# Chemoprofiling of *Alhagi pseudalhagi* (M. Bieb.) Desv. Ex B. Keller and Shap. Roots

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**Abstract**

Plants contain various bioactive compounds which are used for curing of various human diseases. These bioactive compounds are broadly divided into two categories i.e., Primary and Secondary compounds. These primary metabolites are generally used for growth and development processes while as secondary metabolite compounds are widely used for processes other than growth and development i.e. Defense, attraction of pollinators etc. The present study involves the extraction and characterization of chemicals present in *Alhagi pseudalhagi* roots. The Roots of the selected medicinal plant were washed, air dried and then powdered. The aqueous, Dichloromethane, 50% Ethanol and Chloroform extract of roots were used for the qualitative phytochemical analysis to find out the phytochemical constituents in the plants. The Major secondary phytochemicals like Flavonoids, Alkaloids, Saponins and Phenols were quantified. The characterization and identification of chemicals in Dichloromethane, 50% Ethanol and Chloroform extracts was done by using Gas Chromatography- Mass Spectrometry technique.

The results of the present study showed the presence of various classes of secondary metabolites in the roots of this plant. The quantified metabolites per 100 grams of dry sample were as, Flavonoids 8.80 ± 1.59; Alkaloids 3.97 ± 0.07; Saponins 4.82 ± 0.93 and Phenols 1.10 ± 0.21. The important compounds identified in different extracts by Gas Chromatography- Mass Spectrometry were as Methyl salicylate; Phenol, 2,4-bis (1,1-dimethylethyl)-; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Campestrol; β-Sitosterol; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; 4,5-Diamino-2-hydroxypyrimidine; Apigenin 7-β-rutinoside; and Ergosa-5,22-dien-3-ol, acetate, (3 β, 22E)-.

**Keywords:** Secondary Metabolites, GC-MS, phytochemical analysis

## 1. Introduction

Nature has made available all those plants which can be used for the cure of various diseases that continually plague mankind [1]. Those plants which are used for curing diseases are the sources of many important drugs; such drug providing plants are known as drug plants or medicinal plants [2]. Medicinal plants are also defined as, any plants which contain substances that can be used for the therapeutic purposes in one or more of its organ or substances which are precursors for the synthesis of useful drugs [3]. Furthermore, it was noted that a plant become a medicinal plant only when its biological activity has been ethnobotanically reported or scientifically established [4-5]. Ethnobotanical investigations has provided the basic platform for searches on these traditionally used medicinal plants used against diverse diseases [6-7]. Scientific research into these ethno-medicinally important plants has led to the development of many valuable drugs through isolation, identification, purification, characterization, and standardization [8].

*Alhagi pseudalhagi* (M. Bieb.) Desv. Ex B. Keller & Shap. is a small shrub, armed with copious hard spines. Spines 1-2.5 cm long. Leaves simple, drooping from the base of the spines. Flowers small, 1-6 borne on a spine, shortly pedicellate, red paplionaceous flowers in auxillary racemes forming panicles. Its Root decoction is ethnomedicine used for Kidney and biliary stones. So the present study was focused on the chemical composition of Roots of this plant.

## 2. Materials and Methods

### 2.1 Collection and Identification

**Fig 1:** Flower and Collection of *Alhagi pseudoalhagi* (M. Bieb.) Desv Ex B Keller & Shap

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 (MS), India. During the collection, Roots which were infected or having any diseased condition were removed accordingly and fresh plant material was used for experimentation. The roots of this collected plant were shade dried and then converted to powder form for further studies.

**2.2 Extraction**

The extraction was done by Soxhlet method [9] using three solvents viz. Chloroform, 50% Ethanol, and Dichloromethane. After extraction in Soxhlet apparatus for 24 hours the extracts were filtered and used for preliminary and GC-MS analysis by concentrated the extracts to 5ml using rotatory vacuum evaporator at room temperature which were then used for GC - MS analysis.

**2.3 Qualitative and Quantitative analysis**

The Standard procedure for Qualitative and quantitative analysis was followed [10].

**2.4 GC-MS analysis**

The standard procedure was followed for the GC-MS analysis [11].

The analysis was carried out using gas chromatography-high resolution mass spectrometer. 2µl of the prepared extracts was employed for GC-MS analysis. The GC-MS analysis was carried using Alegent 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 $^0$C. the Oven temperature were programmed from 50 $^0$C to 280 $^0$C at 10 0C/minute to 200 $^0$C then 10 $^0$C/ 3 minutes to 250 $^0$C ending with a 5 minutes isothermal at 280 $^0$C. The sample was injected in split mode as 10:80.

**2.5 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 molecular formula of the identified molecules were ascertained.

