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## Investigations on secondary metabolites of *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Leaves using GC-MS

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### Abstract

**Objective:** To characterize the Phytoconstituents especially Secondary metabolites of *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Leaves Using GC-MS.

**Methods:** Qualitative phytochemical tests showed presence of various compounds which were then verified by GC-MS analysis of *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Leaves in Chloroform, 50% Ethanol and Dichloromethane solvents.

**Results:** The GC-MS results of Chloroform extract showed the presence of fourteen compounds, ten compounds in 50% Ethanol extract and seventeen compounds in Dichloromethane extract. Some fatty acids, hydrocarbons and secondary metabolites were found in the extracts. The most important metabolites identified in Chloroform and Dichloromethane extract are Phenol,2,4-bis (1,1-dimethylethyl)-; and Phytol ; while as in 50% Ethanol extract metabolites like Desulphosinigrin;  $\beta$ -D-Mannopyranoside, 1-0-(10-undecenyl)-; 3,3'-Dimethyl-1'-hydroxy-5,8-dimethoxy-2,2'-binaphthalene-1,4,5',8'-tetrone and Stigmasterol.

**Conclusion:** The compounds responsible for Anticancer, Antioxidant, Anti-inflammatory and Antimicrobial were found to be present in the leaves.

**Keywords:** Secondary metabolites, *Alhagi pseudalhagi*, GC-MS

### 1. Introduction

Alhagi is a Latin name derived from Arabic word 'Alhag' meaning old wise man (Boulus, 2000) [4]. Genus Alhagi is comprised of nine species and belongs to family Fabaceae (Anonymous, 2001) [2]. *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. is a perennial plant which is widely distributed in the European and Southern parts of Russia and Central Asia (Sultan *et al.*, 2011) [31]. It is also distributed throughout India (Yadav, 2014) [34]. It is commonly called as 'Damasa' in Marathi and it grows from a massive rhizome system which may extend to six feet into the ground (Srivastava *et al.*, 2014) [29].

The plant has wide range of folkloric claims as whole plant is used as a laxative, diuretic and expectorant in Rajasthan. Leaves are smoked in the treatment of asthma (Sebastian and Bhandari, 1984) [24] administered orally in fever (Shah *et al.*, 1981) [28]. Whole plant decoctions are used in stomach ulcers and to reduce water loss (Sinel'nikov, 1965) [27], for hemorrhoids and in wound dressing (Altmysheva, 1976) [3] as an antipyretic (Gurbanov *et al.*, 1976) [13] and in the treatment of cancer (Zhang *et al.*, 2010) [36]. Oil from leaves is used for rheumatism (Chopra *et al.*, 1956) [6]. The sweet exudation of the leaves and branches is restorative, aphrodisiac, aperient, chalogogue, expectorant, and a blood purifier (Kapoor....ethnobotany). The present study aims at the identification of secondary metabolites from Alhagi pseudalhagi leaves.

### 2. Materials and methods

#### 2.1 Collection and Identification

The plants were collected from Gandhigram village, Akot Taluka, Akola of Vidarbha region, Maharashtra which were morphologically identified and authenticated by taxonomist Professor Dr. S.P. Rothe. The voucher specimens were deposited in the herbarium of Department of Botany, Vidyabharati Mahavidyalaya, Camp, Amravati, Maharashtra, India. During the collection, leaves which were infected, etiolated or having any diseased condition were removed accordingly and fresh plant material was used for experimentation. The leaves of this collected plant were shade dried and then converted to powder form for further studies.

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## 2.2 Extraction

The extraction was done by Soxhlet method (Nikhil, *et al.*, 2010) [19] using three solvents viz. Chloroform (polar), 50% Ethanol (Semi-polar), and Dichloromethane (non-polar). After extraction in Soxhlet apparatus for 24 hours the extracts were filtered and concentrated to 5ml using rotatory vacuum evaporator at room temperature. These crude samples were then used for GC - MS analysis.

## 2.3 GC-MS analysis

The analysis was carried out using gas chromatography-high resolution mass spectrometer. 2 µl of the prepared extracts was employed for GC-MS analysis. The GC-MS analysis was carried using Alegant Hp 7880 with column of 30 meter length, with 0.25 mm internal diameter and 0.32 thickness. Helium gas was used as carrier gas at constant flow rate of 1ml/minute. Injector temperature was set at 50 °C. the Oven temperature were programmed from 50 °C to 280 °C at 10 °C/minute to 200 °C then 10 °C/ 3 minutes to 250 °C ending with a 5 minutes isothermal at 280 °C. The sample was injected in split mode as 10:80.

