50 | Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydroxy-, methyl ester |

Peak No: Number of peak occurring subsequently in the chromatogram of sample. 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). Area: Area covered by a peak in chromatogram which is proportional to the amount of compound present. Area % is calculated by dividing the area of each peak by total area and multiply it by 100

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

| Peak no. | Retention time | Area | Area% | 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        | 8.052          | 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       | 10.857         | 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-Cyclohexene-carboxylic Acid |
| 15       | 12.950         | 39882| 2.77  | 7-Hexadecene, (Z)-                             |
| 16       | 13.456         | 19010| 13.18 | Dibutyl phthalate                              |
| 17       | 13.862         | 12165| 0.84  | Dodecanonic 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, pentalfluoropropanate    |
| 22       | 16.996         | 12416| 8.64  | Tetracosamethyl-cyclododecasiloxane             |
| 23       | 17.224         | 104388| 7.24  | 9-Tetradecenal, (Z)-                           |
| 24       | 18.977         | 1442145| 8.64  | 9-Tetradecenal, (Z)-                           |

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

| Peak no. | Retention time | Area | Area% | 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-(ethenyl)-                        |
| 4        | 9.723          | 44302| 6.54  | 5-Tetradecene, (E)-                            |
| 5        | 11.427         | 50017| 7.40  | 5-Tetradecene, (E)-                            |
| 6        | 12.444         | 16941| 2.50  | Hexestrol, O-trifluoroacetyl-                   |
| 7        | 12.410         | 17360| 2.56  | Spiro-1-(cyclohex-2-ene)-2-(5'-oxabicyclo[2.1.0]pentane), 1',4',2,6,6-pentamethyl-Hexestrol |
| 8        | 12.700         | 13987| 2.06  | Hexestrol                                      |
| 9        | 12.951         | 44074| 6.50  | 9-Octadecene, (E)-                             |
| 10       | 13.456         | 21762| 32.12 | 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester |
| 11       | 13.942         | 116224| 17.15 | Benzene, nitro-, benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester |
| 12       | 13.428         | 33689| 4.97  | 9-Eicosene, (E)-                               |
| 13       | 15.584         | 23734| 4.04  | Z-5-Nonadecene                                 |
| 14       | 24.125         | 677574| 100.00| Tetracosamethyl-cyclododecasiloxane             |
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)-, 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]. Bajania 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 Vadhavazhi 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. Phytochemicals 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. | Retention time | Area | Area% | Name |
|---------|----------------|------|-------|------|
| 1       | 6.006          | 18779 | 2.18  | Cyclobutane, 1,2-bis (1-methylethyl)-, 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-Hexadecane, (Z)- |
| 7       | 13.262         | 13497 | 1.57  | 3-Tridecane |
| 8       | 13.454         | 295847 | 34.40 | 1,2-Benzenedicarboxylic acid, butyl-2-methylpropyl ester |
| 9       | 13.940         | 106510 | 12.38 | Benzopenpranoic acid, 3,5-bis (1,1-diethylmethyl)-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])methyly]silane |
| 12      | 15.582         | 30770 | 3.58  | 1,7-Dimethyl-4-(1-methylthyl) cyclodcane |
| 13      | 18.206         | 860116 | 100.00 | Carbamic acid, methylnitroso-, 1-naphthalenyl |

Table 5: Compounds identified in the Aegle marmelos bark

| Peak no. | Retention time | Area | Area% | Name |
|---------|----------------|------|-------|------|
| 1       | 6.701          | 33134 | 4.26  | Benzene, nitro- |
| 2       | 9.724          | 26666 | 3.43  | 6-Dodecane, (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-Octadecane, (E)- |
| 7       | 13.456         | 205078 | 26.35 | 1,2-Benzenedicarboxylic acid, butyl-2-methylpropyl ester |
| 8       | 13.941         | 96234 | 12.36 | Benzopenpranoic acid, 3,5-bis (1,1-diethylmethyl)-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-methylthyl)-2,3-dihydrofuro[3,2-g] chromen-7-one |
| 12      | 16.733         | 33654 | 4.32  | (S)-7-Hydroxy-8,8-dimethyl-7,8-dihydropyran (3,2-g) chromen-2 (6H)-one |

Table 6: Compounds identified in the Aegle marmelos fruit pulp

| Peak no. | Retention time | Area | Area% | Name |
|---------|----------------|------|-------|------|
| 1       | 6.121          | 12707 | 0.63  | Benzene, 1-bromo-2-methyl- |
| 2       | 6.701          | 41879 | 2.08  | Benzene, nitro- |
| 3       | 7.882          | 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-diza- |
| 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-Octadecane, (E)- |
| 9       | 13.456         | 209997 | 10.43 | Dibutyl phthalate |
| 10      | 13.941         | 111967 | 5.56  | Benzopenpranoic acid, 3,5-bis (1,1-diethylmethyl)-4-hydroxy-, methyl ester |
| 11      | 14.326         | 41176 | 2.05  | 9-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- |
| 14      | 18.387         | 73670 | 3.66  | 2H-1-Benzencyclopropane-2-one, 7-[(3,7-dimethyl-2,6-octadienyl)oxy]-, (E)- |
| 17      | 19.083         | 40266 | 2.00  | Cyclobarbitol |
| 18      | 19.508         | 889357 | 44.19 | 2,6-Dimethyl-3,5,7-octatriene-2-ol, E, E- |
| 19      | 20.126         | 2012616 | 100.00 | Carbamic acid, methylnitroso-1-naphthalenyl |
This group of alkaloids is essentially limited to its mythological importance. Charak, the "father of medicine" from ancient Indian history, also mentioned the importance of this plant 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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