International Journal of Advance Research in Science and Engineering
Volume No.07, Special Issue No.04, March 2018
www.ijarse.com
IJARSE
ISSN: 2319-8354

Chemical constituents of Camel thorn *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Stem

Wagay N.A.*[1], Yatoo Ghulam Mohiuddin[2], Shah Rafiq[3]
[1]*Botany Research Laboratory, Vidyabharati Mahavidyalya College, Camp, Amravati, Maharashtra, **India**-444602*
[2]*Plant Tissue Culture Laboratory, Department of Botany, S. G. B. Amravati, University, Amravati, Maharashtra, **India**- 444602*
[3]*Plant Tissue Culture Research Laboratory, Department of Botany, University of Kashmir, Hazratbal, Srinagar, J&K, India-* 190006
Corresponding Author: Dr. Nasir Aziz Wagay, Research Scholar

ABSTRACT
Alhagi pseudalhagi (M. Bieb.) Desv. ex B. Keller & Shap. is rarely found in India. It has been reported by various authors for its potential to cure various ailments. In the present study stem of this plant has been analyzed for its chemical constituents. Analytical, Qualitative, Quantitative and GC-MS analysis was carried out for the understudy plant part. Solvents with different polarities were used for the extraction of chemical constituents and those extracts were analyzed by Gas Chromatography- Mass Spectrometric analysis. 17, 9 and 7 compounds were identified in solvents Chloroform, Dichloromethane and 50 % Ethanol respectively among which seven are secondary metabolites. All the seven secondary metabolites are effective against various diseases and have vast pharamacological importance.

I INTRODUCTION

Basic medicinal and pharmacological properties of plants are often related to phytochemicals or bioactive compounds. Phytochemicals are produced by these plants for various processes like structure and maintenance, protection against the biotic and abiotic environmental challenges, ecological roles etc. Higher plants has a special feature of producing most and large number of phytochemicals (Castello *et al.*, 2002). Some chemicals among these phytochemicals have vast pharmacological properties which can be used for curing various ailments and the most important of these phytochemicals are among alkaloids, flavonoids, saponins and phenolic compounds (Hill, 1952; Pandey *et al.*, 2013).

Alhagi pseudalhagi (M. Bieb.) Desv. ex B. Keller & Shap. is a perennial shrub of family Fabaceae commonly called as 'Camel thorn'. Stem has a slightly bad taste and is used as aperient, expectorant, diuretic, aphrodisiac, purifies blood; good in vomiting, small pox eruption and piles (Yadav *et al.*, 2014). Decoctions of whole plant is cholegogic and astringent for cholitis, gastritis, stomach ulcers; to reduce water loss; for hemorrhoids & wound dressing; for dysentery, nasopharynx diseases, angina, extremity eczema and also acts as an antipyretic (Sultan *et al.*, 2011). The entire plant is used to treat cold, fever, the damp-heat of the stomach and abdominal as well as the enteritis (Zhang, 2010). The plant extracts have shown good results after being tested

for various cell lines for their cytotoxic activity (Behzad S. *et al.*,2014); anti-ulcerogenic activity (Gharib Naseri *et al.*, 2007). Phytochemical study of Roots has already been carried out by Wagay *et al.*, 2017 and chemical study of leaves has also been carried out by Wagay *et al.*, 2016. This study was aimed to study the chemical constituents present in the Stem of *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. and active secondary metabolites present in it.

II MATERIALS AND METHODS

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. The Stem of this collected plant was shade dried and then converted to powder form for further studies.

The procedures recommended in Indian Pharmacopoeia (Anonymous, 1966) and Gupta *et al*., 2013 were followed for analytical or physiochemical study.

The extraction was done by using 10 grams of plant powder and 180ml of solvent viz. Chloroform, 50% Ethanol, and Dichloromethane in the Soxhlet apparatus. The heating mantle temperature was set at evaporating temperature of the solvent and the cycles of soxhletion was done for about 6 hours. After extraction in Soxhlet apparatus for 6 hours the extracts were filtered and concentrated to 5ml using rotatory vacuum evaporator at room temperature. These concentrated samples were then used for further analysis.

The standard procedure for Qualitative analysis and quantitative analysis was followed (Wagay *et al.*, 2015).

The GC-MS 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 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 ^{0}C/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.

Interpretation on mass spectrum of GC-MS was done using the National Institute Standard and Technology (NIST) database. The mass spectrum of 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 by this analysis.