## 2.4 Identification of compounds

Interpretation on mass spectrum of GC-MS was done using the National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of unknown compounds was compared with the spectral data of known compounds present in spectral libraries (NIST). The name, molecular weight and structure of the components of the test materials were ascertained.

## 3. Results

The extraction of *Alhagi pseudalhagi* leaves in three different solvent gives valuable information about its chemical constituents. The GC-MS data reveals the presence of fourteen compounds in Chloroform extract, ten compounds in 50% Ethanol extract and seventeen compounds in Dichloromethane extract. In all the analyzed extracts, hydrocarbons, fatty acids and secondary metabolites were found to be present. The most important metabolites identified in Chloroform and Dichloromethane extract are Phenol,2,4-bis (1,1-dimethylethyl)-; and Phytol ; while as in 50% Ethanol extract metabolites like Desulphosinigrin; β-D-Mannopyranoside, 1-0-(10-undecenyl)-; and Stigmasterol were present.

Figures 1, 2, & 3 are chromatograms showing the peaks and retention time and Tables 1, 2, & 3 shows the retention time, name of compound, molecular weight, and molecular formula. This was followed by Table no. 4 showing the secondary metabolites present in leaves of *Alhagi pseudalhagi* in which name of compound, its Category and biological activity of that compound is displayed.

## 4. Discussion

Nowadays, interest for study of organic compounds from plants and their activity has increased. This increasing interest in the phytochemical compounds is due to nutritional incidence and their role in health and disease (Steinmetz *et al.*, 1991). Different phytochemicals have been found to have broad range of activities, which may be helpful in protection against Chronic diseases. (Liu, 2003) The combination of separation technique (GC) with the best identification technique (MS) made GC-MS an ideal technique for qualitative and quantitative analysis for volatile and semi-volatile compounds. Hence this technique is used mostly in the analysis of phytochemicals.

The qualitative phytochemical tests (Wagay *et al.*, 2015) [33] have revealed the presence of various compounds in the leaves of *Alhagi pseudalhagi*. The GC-MS analysis supports the same and shows the presence of various phytochemicals among which some are categorized as secondary metabolites. This confirms its importance as ethnomedicinally important plant and as well as can be used in pharmaceutical industry. Similar studies have also been done by Ekade and Manik, 2014 [8]; Ghazali *et al.*, 2014 [11]; Abirami and Rajendran, 2012 [1]; Salvamangai and Bhaskar, 2012 [23]; etc. In the present investigation, Leaves of this understudy plant were found to contain Hexadecanoic acid, methyl ester; 9,12,15-Octadecatrienoic acid, methyl ester; Dodecanoic acid; d-Mannose; 1,3-Triacontanediol; 9,12,15-Octadecatrienoic acid,(Z,Z,Z)-; etc and some secondary metabolites under categories of Terpenoids are Phytol, and Lycopene, 1,2-dihydro-1-hydroxy-, Flavonoids are 2H-1-Benzopyran-7-ol,3-(2,4-dimethoxyphenyl)-3,4-dihydro; and Coumaran-7-ol-3-one, 2-(4-methoxybenzylidene)-6-methoxy;3,3'-Dimethyl-1'-hydroxy-5,8-dimethoxy-2,2'- binaphthalene-1,4,5',8'-tetrone. Steroid is Stigmasterol; Glycoside is Desulphosinigrin; Phenols is Phenol, 2, 4-bis (1,1-dimethylethyl)-. All these phytochemicals with their biological activity are placed in Table no. 4.

Similar compound to Coumaran-7-ol-3-one, 2-(4-methoxybenzylidene)-6-methoxy has been found to have antimicrobial, antiviral, anticancer, anti-inflammatory and antioxidant properties (Zwergel *et al.*, 2013) [37]. 3,3'-Dimethyl-1'-hydroxy-5,8-dimethoxy-2,2'- binaphthalene-1,4,5',8'-tetrone has not been reported for any biological activity yet while as its same parent molecules have been found to possess anticancerous activity (Sagar *et al.*, 2010; Shantanu Karkare, *et al.*, 2013) [22, 26]. β-D-Mannopyranoside, 1-0-(10-undecenyl)- is also a new compound whose biological activity is yet to be ascertained.