## 3. Results

### 3.1 Qualitative Phytochemical Analysis

**Table 1:** Qualitative Phytochemical Screening of *Alhagi pseudalhagi* (M. Bieb.) Desv ex B. Keller & Shap

| Sr. No. | Constituents | Chemical tests | Root | | | |
|---|---|---|---|---|---|---|
| | | | Water | DCM | Ethanol | Chloroform |
| 1 | Alkaloids | Wagners test | + + + | + + + | + + + | - - - |
| | | Mayers test | + + + | + - - | + + + | + - - |
| 2 | Flavonoids | Sodium hydroxide test | + + + | + - - | + + + | + + + |
| | | Lead acetate test | - - - | - - - | - - - | + + + |
| 3 | Glycosides | Killer killiani test | - - - | - - - | - - - | - - - |
| | | Fehlings test | - - - | - - - | - - - | - - - |
| 4 | Phenols | Phenols test | - - - | - - - | - - - | - - - |
| 5 | Saponins | Froathing / Foam Test | + + + | + + + | + + + | - - - |
| 6 | Steroids | Salkowaski test | + + + | + + + | - - - | - - - |
| | | LB test | - - - | + + + | - - - | - - - |
| 7 | Tannin | Ferric chloride test | - - - | - - - | + + + | - - - |
| 8 | Terpenoids | Salkowaski test | + + + | + + + | + + + | - - - |

'+'= Present and '–'= Absent

The qualitative phytochemical screening of *Alhagi pseudalhagi* (M. Bieb.) Desv ex B. Keller & Shap. in all the undertaken four extracts i.e., Water, Dichloromethane, 50% Ethanol and Chloroform showed that there is presence of phytoconstituents like Alkaloids, Flavonoids, Phenols, Saponins, Steroids and Terpenoids. However, Glycosides were not detected in any extracted part while as Flavonoids and Terpenoids were detected in most of the extracts.

### 3.2 Quantitative Phytochemical Analysis

The crude content of major phytochemical compounds in *Alhagi pseudalhagi* (M. Bieb.) Desv ex B. Keller & Shap. were determined. It was found that among all the five tested phytochemicals, the plant showed higher level of Flavonoids followed by Alkaloids and Saponins while as crude content of Phenols was lesser than Flavonoids, Alkaloids and Saponins.

### 3.3 GC-MS Analysis

**Table 2:** Quantitative Phytochemical analysis of *Alhagi pseudalhagi* (M. Bieb.) Desv ex B. Keller & Shap.

| S. No. | Phytochemicals | % of Crude Content (g/ 100 gms of Root dry sample) |
|---|---|---|
| 1 | Flavonoids | 8.80 ± 1.59 |
| 2 | Alkaloids | 3.97 ± 0.07 |
| 3 | Saponins | 1.82 ± 0.33 |
| 4 | Phenols | 1.10 ± 0.21 |

Percentage mean (n=3) ± SD

Figures 2, 3 & 4 are chromatograms showing the peaks and retention time and Tables 3, 4, & 5 shows the retention time, name of compound, molecular weight, and molecular formula.

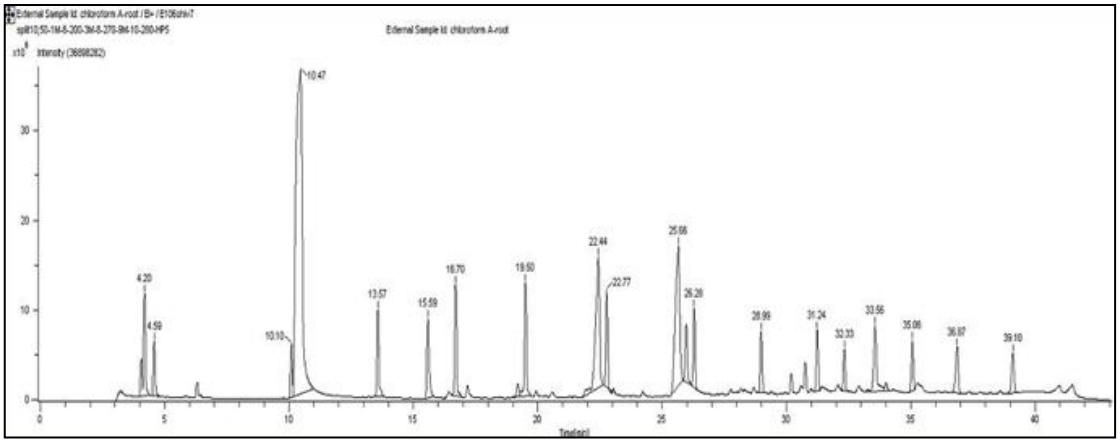


**Fig 2:** Chromatogram of Chloroform extract *Alhagi pseudalhagi* (M. Bieb.) Desv ex B. Keller & Shap. Roots

**Table 3:** Compounds identified in the Chloroform extract of *Alhagi pseudalhagi* (M. Bieb.) Desv ex B. Keller & Shap. Roots.

