Phytochemical Screening and GC-MS Chemical Profiling of Ethyl Acetate Extract of Seed and Stem of *Anethum sowa* Linn.

Muhammad Abdullah Al-Mansur[1], M. Mahboob Ali Siddiqi[2], Md. Ahedul Akbor[1] and Koushik Saha[3]

[1]Institute of National Analytical Research and Services (INARS), BCSIR, Dhaka-1205, Bangladesh
[2]Institute of Natural Sciences, United International University, Dhaka-1209, Bangladesh
[3]Department of Chemistry, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

(Received: September 20, 2017; Accepted: October 28, 2017; Published (web): December 23, 2017)

ABSTRACT: Tahe phytochemical constituents from the ethyl acetate extracts of seed and stem of *Anethum sowa* were identified by qualitative and gas chromatography-mass spectroscopy (GC-MS). Qualitative analyses exhibited the presence of alkaloids, flavonoids, tannins, carbohydrate, steroids and terpenoids in both extracts. In GC-MS analysis of *A. sowa* 6 notable peaks (3,4,4a,5,6,7,8,9-Octahydro-2H-benzocyclohepten-2-one, 2,2,4,6,7-Pentamethyl-1,2,3,4-tetrahydro quinoline, 5-Ethyl-2-methyl-pyridin-4-amine, 2-(2-Furyl) pyridine, 9-Ethyl 9-borabicyclo-[3.3.1]-nonane and 7-Methylenebicyclo-[4.2.0]-octane) and 5 significant peaks (3-Cyclopentyl-1-propyne, 2,2,4,6,7-Pentamethyl-1,2,3,4-tetrahydroquinoline,3,4,4a,5,6,7,8,9-Octahydro-2H-benzocyclohepten-2-one, 1,5-Naphthy-ridin-2-amine and Octahydro-4,7-methano-5H-inden-5-one) with comparatively higher peak area (%) among 26 and 23 compounds were detected from the ethyl acetate extract of stem and seed respectively. The study encapsulates the information regarding the phytochemical constituents present in the extracts which may have pharmacological importance.

Key words: *Anethumsowa*, gas chromatography-mass spectroscopy (GC-MS), ethyl acetate extract, phytochemical constituents.

INTRODUCTION

A greater portion of nature is covered with plant kingdom. Plants referred to medicinal plants are a significant segment of inherent medical systems all over the world due to the presence of important natural products. There have been augmented waves of fascination in the area of research in natural products chemistry. This phase can be ascribed to several considerations comprising unmet therapeutic needs, the stunning assortment of both chemical structures and biological activities of naturally occurring secondary metabolites, the utility of novel bioactive natural compounds as biochemical probes.

Correspondence to: Muhammad Abdullah Al-Mansur
E-mail: nayeembcsir@gmail.com
Tel: +88-01715010829

Dhaka Univ. J. Pharm. Sci. **16**(2): 187-194, 2017 (December)

the development of novel and sensitive techniques to detect biologically active natural products and improved techniques to isolate, purify and structurally characterize these active constituents.[1] Our investigation is focused on the investigation onto phytochemical constituents in ethyl acetate extract of seed and stem of *Anethum sowa* using gas chromatography-mass spectroscopic (GC-MS) approach.

Anethum sowa Linn. (Common name- Dill; Bengali- Shulfa; Family-Apiaceae), an annual or a biennial cold weather glabrous and aromatic herb, reaches up to 1 m in height. In Bangladesh, it is abundantly cultivated in the northern part of the country and throughout India mainly in Punjab, Uttar Pradesh, Gujarat, Maharasshtra, Assam and West Bengal. It is often found with weed of cultivation and

even as an escape in irrigated fields. Its seed has insecticidal, ovicidal and synergistic activity and is also known to contain of dillapiol and also contains essential oil having antioxidant and antimicrobial activity.[2]

The aim of the present work was to phytochemically screen the plant metabolites present in the ethyl acetate extract of seed and stem qualitatively by applying phytochemical tests and quantitatively by gas chromatography-mass spectroscopic (GC-MS) analysis. In GC-MS analysis, the percent area represents the percentage wise amount of the respective compound.