## 5. Acknowledgement

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**Table 1:** Compounds identified in Chloroform Extract

S. No.	RT	Name of compound	MW	MF
1.	6.71	1-Dodecene	168	C <sub>12</sub> H <sub>24</sub>
2.	9.99	1-Tetradecene	196	C <sub>14</sub> H <sub>28</sub>
3.	12.06	Phenol,2,4-bis (1,1-dimethylethyl)-	206	C <sub>14</sub> H <sub>22</sub> O
4.	13.09	1-Hexadecene	224	C <sub>16</sub> H <sub>32</sub>
5.	15.90	1-Hexadecene	224	C <sub>16</sub> H <sub>32</sub>
6.	17.92	Hexadecanoic acid, methyl ester	270	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>
7.	19.28	1-Nonadecene	266	C <sub>19</sub> H <sub>38</sub>
8.	19.93	n- Hexadecanoic acid	256	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
9.	21.27	9,12,15-Octadecatrienoic acid, methyl ester	292	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>
10.	21.59	Phytol	296	C <sub>20</sub> H <sub>40</sub> O
11.	22.36	9,12,15-Octadecatrienoic acid,(Z,Z,Z)-	278	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>
12.	22.75	3-Eicosene, (E)-	280	C <sub>20</sub> H <sub>40</sub>
13.	25.42	1-Docosene	308	C <sub>22</sub> H <sub>44</sub>
14.	27.86	1-Octacosanol	410	C <sub>28</sub> H <sub>58</sub> O
15.	30.03	17-Pentatriacontene	490	C <sub>35</sub> H <sub>70</sub>

**Table 2:** Compounds identified in 50% Ethanol Extract

S. No.	RT	Name of compound	MW	MF
1.	12.58	Dodecanoic acid	200	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>
2.	14.49	d-Mannose	180	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>
3.	15.39	Tetradecanoic acid	228	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>
4.	15.69	Desulphosinigrin	279	C <sub>10</sub> H <sub>17</sub> NO <sub>6</sub> S
5.	16.36	β-D-Mannopyranoside, 1-0-(10-undecenyl)-	332	C <sub>17</sub> H <sub>32</sub> O <sub>6</sub>
6.	18.65	n-Hexadecanoic acid	256	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
7.	27.53	Stigmasterol	412	C <sub>29</sub> H <sub>48</sub> O
8.	28.37	2H-1-Benzopyran-7-ol,3-(2,4-dimethoxyphenyl)-3,4-dihydro	286	C <sub>17</sub> H <sub>18</sub> O <sub>4</sub>
9.	29.44	Octadecanal,2-bromo-	346	C <sub>18</sub> H <sub>35</sub> BrO
10.	31.83	Coumaran-7-ol-3-one, 2-(4-methoxybenzylidene)-6-methoxy	298	C <sub>17</sub> H <sub>14</sub> O <sub>5</sub>

**Table 3:** Compounds identified in Dichloromethane Extract

S. No.	RT	Name of compound	MW	MF
1.	6.62	2-Decene,(Z)-	140	C <sub>10</sub> H <sub>20</sub>
2.	14.85	1-Tetradecene	196	C <sub>14</sub> H <sub>28</sub>
3.	17.16	Phenol,2,4-bis (1,1-dimethylethyl)-	206	C <sub>14</sub> H <sub>22</sub> O
4.	18.41	1-Hexadecene	224	C <sub>16</sub> H <sub>32</sub>
5.	21.61	1-Hexadecene	224	C <sub>16</sub> H <sub>32</sub>
6.	22.31	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	C <sub>20</sub> H <sub>40</sub> O
7.	22.97	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	C <sub>20</sub> H <sub>40</sub> O
8.	24.67	n-Hexadecanoic acid		
9.	25.03	Hexadecanoic acid, ethyl ester	284	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>
10.	27.61	Phytol	296	C <sub>20</sub> H <sub>40</sub> O
11.	28.25	9,12,15-Octadecatrienoic acid,(Z,Z,Z)-	278	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>
12.	28.44	9,12,15-Octadecatrienoic acid, ethyl ester,(Z,Z,Z)-	306	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>
13.	28.83	1-Docosene	308	C <sub>22</sub> H <sub>44</sub>
14.	36.96	Octadecane,3-ethyl-5-(2-ethylbutyl)	366	C <sub>26</sub> H <sub>54</sub>
15.	37.79	Heptacosane	380	C <sub>27</sub> H <sub>56</sub>
16.	39.87	3,3'-Dimethyl-1'-hydroxy-5,8-dimethoxy-2,2'-binaphthalene-1,4,5',8'-tetrone	418	C <sub>24</sub> H <sub>18</sub> O <sub>7</sub>
17.	40.52	1,3-Triacontanediol	454	C <sub>30</sub> H <sub>62</sub> O <sub>2</sub>
18.	41.78	Lycopene, 1,2-dihydro-1-hydroxy-	554	C <sub>40</sub> H <sub>58</sub> O
19.	42.18	2-Hexadecanol	242	C <sub>16</sub> H <sub>34</sub> O