| Sr. No. | RT | Name of Compound | Peak area (%) | MW | MF |
|---|---|---|---|---|---|
| 1. | 4.20 | Benzene, 1,3 dimethyl- | 4.59 | 106 | $C_8H_{10}$ |
| 2. | 4.59 | Benzene, 1,3-dimethyl- | 1.87 | 106 | $C_8H_{10}$ |
| 3. | 6.30 | Cyclopropane, 1-hexyl-2-methyl- | 1.86 | 140 | $C_{10}H_{20}$ |
| 4. | 10.10 | 1- Dodecene | 1.70 | 168 | $C_{12}H_{24}$ |
| 5. | 10.47 | Methyl salicylate | 34.77 | 152 | $C_8H_8O_3$ |
| 6. | 15.59 | Phenol, 2,4-bis (1,1-dimethylethyl) | 3.07 | 206 | $C_{14}H_{22}O$ |
| 7. | 19.50 | 1-Hexadecene | 4.29 | 224 | $C_{16}H_{32}$ |
| 8. | 22.44 | n-Hexadecanoic acid | 8.56 | 256 | $C_{16}H_{32}O_2$ |
| 9. | 25.66 | Trans-13-Octadecanoic acid | 10.34 | 282 | $C_{18}H_{34}O_2$ |
| 10. | 25.97 | Octadecanoic acid | 2.17 | 284 | $C_{18}H_{36}O_2$ |
| 11. | 26.28 | 1-Docosene | 2.64 | 308 | $C_{22}H_{44}$ |
| 12. | 39.10 | Hentriacontane | 1.73 | 436 | $C_{31}H_{64}$ |

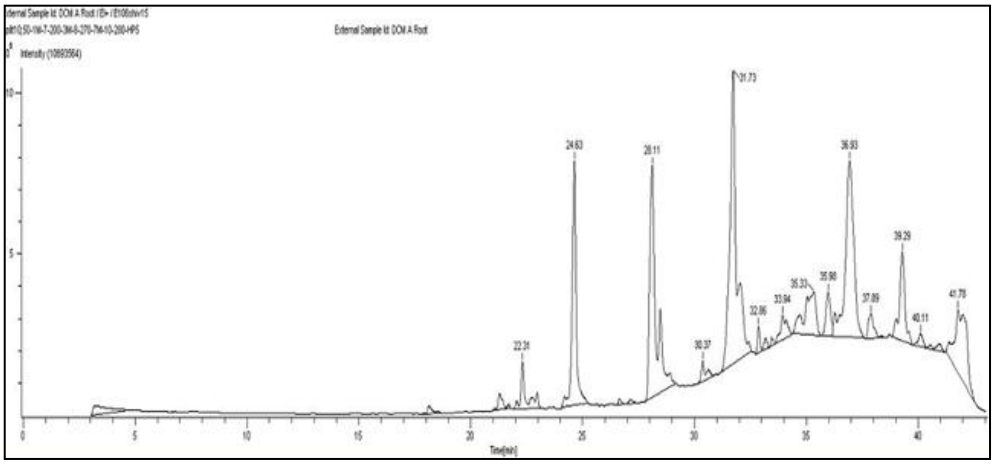


**Fig 3:** Chromatogram of DCM extract of *Alhagi pseudalhagi* (M. Bieb.) Desv ex B. Keller & Shap. Roots

**Table 4:** Compounds identified in the Dichloromethane extract of *Alhagi pseudalhagi* (M. Bieb.) Desv ex B. Keller & Shap Roots.

| Sr. No. | RT | Name of Compound | Peak area (%) | MW | MF |
|---|---|---|---|---|---|
| 1. | 18.14 | Dodecanoic acid | 0.40 | 200 | $C_{12}H_{24}O_2$ |
| 2. | 21.29 | Tetradecanoic acid | 0.97 | 229 | $C_{14}H_{28}O_2$ |
| 3. | 22.31 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 2.79 | 296 | $C_{20}H_{40}O$ |
| 4. | 24.63 | n-Hexadecanoic acid | 11.06 | 256 | $C_{16}H_{32}O_2$ |
| 5. | 28.11 | Cis-Vaccenic acid | 16.10 | 282 | $C_{18}H_{34}O_2$ |

| | | | | | |
|---|---|---|---|---|---|
| 6. | 28.47 | Octadecanoic acid | 3.24 | 284 | $C_{18}H_{36}O_2$ |
| 7. | 31.73 | Heptacosane | 21.22 | 380 | $C_{27}H_{56}$ |
| 8. | 35.33 | Octadecane, 3-ethyl-5-(2-ethylbutyl) | 5.66 | 366 | $C_{26}H_{54}$ |
| 9. | 35.98 | Campestrol | 2.24 | 400 | $C_{28}H_{48}O$ |
| 10. | 36.93 | Ethanol,2-(9-octadecynyloxy),(Z) | 16.49 | 312 | $C_{20}H_{40}O_2$ |
| 11. | 39.29 | β-Sitosterol | 5.45 | 414 | $C_{29}H_{50}O$ |
| 12. | 41.78 | β-Sitosterol | 9.06 | 414 | $C_{29}H_{50}O$ |