MATERIALS AND METHODS

Plant collection, identification and authentification. Fresh stem and seed of *A. sowa* were collected from BCSIR campus, Dhaka and identified by the taxonomist of Bangladesh National Herbarium, Dhaka, where a voucher specimen (DACB No. = 31282) has been deposited.[2]

Extraction and processing. Freshly collected stem and seed of *A. sowa* were dried in open air and powdered by using a grinding machine. The air-dried and powdered material of seed (250g) was soaked by ethyl acetate (2.5 litres× 3) at room temperature for 2 days against each soaking. Consequently gummy mass of ethyl acetate extract (2.01 g) was obtained from the filtrate using rotary evaporator under reduced pressure. By the same process, the air-dried and powdered material of stem (250g) was soaked in ethyl acetate (2.5 litres×3) at room temperature for 2 days for each soaking. Consequently, gummy mass of ethyl acetate extract (1.45 g) was separated by filtration followed by evaporation of solvent using rotary evaporator under reduced pressure.[2] The ethyl acetate extracts of seed and stem were subjected to qualitative phytochemical screening and GC-MS analysis.

Chemical reagents for screening of phytochemical constituents. Chemicals and reagents like ethyl acetate, sulphuric acid, Mayer's reagent, Hager's reagent, Wagner's reagent, acetic anhydride, lead acetate, alcoholic solution of α-napthol, ammonia solution, sodium chloride, gelatine solution, chloroform, Fehling's solution A and B, sodium hydroxide, hydrochloric acid, mercuric chloride, potassium iodide (KI) and benzene were used to analyze phytochemicals present in the ethyl acetate extract of seed and stem of *A. sowa*. All solvents were of analytical grade and collected from commercial sources (E. Merck (Germany), BDH (England) and Sigma Aldrich (Germany).

Methodology for screening of phytochemical constituents. Specific chemical tests were carried out for phytochemical constituents. Standard procedures were followed to identify the constituents as described by Harborne[3], Trease and Evans[4] and Sofwara[5] to confirm the presence of various constituents in the ethyl acetate extracts only.

Instrumentation for GC-MS analysis. The GC-MS analysis was performed on a GC-MS-QP 2010 Ultra instrument equipped with AB innowax column (30 × 0.25 mm id, film thickness 0.25 μm). Initially, oven temperature was maintained at 120^0C for 1 minute and temperature was gradually increased up to 270°C for 25 minutes and 0.5μl of sample was injected for analysis. Helium at 1.15 ml/min was the carrier gas. The sample injector and mass transfer line temperature were set at 200°C and 250°C and split ratio was 200 throughout the experiment periods. The ionization mass spectroscopic analysis was done with 70 eV. Mass spectra were recorded across the range of 50 m/z to 650 m/z for the duration of 40.75 minutes. Identification of components was based on comparison of their mass spectra with those of Wiley and NIST Libraries, Adams[6] and by comparison of their retention indices with literature values.[7]

RESULTS AND DISCUSSION

Analysis of phytochemical constituents. The phytochemical investigation of the ethyl acetate extract of seed and stem of *A. sowa* revealed the presence of carbohydrates, flavonoids, tannins, steroids, glycosides, alkaloids, anthraquinone, and terpenoids. On the contrary, only saponins are absent in extract of stem and seed. In addition,

carbohydrates, flavonoids, tannins, alkaloids, steroids and terpenoids are present whereas glycosides, anthraquinone are absent in extract of stem.