**Table 4:** Secondary metabolites found in Leaves of *Alhagi pseudalhagi*

S. No.	Name of Compound	Category	Activity
1.	Phenol,2,4-bis (1,1-dimethylethyl)-	Phenol	Anti-bacterial (Kappuswamy <i>et al.</i> , 2013) [17]
2.	Phytol	Terpenoid/ Diterpene	Antimicrobial, anti-inflammatory, diuretic (Rajaswari <i>et al.</i> , 2012) [21] Anti-cancer (Himaja <i>et al.</i> , 2014) [14] Cancer preventive (Sermakaani <i>et al.</i> , 2012) [25]
3.	Desulphosinigrin	Glycoside/ Glucosinolate	Aid in phloem mobility of plants. (Brudenell <i>et al.</i> , 1999) [5]
4.	Stigmasterol	Steroid	Antioxidant & reduce blood cholesterol level (Zawistowski, 2010) [35] Anti-osteoarthritic (Gabay <i>et al.</i> , 2010) [10]; Cyto-toxicity activity (Huang <i>et al.</i> , 2009) [9]; Anti tumour activity (Kasahara <i>et al.</i> , 1994) [16] Ekade <i>et al.</i> , 2014; [8] anti-HIV reverse transcriptase (De Oliveira <i>et al.</i> , 2014) [7]
5.	2H-1-Benzopyran-7-ol,3-(2,4-dimethoxyphenyl)-3,4-dihydro	Flavonoid / Coumarin	Used in perfumes and aroma enhancer (Grover <i>et al.</i> , 2013) [12] Anthelmintic, anti-inflammatory, anti-diarrheal (Muthuchelian <i>et al.</i> , 2011) [20]
6.	Coumaran-7-ol-3-one, 2-(4-methoxybenzylidene)-6-methoxy	Flavonoid / Coumarin	Antimicrobial, Antiviral, Anticancer, Anti-inflammatory, Antioxidant (Zwergel <i>et al.</i> , 2013) [37]
7.	3,3'-Dimethyl-1'-hydroxy-5,8-dimethoxy-2,2'-binaphthalene-1,4,5',8'-tetrone	Flavonoid/ Naphthoquinone	Anti-cancer (Sagar <i>et al.</i> , 2010; Shantanu Karkare, <i>et al.</i> , 2013) [22, 26]
8.	Lycopene, 1,2-dihydro-1-hydroxy-	Terpenoid	Antioxidant (Fadupin <i>et al.</i> , 2012) [9] Anticarcinogenic (TerraTox™)