III RESULTS

Morphological Characters

Alhagi pseudalhagi (M. Bieb.) Desv. ex B. Keller & Shap. is a shrub of family Fabaceae commonly called as ‘Camel Thorn’ or ‘Jawasa’. It is armed with 1-2.5 cm long spines. Leaves are drooping from the base of spines or branches,smooth and simple, oblong, obtuse, glabrous, leathery, with rounded apex. Flowers are shortly pedicillate, small, 1-6 in number, borne on a spine, red papillonaceous and form panicles by auxillary

racemes. Calyx is glabrous and 2-3 mm long. Corolla is Reddish and length about three times as that of Calyx. Pod is greenish gray and hard, falcate or straight, torrulose, 2.5 cm long containing 6-8 sub-reniform seeds. It has flowering period of October to March.


Figure No. 1 & 2: Habit and flower of Alhagi pseudalhagi (M. Bieb.) Desv. ex B. Keller & Shap.

Analytical Analysis

Table No 1: Analytical values of *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Stem

S. No.	**Parameter studied**		**%age value (w/w)**
1.	Moisture content		8.98 ± 0.34
2.	Total Ash		7.24 ± 0.59
3.	Acid soluble ash		82.27 ± 1.75
4.	Acid insoluble ash		15.73 ± 0.88
5.	Water soluble ash		35.90 ± 0.43
6.	Water insoluble Ash		63.23 ± 0.37
7.	**Extractive values in**	Chloroform	4.39 ± 0.16
		50% Ethanol	10.49 ± 0.46
		Dichloromethane	3.79 ± 0.36

Note: Percentage mean (n=3) ± SD

Qualitative Phytochemical Analysis

Table No. 2 : Qualitative Phytochemical Screening of *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Stem

S. No.	Constituents	Chemical tests	STEM			
			W	D	E	C
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	Tannins	Ferric chloride test	- -	- -	+ +	- -
8	Terpenoids	Salkowaski test	- -	- -	+ +	- -

Note '+'= Present and '–'= Absent

Where, W= Water; D= Dichloromethane; E= 50% Ethanol; C= Chloroform respectively.

Quantitative Phytochemical analysis

Table No. 3: Quantitative Phytochemical analysis of *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Stem

S. No.	Phytochemicals	(g/100 gms of dry sample)
1	Flavonoids	6.99 ± 0.72
2	Alkaloids	8.36 ± 1.33
3	Saponins	3.69 ± 0.90
4	Phenols	2.05 ± 0.72

Note: Percentage mean (n=3) ± SD

Phenols: expressed as gallic acid equivalents (GAE/g)

Flavonoids: expressed as quercetin equivalent (mg/g)

Gas Chromatography- Mass Spectrometric Analysis

International Journal of Advance Research in Science and Engineering
Volume No.07, Special Issue No.04, March 2018
www.ijarse.com
IJARSE
ISSN: 2319-8354

Figure 3: Chromatogram of Chloroform extract of *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Stem

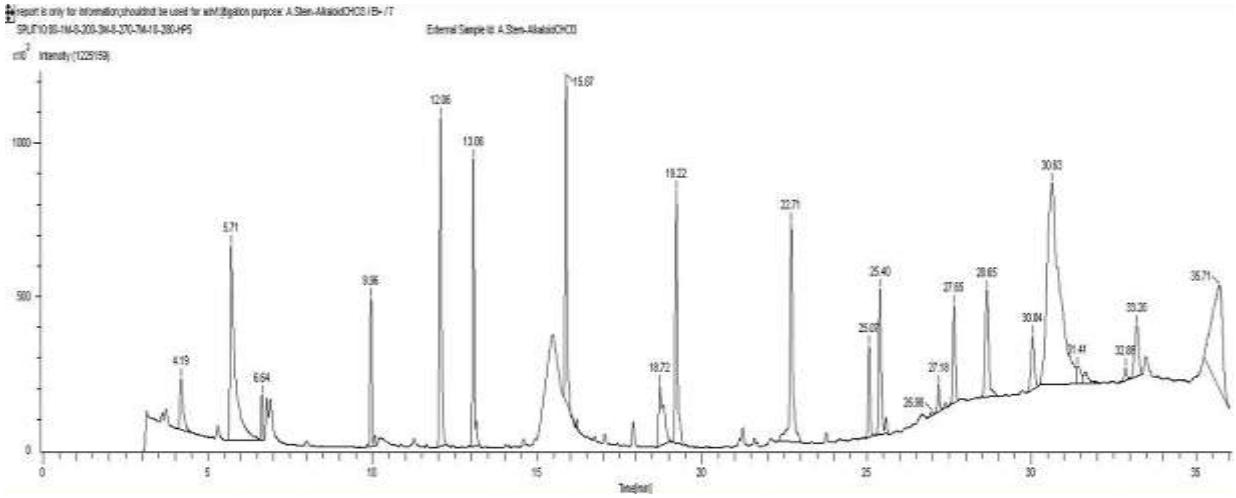

Table No. 4: Compounds identified in the Chloroform extract of *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Stem