From the previous study, it is evident that carbohydrates may possibly increase the potency of the therapeutically important ingredients. Hence, a finer curative result may be gained from the combination of active principles in each plant than by single isolated constituent.[8,9] Additionally, tannins have antidiarrheal impact and these substances may precipitate proteins on the enterocytes reducing peristaltic movement and intestinal secretion.[10] Furthermore, saponins have expectorant, cardiotonic and hypoglycemic activity.[11-14] Besides, glycosides have laxative, diuretic and antiseptic properties.[15-17] Moreover, flavonoids demonstrate significant antimicrobial[18], hypoglycemic and anti-diabetic,[19] anti-inflammatory,[20] antioxidant,[21,22], anti-tumour[23] and free radical-scavenging activities. From the phytochemical study it was revealed that the ethyl acetate extract of seed and stem may have anti-inflammatory, antioxidant agents associated with free radical-scavenging action due to the presence of flavonoids and antidiarrheal activity owing to tannins. In addition, the presence of terpenoids indicates that the ethyl acetate extract may have cytotoxicity activity.[24]

Table 1. The phytochemical investigation of the powder and ethyl acetate extract of seed and stem of *A. sowa.*

Chemical class of constituents	Test	Ethyl acetate extract of seed	Ethyl acetate extract of stem
Carbohydrates	Molisch's test	+	+
Flavonoids	a)Alkaline reagent test	+	+
	b) Lead acetate test	+	+
Tannins	a) Gelatin-salt block test	+	+
	b) Lead acetate test	+	+
Steroids	a) Salkowski test	+	+
	b) Liebermann-Burchard's test	+	+
Saponins	a) Frothing	-	-
	b) Emulsification	-	-
Glycosides	a) Sodium hydroxide reagent	-	-
	b) Test for glycosides with glucose as the glycone	-	-
Alkaloids	a) Mayer's reagents	+	+
	b) Hager's test	+	+
	c) Wagner's test	+	+
Terpenoids	a) Salkowski test	+	+
	b) Liebermann-Burchard's test	+	+
Anthraquinone	Borntrager'stest	+	-

+=present; - = absent.

GC-MS analysis of the extracts. In the GC-MS analyses of *A. sowa*, 26 compounds were identified from the ethyl acetate extract of stem and 23 compounds from that of seed. The recognition of phytochemicals is determined by the peak area, molecular weight and molecular formula. The chromatogram (Figure 1) of ethyl acetate extract of stem represents 6 notable peaks. Among these, 3,4, 4a,5,6,7,8,9-Octahydro 2H-benzocyclohepten-2-one with retention time 6.135 has the highest peak area (17.098%). In addition, 2,2,4,6,7-Pentamethyl-1,2,3,4-tetrahydro quinoline giving retention time 8.654 refers peak area (10.51 %) while 5-Ethyl-2-methyl-pyridin-4-amine with retention time 10.328 exhibits peak area increased by 4.69% compared to 2,2,4,6,7-Pentamethyl-1,2,3,4-tetrahydro quinoline. Moreover 7-Methylenebicyclo-[4.2.0]-octane showed retention time 14.797 with peak area 8.302% which

is almost equally found against 9-Ethyl-9-borabicyclo-[3.3.1]-nonane with retention time 18.991. Lastly 2-(2-Furyl) pyridine reveals 11.408% peak area at retention time 24.2. The chemical structures of the most prevalent compounds of ethyl acetate extract of stem of *A. sowa* have been shown in table 3.