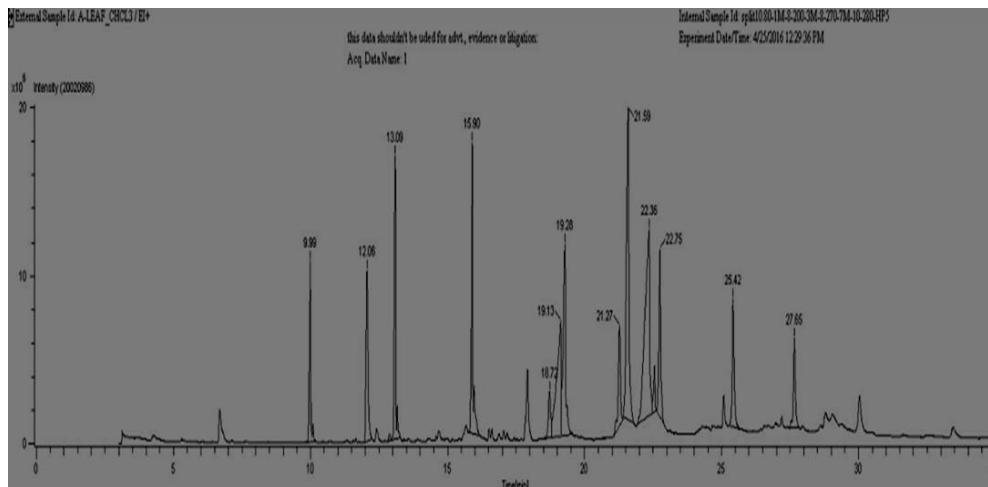


Fig 1: Chromatogram of Chloroform Extract

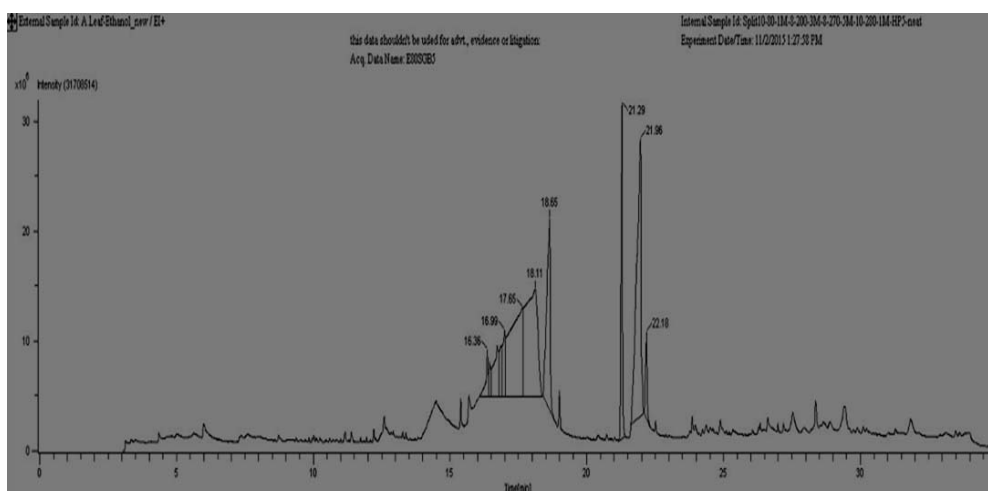


Fig 2: Chromatogram of 50% Ethanol Extract

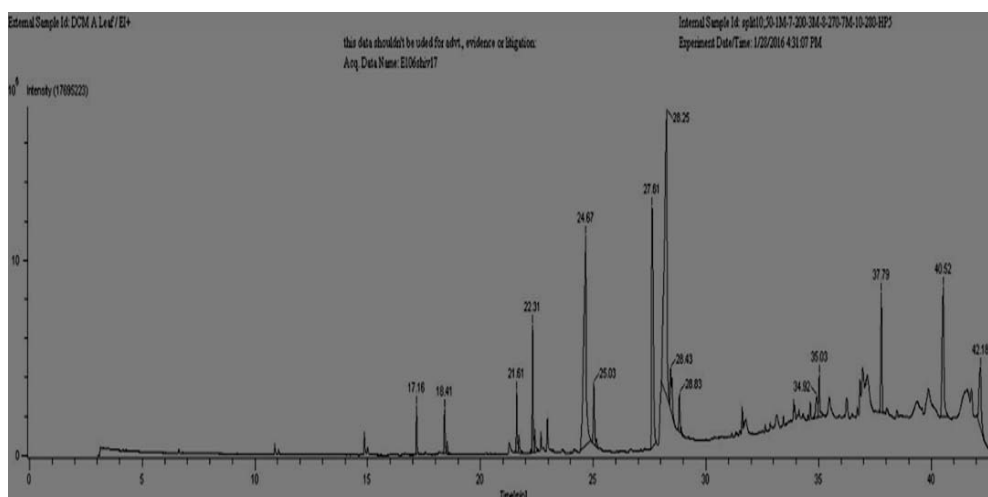


Fig 3: Chromatogram of Dichloromethane Extract

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