S. No	RT	Name of compound	Peak Area %	MW	MF
1.	4.19	2-Pentenoic acid, 2-methoxy-4-methyl, methyl ester	1.66	158	$C_8H_{14}O_3$
2.	5.71	Butanedioc acid, hydroxyl, dimethyl ester	9.26	162	$C_6H_{10}O_5$
3.	6.64	1-Dodecene	0.74	168	$C_{12}H_{24}$
4.	6.79	2-Propoxy-succinic acid, dimethyl ester	0.56	204	$C_9H_{16}O_5$
5.	9.96	1-Tetradecene	2.78	196	$C_{14}H_{28}$
6.	12.06	Phenol,2,4-bis(1,1-diethylethyl)-	6.97	206	$C_{14}H_{22}O$
7.	13.06	1-Tetradecene	5.47	196	$C_{14}H_{28}$
8.	15.45	3-O-Methyl-d-glucose	15.67	194	$C_7H_{14}O_6$
9.	15.87	1-Nonadecene	5.99	266	$C_{19}H_{38}$
10.	19.22	1-Nonadecene	6.03	266	$C_{19}H_{38}$
11.	22.71	1-Nonadecene	6.30	266	$C_{19}H_{38}$
12	25.07	Stigmasterol	1.77	412	$C_{29}H_{48}O$
13.	25.40	1-Docosene	3.65	308	$C_{22}H_{44}$
14.	25.60	S-indacene, 1,2,3,5,6,7- hexahydro-1,1,5,5-tetramethyl-4,8-bis (3-methylbutyl)-	0.22	354	$C_{26}H_{42}$
15.	27.65	Octatriacontyl pentafluropropoinate	2.24	696	$C_{41}H_{77}F_5O_2$
17.	33.20	5α–Pregnane-12,20-dione	2.05	316	$C_{21}H_{32}O_2$

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Figure No. 4: Chromatogram of Dichloromethane extract of *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Stem