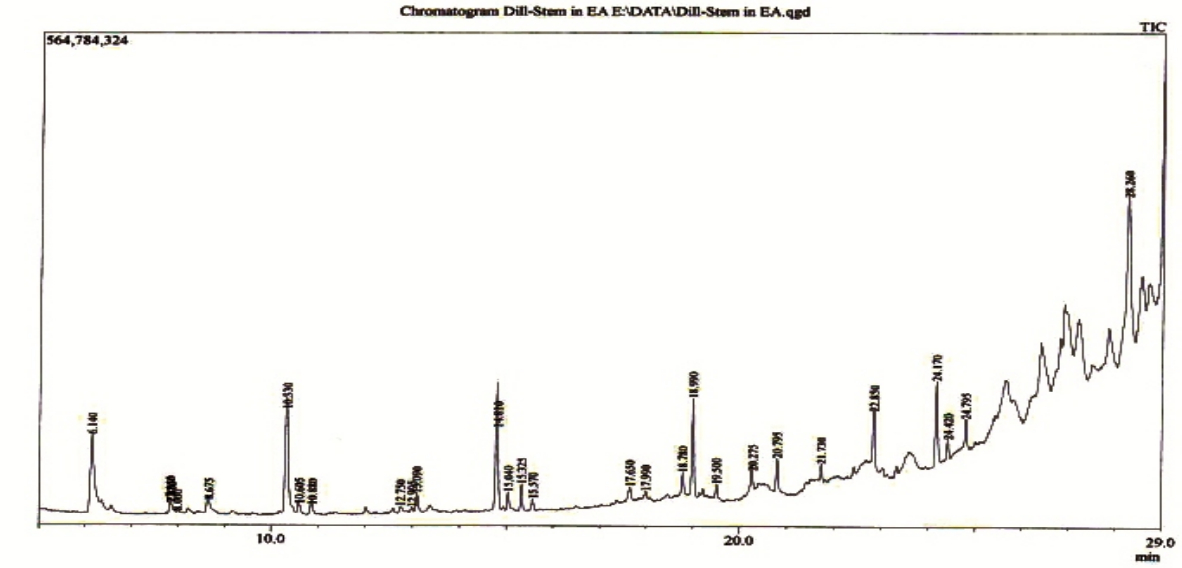


Figure 1. Chromatogram (GC/MS) of the ethyl acetate extract of stem of *A. sowa*.

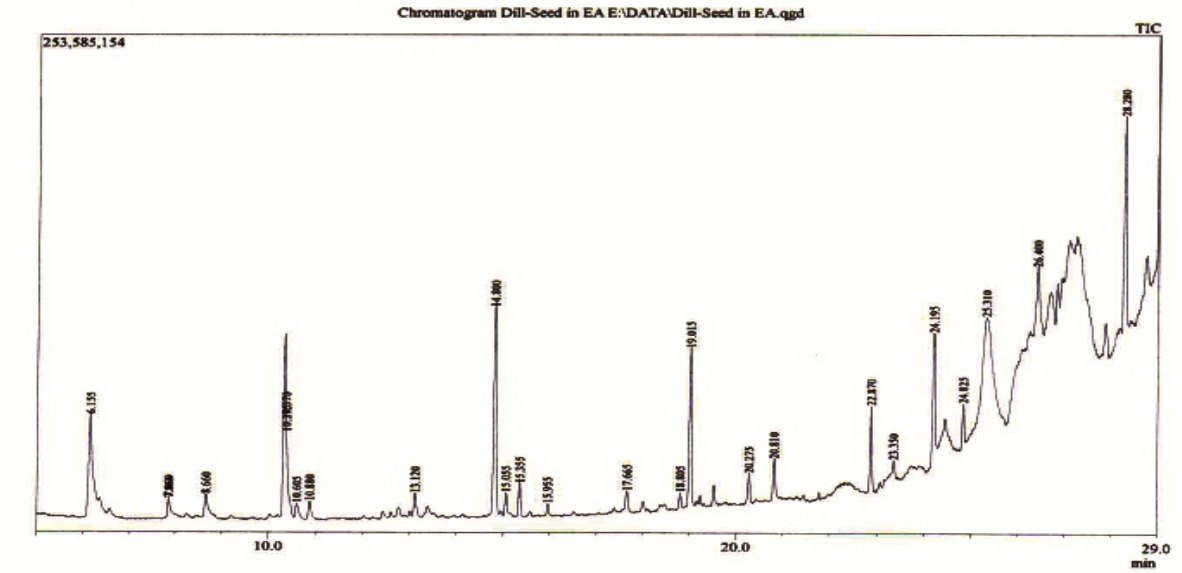


Figure 2. Chromatogram (GC/MS) of the ethyl acetate extract of seed of *A. sowa*.