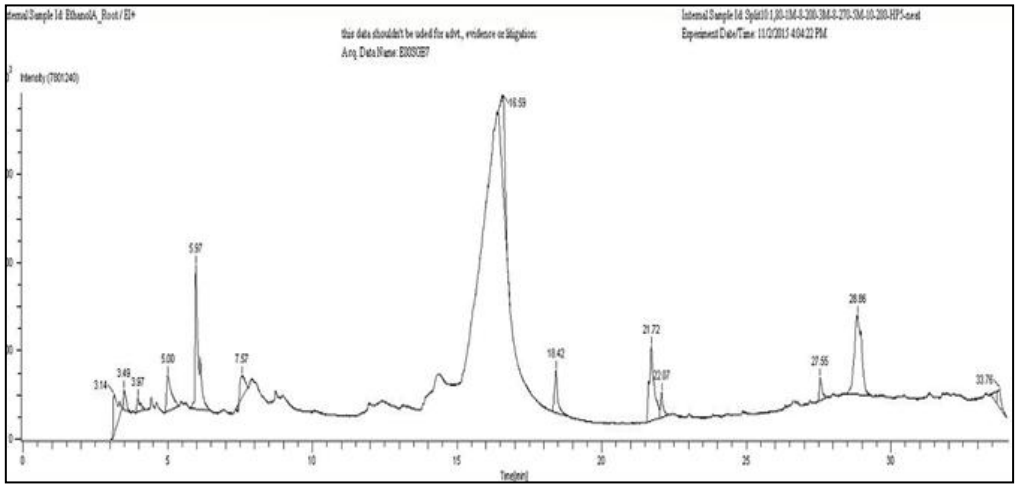

**Fig 4:** Chromatogram of 50% Ethanol extract of *Alhagi pseudalhagi* (M. Bieb.) Desv ex B. Keller & Shap. Roots

**Table 5:** Compounds identified in the 50% Ethanol extract of *Alhagi pseudalhagi* (M. Bieb.) Desv ex B. Keller & Shap. Roots.

| S. No. | RT | Name of Compound | Peak area (%) | MW | MF |
|---|---|---|---|---|---|
| 1. | 3.49 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 2.01 | 144 | $C_6H_8O_4$ |
| 2. | 5.00 | 4,5-Diamino-2-hydroxypyrimidine | 6.33 | 126 | $C_6H_6N_4O$ |
| 3. | 5.97 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 17.64 | 144 | $C_6H_8O_4$ |
| 4. | 7.57 | 2-Furanocarboxyldehyde, 5-(hydroxyl methyl)- | 3.80 | 126 | $C_6H_6O_3$ |
| 5. | 16.59 | 3-O-Methyl-d-glucose | 13.53 | 194 | $C_{17}H_{14}O_6$ |
| 6. | 18.42 | n- Hexadecanoic acid | 5.06 | 256 | $C_{16}H_{32}O_2$ |
| 7. | 22.07 | Octadecanoic acid | 2.38 | 284 | $C_{18}H_{36}O_2$ |
| 8. | 27.55 | Apigenin 7- β-rutinoside | 2.37 | 578 | $C_{27}H_{30}O_{14}$ |
| 9. | 28.86 | Ethanol, 2,9-(octadecenyloxy)-, (Z)- | 21.58 | 312 | $C_{20}H_{40}O_2$ |
| 10. | 33.76 | Ergosa-5,22-dien-3-ol, acetate, (3 β, 22E)- | 2.63 | 440 | C30H48O2 |

## 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 [12]. 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 phytocompounds.

The qualitative phytochemical tests have revealed the presence of various compounds in the roots of *Alhagi pseudalhagi*. The GC-MS analysis supports the same and shows the presence of various phytocompounds among which some are categorized as secondary metabolites. This confirms its importance as ethnomedicine important plant and as well as can be used in pharmaceutical industry [13-17]. In the present investigation, Roots 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 is Phytol, Flavonoids are 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- and Apigenin 7-β-rutinoside; Steroid are Campesterol, β-Sitosterol and Ergosa-5,22-dien-3-ol, acetate, (3 β, 22E)-; Phenols are Methyl salicylate and Phenol, 2, 4-bis (1,1-dimethylethyl).

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