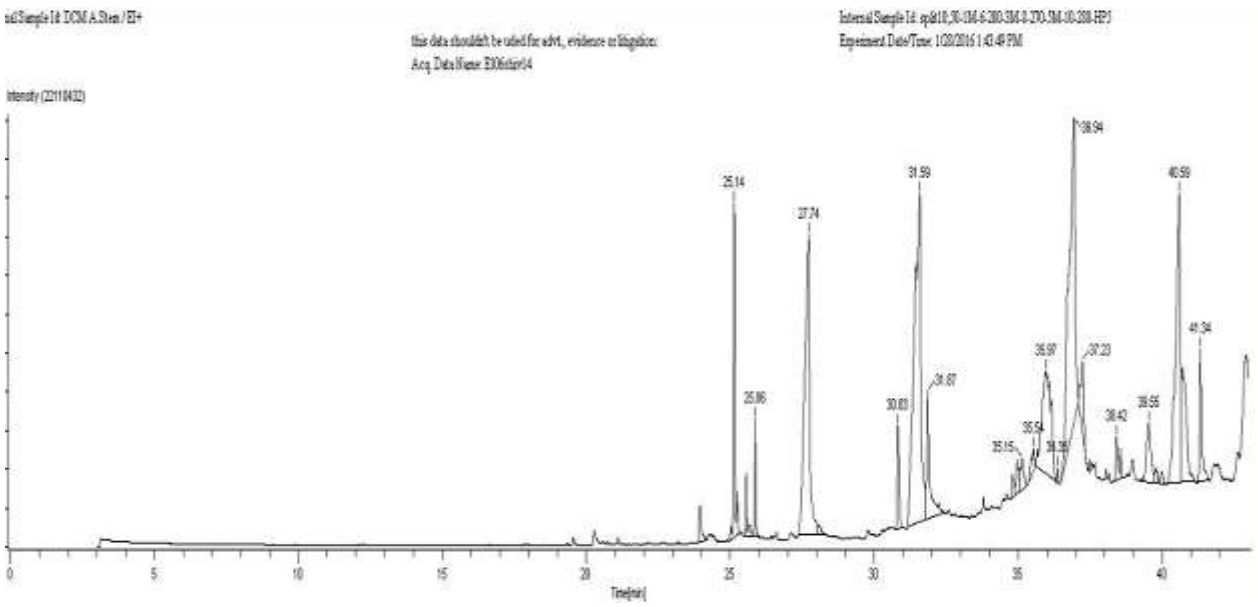


Table No. 5: Compounds identified in the Dichloromethane extract of *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Stem.

S. No.	RT	Name of compound	Peak Area %	MW	MF
1.	19.54	2(4H)-Benzofuranone,5,6,7,7a-terahydro-4,4,7a-trimethyl-(R)-	0.22	180	$C_{11}H_{16}O_2$
2.	23.95	Tetradecanoic acid	0.62	228	$C_{14}H_{28}O_2$
3.	25.14	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	3.80	296	$C_{20}H_{40}O$
4.	27.70	n-Hexadecanoic acid	12.43	256	$C_{16}H_{32}O_2$
5.	31.86	Octadecanoic acid	3.21	284	$C_{18}H_{36}O_2$
6.	35.98	3.3'-Dimethyl-1'-hydroxy-5,8-dimethoxy-2,2'-binapthalene-1,4,5',8'-tetrone	9.26	418	$C_{24}H_{18}O_7$
7.	36.92	Vitamin E	20.29	430	$C_{29}H_{50}O_2$
8.	41.33	Heptacosane	2.30	380	$C_{27}H_{56}$
9.	42.93	ϒ -Sitosterol	3.96	414	$C_{29}H_{50}O$

Figure No. 3: Chromatogram of 50% Ethanol extract of *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Stem

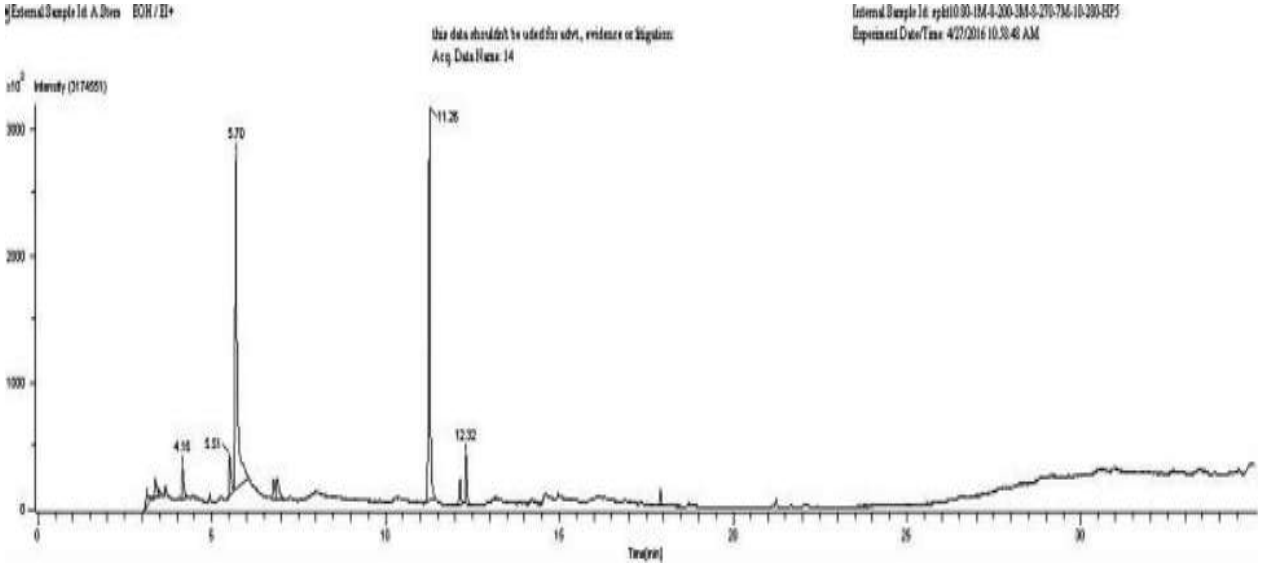


Table No. 6: Compounds identified in the 50% Ethanol extract of *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Stem.