From the GC-MS analyses of *A. sowa*, it is noticed that the ethyl acetate extract of seed provided 23 compounds. The chromatogram (Figure 2) of ethyl acetate extract of seed represents 5 significant peaks. Among them, 3-Cyclopentyl-1-propyne with retention time of 6.149 has peak area of 14.137%. In addition, 2,2,4,6,7-Pentamethyl-1,2,3,4-tetra-hydro quinoline at retention time 8.668 refers peak area of 7.321% which was almost half of 3-Cyclopentyl-1-propyne. Moreover 3,4,4a,5,6,7,8,9-Octahydro-H-

benzocyclohepten -2-one with retention time 19.013 exhibited peak area 8.105 % which is almost 1% higher than 1,5-Naphthyridin-2-amine. Furthermore Octahydro-4, 7-methano-5H-inden-5-one showed retention time of 25.296 with the highest peak area of 24.673% among all compounds found in the ethyl acetate extract of seed of *A. sowa*. The chemical structures of the most prevalent compounds of ethyl acetate extract of seed of *A. sowa* have been shown in table 5.

Table 2. **Chemical constituents present in the ethyl acetate extract of stem as determined by GC-MS.**

No	Name of compound	Retention time	Molecular weight	Molecular formula	Peak area	Peak area (%)
1	2H-Benzocyclohepten-2-one, 3,4,4a,5,6,7,8,9-octahydro	6.135	164	$C_{11}H_{16}O$	56785716	17.098
2	Quinoline, 2,2,4,6,7-pentamethyl-1,2,3,4-tetrahydro	8.654	203	$C_{14}H_{21}N$	34906103	10.510
3	5-Ethyl-2-methyl-pyridin-4-amine	10.328	136	$C_8H_{12}N_2$	50212888	15.119
4	Limonene	10.588	136	$C_{10}H_{16}$	7755796	2.335
5	2-(2-Hydroxyphenoxy) -1-phenylethanol	10.855	230	$C_{14}H_{14}O_3$	8909854	2.683
6	Benzenemethanol, 3-hydroxy	12.751	124	$C_7H_8O_2$	9255811	2.787
7	2,5-Cyclohexadiene-1,4-dione, 3-hydroxy-2-methyl-5-(1-methylethyl)	12.989	180	$C_{10}H_{12}O_3$	1919963	0.578
8	Succinic acid, di (but-3-yn-2-yl) ester	13.094	222	$C_{12}H_{14}O_4$	17690896	5.327
9	7-Methylenebicyclo [4.2.0] octane	14.797	122	C_9H_{14}	27572684	8.302
10	Cyclonon-4-ynone	15.032	136	$C_9H_{12}O$	9330595	2.809
11	3-Pyridinamine, N-methyl-2-nitro	15.325	153	$C_6H_7N3O_2$	12194283	3.672
12	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene	15.565	204	$C_{15}H_{24}$	12314843	3.708
13	2(5H)-Furanone, 4-methyl-3-(2-methyl-2-propenyl)	17.485	152	$C_9H_{12}O_2$	354536	0.107
14	7,12-Dihydro-6,7-bis(4-hydroxphenyl)-6H-[1,2,4] triazolo [1,5,1,2] pyrimido [5,4-c] chromen-2 o1	17.991	426	$C_{24}H_{18}N_4O_4$	801040	0.241
15	2,5-Cyclohexadiene 1,4-dione, 3-hydroxy-2-methyl-5 (1-methylethyl)	18.784	180	$C_{10}H_{12}O_3$	12400745	3.610
16	9-Borabicyclo [3.3.1] nonane, 9-ethyl	18.991	150	$C_{10}H_{19}B$	27074161	8.152
17	Spiro [2.2]pentane-1-carboxylic acid, 2-cyclopropyl-2-methyl	19.508	166	$C_{10}H_{14}O_2$	6402976	1.928
18	1-Nitro-bicyclo [6.1.0] nonan-2-one	20.257	183	$C_9H_{13}NO_3$	20237842	5.563
19	2H-1b,4-Ethanopentaleno [1,2-b]oxirene, hexahydro-, (1a alpha, 1b, beta, 4,beta, 4a alpha, 5a,alpha.)	20.795	150	$C_{10}H_{14}O$	13878865	4.179
20	cis-beta-Farnesene	21.465	204	$C_{15}H_{24}$	287133	0.086
21	2H-Benzocyclohepten-2-one, 3,4,4a,5,6,7,8,9-octahydro-4a-methyl-(S)	22.843	178	$C_{12}H_{18}O$	17407073	5.241
22	2-(2-Furyl) pyridine	24.200	154	C_9H_7NO	46846641	11.408
23	β-bisabolene	24.413	204	$C_{15}H_{24}$	17268013	4.036
24	Cyclohexene. 4-isopropenyl-1-methoxyme	24.796	186	$C_9H_{14}O_2S$	16092684	4.845
25	Naphthalene,1,2,3,4,4a,5,6,8a –octahydro-4a,8-dimethyl-2-(1-methylethenyl)-[2R-(2 alpha,4a alpha,8a beta)]	28.268	204	$C_{15}H_{24}$	11997604	2.727
26	Norcymserine,N-[2-phtenethyl]	7.844	202	$C_{12}H_{10}O_3$	2687023	0.607

Table 3. **Chemical structures of the most prevalent compounds of ethyl acetate extract of stem of *A. sowa:***

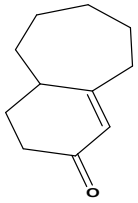
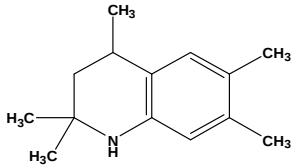
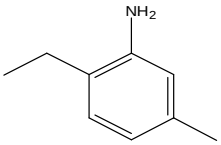
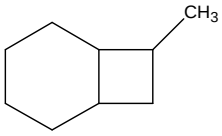
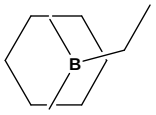
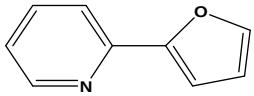
Name of compound	Chemical structure of the compound
3,4,4a,5,6,7,8,9-Octahydro 2H-benzocyclohepten-2-one	
2,2,4,6,7-Pentamethyl-1,2,3,4-tetrahydro quinoline	
5-Ethyl-2-methyl-pyridin-4-amine	
7-Methylenebicyclo-[4.2.0]-octane	
9-Ethyl-9-borabicyclo-[3.3.1]-nonane	
2-(2-Furyl)-pyridine	

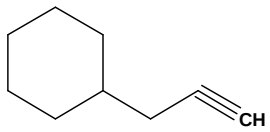
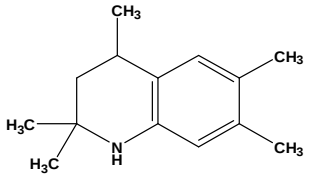
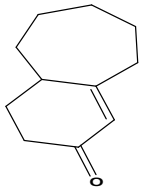
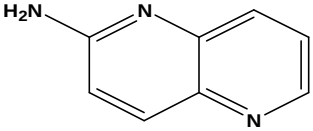
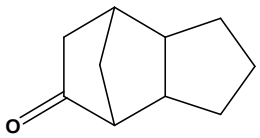
Table 4. Chemical constituents present in the ethyl acetate extract of seed using GC-MS analysis.

No	Name of the Compound	R. time	Molecular weight	Molecular formula	Peak area	Peak area (%)
1	3-Cyclopentyl-1-propyne	6.149	108	C_8H_{12}	45044690	14.137
2	6-(2-Ethoxy-phenyl)-5 nitro-piperidin-2-one	7.863	264	$C_{13}H_{16}N_2O_4$	2646183	0.824
3	Quinoline, 2,2,4,6,7-pentamethyl-1,2,3,4-tetrahydro	8.668	203	$C_{14}H_{21}N$	23328789	7.321
4	5-Ethyl-2-methyl-pyridin-4-amine	10.605	136	$C_8H_{12}N_2$	4790942	1.504
5	3-Pyridinamine, N-methyl-2-nitro	10.605	153	$C_6H_7N_3O_2$	1902397	0.597
6	5-Ethyl-2-methyl-pyridin-4-amine	10.880	136	$C_8H_{12}N_2$	5715519	1.794
7	1-Methyl-2-trimethyloxycyclohexene	13.107	198	$C_{11}H_{22}OSi$	10367603	3.254
8	2H-Benzocyclohepten-2-one, 3,4,4a,5,6,7,8,9-octahydro	14.800	164	$C_{11}H_{16}O$	38204660	11.990
9	3-Pyridinamine, N-methyl-2-nitro	15.056	153	$C_6H_7N_3O_2$	4716306	1.480
10	5-Decene, 4-ethynyl-, (E)	15.344	164	$C_{12}H_{20}$	6145828	1.929
11	2,5-Cyclohexadiene-1,4-dione, 3-hydroxy-2-methyl-5-(1-methylethyl)	15.961	180	$C_{10}H_{12}O_3$	2714045	0.838
12	1-Cyclohexene-1-methanol	17.666	112	$C_7H_{12}O$	3918445	1.230