S. No.	RT	Name of compound	Peak Area %	MW	MF
1.	4.16	Butanedioic acid, dimethyl ester	3.37	146	$C_6H_{10}O_4$
2.	5.51	Levoglucosenone	4.44	126	$C_6H_6O_3$
3.	5.70	3-Acetoxy-3-hydroxypropionic acid, methyl ester	40.04	162	$C_6H_{10}O_5$
4.	6.78	2-Propoxy-succinic acid, dimethyl ester	1.87	204	$C_9H_{16}O_5$
5.	6.89	2-Propoxy-succinic acid, dimethyl ester	3.79	204	$C_9H_{16}O_5$
6.	11.26	Citric acid, trimethyl ester	34.05	234	$C_9H_{14}O_7$
7.	12.32	Trimethyl citrate	3.99	234	$C_9H_{14}O_7$

IV DISSCUSSION

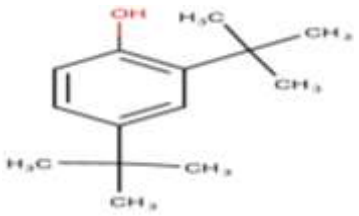
The present study was aimed to investigate the above said phytochemicals by using different solvents with different polarities for plant *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. (Fig.1 & 2). *A. pseudoalhagi* was divided into three parts viz., Roots, Stem, and Leaves. Stem was selected for the present study which was extracted in four solvents viz., Water, Dichloromethane, 50 % Ethanol and Chloroform for qualitative analysis. The quantitative analysis of Flavonoids, Alkaloids, Saponins and Phenols was done in the present study for the stem of *A. pseudoalhagi.* Three different solvents Chloroform, Dichloromethane and 50 % Ethanol were used for GC-MS analysis. GC-MS results of *A. pseudoalhagi* Stem in Chloroform are depicted in Fig. 4.4.4 and Table 4.4.12; Dichloromethane (Fig. 4.4.5 and Table 4.4.13), and 50% Ethanol (Fig. 4.4.6 and

Table 4.4.14). The identified compounds were 17, 9 and 7 in solvents Chloroform, Dichloromethane and 50 % Ethanol respectively. Among the identified compounds in the stem of *A. pseudoalhagi* one is phenol; one is terpenoid; two are Flavonoids; three are steroids while as the other identified compounds are hydrocarbons, Fattyacids, methyl esters etc.

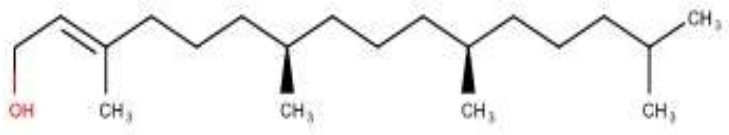
Table No. 7: Secondary metabolites present in *Alhagi pseudalhagi* (M. Bieb.) Desv. ex B. Keller & Shap. Stem and their biological activities reported

S. No.	Name of Compound	Category	Biological activity
1.	Phenol,2,4-bis (1,1-dimethylethyl)-	Phenol	Anti-bacterial activity (Kuppuswamy *et al.,* 2013), Antifungal activity and Antioxidant (Raja *et al.,* 2011) Antiinflammatory activity (Sanitha Phillips *et al.,* 2015) Anticancerous (Pereira *et al.*, 2009).
2.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	Terpenoid/ Diterpene	Anti-inflammatory (Ogunlesi *et al.,* 2009), Antimicrobial, diuretic (Rajeswari *et al.,* 2012) Anti-cancer(Himaja *et al.,* 2014), Cancer preventive (Sermakkani *et al.,* 2012), Joint dislocation, Hernia and Antimalarial (Dandekar *et al.,* 2015).
3.	2(4H)-Benzofuranone,5,6,7, 7a-terahydro-4,4,7a-trimethyl-(R)-	Coumaran / Flavonoid	Antihelminthic, Anti-inflammatory, Antidiarrhoeal (Vijisaral *et al*., 2014).
4.	ϒ-Sitosterol	Steroid	Anticancerous (Scholtysek *et al*., 2009); Anti breast cancer (Bruce J., 2013) Antioxidative (Wang *et al.,* 2002) Anti-inflammatory (Sosa *et al.,* 2016); Uterotonic, Estrogenic, (Duke, 2002).
5.	5α–Pregnane-12,20-dione	Steroid	Anticancerous (Bradford and Awad, 2007; Woyengo *et al.,* 2009) Antibacterial activity (Epand *et al.,* 2007)
6.	Stigmasterol	Steroid	Antioxidant & reduce blood cholesterol level (Zawistowaski, 2010) Anti-osteoarthitic (Gabay *et al.,* 2010); Cyto-toxicity activity (Huang *et al.,* 2009); Anti tumour activity (Kasahara *et al.,* 1994); anti-HIV reverse transcriptase (De Oliveira *et al.,* 2014); Thyroid inhibiting properties, Precursor of progesterone, Antimicrobial, Anti-asthama, Anti inflammatory, Diuretic (Dandekar *et al.,* 2015); inhibit Na^+ K^+ pump ATPases and influence prostrate metabolism and growth (Bombardelli and Morazzoni, 1997).
7.	3,3'-Dimethyl-1'-hydroxy-5,8-dimethoxy-2,2'- binapthalene-1,4,5',8'-tetrone (Diospyrin)	Flavonoid/ Napthoquinone	Anti-cancer activity (Sagar *et al.,* 2010)