Table contd.

13	Preg-4-en-3-one, 17. Alpha.-hydroxy-17. beta.-cyano	19.114	313	$C_{20}H_{27}NO_2$	134246	0.041
14	2H-Benzocyclohepten-2-one, 3,4,4a,5,6,7,8,9-octahydro	19.013	164	$C_{11}H_{16}O$	25824311	8.105
15	(1,3-Dimethyl-2-methylene-cyclopentyl)-methanol	20.283	140	$C_9H_{16}0$	12875477	4.041
16	2H-Benzocyclohepten-2-one, 3,4,4a,5,6,7,8,9-octahydro	20.812	164	$C_{11}H_{16}O$	7849200	2.463
17	9-Borabicyclo [3.3.1] nonane,9-ethyl-	22.871	150	$C_{10}H_{19}B$	12172867	3.820
18	Spiro [2.9]dodeca-4, 8-diene	22.871	162	$C_{12}H_{18}$	3109373	0.976
19	1,5-Naphthyridin-2-amine	24.192	145	$C_8H_7N_3$	24220032	7.601
20	2-Furanacetaldehyde, alpha-isopropylidene	24.825	150	$C_9H_{10}O_2$	9834222	3.086
21	4,7-Methano-5H-inden-5-one, octahydro	25.296	150	$C_{10}H_{14}0$	78617338	24.673
22	2H-Benzocyclohepten-2-one, 3,4,4a,5,6,7,8,9-octahydro-4a-methyl-(S)	26.387	164	$C_{11}H_{16}O$	2701114	0.826
23	2(1H)-Naphthalenone, 3,4,4a,5,8,8a-hexahydro-4a-methyl-, trans	28.281	164	$C_{11}H_{16}O$	19875495	5.733

Table 5. Chemical structures of the most prevalent compounds of ethyl acetate extract of seed of *A. sowa*.

Name of compounds	Chemical structure of compounds
3-Cyclopentyl-1-propyne	
2,2,4,6,7-Pentamethyl-1,2,3,4-tetrahydro quinoline	
3,4,4a,5,6,7,8,9-Octahydro 2H-benzocyclohepten-2-one	
1,5-Naphthyridin-2-amine	
Octahydro-4,7-methano-5H-inden-5-one	

CONCLUSION

This study accentuates the presence of many secondary metabolites in the aerial parts of *A. sowa* as well as provides an overview of the different classes of molecules that may have pharmacological importance. So, further studies are needed on these

phytochemical constituents in order to isolate and elucidate the structure of these compounds with different biological activities.

ACKNOWLEDGEMENTS

We are grateful to Institute of National Analytical Research and Services (INARS), BCSIR, Dhaka, Bangladesh for giving us the opportunity to use gas chromatography-mass spectrophotometer (GC-MS).