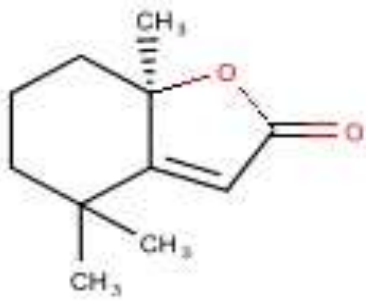
Molecular structures of the identified secondary metabolites from *Alhagi pseudalhagi* Stem

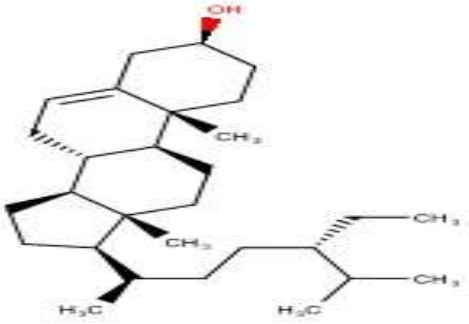


Phenol,2,4-bis (1,1-dimethylethyl)-

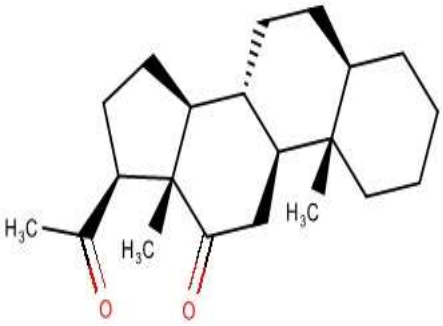


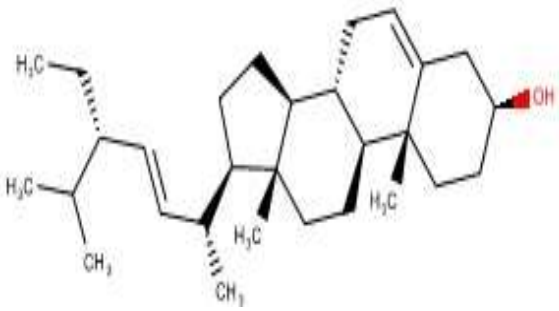
3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)



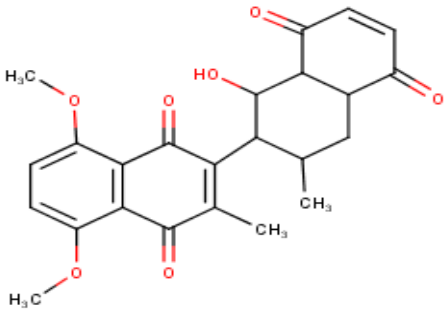


2(4H)-Benzofuranone,5,6,7, 7a-terahydro-4,4,7a-trimethyl-(R)- ϒ -Sitosterol





5α–Pregnane-12,20-dione Stigmasterol



3,3’-Dimethyl-1’-hydroxy-5,8-dimethoxy-2,2’- binapthalene-1,4,5’,8’-tetrone (Diospyrin)

V ACKNOWLEDGEMENTS

The authors express sincere thanks to Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT), Mumbai for supporting instrumental analysis for this research work. The authors are also thankful to Dr. V.R. Deshmukh, Head Department of Botany, VBMV, Camp, Amravati for providing necessary laboratory facilities.

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