REFERENCES

1. Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A. and Armand, R. 2015. The application of medicinal plants in traditional and modern medicine: A review of *Thymus vulgaris*. *Int. J. of Clin. Med*. **6**, 635-642.
2. Al-Mansur, M.A., Saha, K., Sultana, N. and Siddiqi, M.M.A. 2016. Comparative studies on cytotoxic, antibacterial and antioxidant activity among different extracts of seed and stem of *Anethumsowa* L. available in Bangladesh. *World J. of Pharm. Res.* **5**, 01-10.
3. Harborne, J.B. 1973. Phytochemical methods. London chapman and hall, Ltd. Pp. 49-188.
4. Trease, G.E. and Evans, W.C. 1989. Pharmacognosy. 11th Edn. Brailliartiridel can. Macmillan publishers. p. 530.
5. Software, A. 1993. Medicinal plants and traditional medicines in Africa. Spectrum books ltd., Ibadan, Nigeria p. 289.
6. Adams, R.1995. Identification of essential oil components by gas chromatography/mass spectroscopy. Allured publishing Co., Carol Stream, II.
7. Vanden, D.H. and Kratz, P.D. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr*. **11**, 463-471.
8. Frantisek, S. 1991. The natural guide to medicinal herbs and plants. Twickenham, UK: Tiger bark institute. 1-8.
9. Irene, M.V., Mynthia, A.C. and Kenneth, B.M. 1998. Comparative antidiabetic activities of some medicinal plants. *J. Ethnopharmacol*. **22**, 1-2.
10. Yu, Y.L., Leung, L.K. and Bi, Y.R. 2000. Antioxidant activity of flavonoids isolated from *Scutellaria rehderiana*. *J. Am. Chem. Soc*. **77**, 807-813.
11. Clarke, E.G.C. 1975. Isolation and identification of drugs. Vol. 2. London, UK: pharmaceutical press; 905.
12. Finar, I.L. 1989. Organic chemistry: Stereochemistry and the chemistry of natural products. 5th ed. Singapore, Singapore: Longman Group; 517-605.
13. Anila, L., Vijayalakshmi, N.R. and Tian, C. 2000. Beneficial effect of flavonoids from *Sesamumindicum*, *Emblica officinalis* and *Momordicacharantia*. *Phytother Res*. **14**, 592-595.
14. Sui, D.Y., Luz, Z. and Li, S.H. 1994. Hypoglycaemic effect of saponins isolated from leaves of *Acanthopanaxsenticosus*. *Int. J. Diab. Metabol*..**19**, 683-685.
15. Ghislain, O., Sandra, L. and Nele, B. 2012. Applications of glycobiology: Biological and immunological effects of a chemically modified amylose-derivative. London, UK: RSC Publishing, 38.
16. Chakarborty, A., Choudhary, B.K. and Bhattacharya, P. 1995. Clausenol and clausenine - Two carbazole alkaloids from *Clausena anisata*. *Phytochem.***40**, 295-298.
17. Boyce, P.W. and Christy L.P. 2004. Applied pharmacology for the veterinary technicians. 2nd ed. St. Luis, USA: WB Saunders Co. pp. 126-127.
18. Narayana, K.R., Reddy, M.R. and Chaluvadi-Krishna, D.R. 2001. Bioflavonoids classification, pharmacology, biochemical effects and therapeutic potential. *Ind. J. Pharmacol*. **33**, 2-16.
19. Coleman, D.L. 1973. Effect of parabiosis of obese with diabetes and normal mice. *Diabetologia*. **9**, 294-298.
20. Middleton, E.J.R., Kandaswami, C. and Theoharides, T.C. 2000. The effects of plants flavonoids on mammalian cells: Implications on inflammation, heart disease and cancer. *Pharmacol. Rev*. **52**, 673-751.
21. Robak, J. and Marcinkiewiez, D. 1995. Scavenging of reactive oxygen species as the mechanism of drug action. *J. Pharmacol.***47**, 89-98.
22. Parker, L., Rimbach, G. and Virgili, F. 1999. Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (*Pinusmaritima*) bark pycnogenol. *Free Rad. Biol. Med.***27**, 704-724.
23. Ahmad, M., Aktar, M.S. and Malik, T. 2000. Hypoglycemic action of the flavonoids fraction of cuminumseeds. *Phytotherap Res.***14**, 103-106.
24. Ozçelik, B., Kartal, M. and Orhan, I. 2001. Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. *Pharm. Biol*. **49**, 396-402.