

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

Muhammad Abdullah Al-Mansur¹, M. Mahboob Ali Siddiqi², Md. Ahedul Akbor¹ and Koushik Saha³

¹Institute of National Analytical Research and Services (INARS), BCSIR, Dhaka-1205, Bangladesh

²Institute of Natural Sciences, United International University, Dhaka-1209, Bangladesh

³Department of Chemistry, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

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ABSTRACT: The 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-tetrahydroquinoline, 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-Naphthylridin-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.

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, Maharashtra, Assam and West Bengal. It is often found with weed of cultivation and

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

even as an escape in irrigated fields. Its seed has insecticidal, ovicidal and synergistic activity and is also known to contain 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 authentication. 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 -naphthol,

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 Harborne³, Trease and Evans⁴ and Sofwara⁵ 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 μ m). Initially, oven temperature was maintained at 120°C for 1 minute and temperature was gradually increased up to 270°C for 25 minutes and 0.5 μ l 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, Adams⁶ 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, cardiotoxic and hypoglycemic activity.¹¹⁻¹⁴ Besides, glycosides

have laxative, diuretic and antiseptic properties.¹⁵⁻¹⁷ Moreover, flavonoids demonstrate significant antimicrobial¹⁸, hypoglycemic and anti-diabetic,¹⁹ anti-inflammatory,²⁰ antioxidant,^{21,22} anti-tumour²³ 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's test | + | - |

+ = 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.

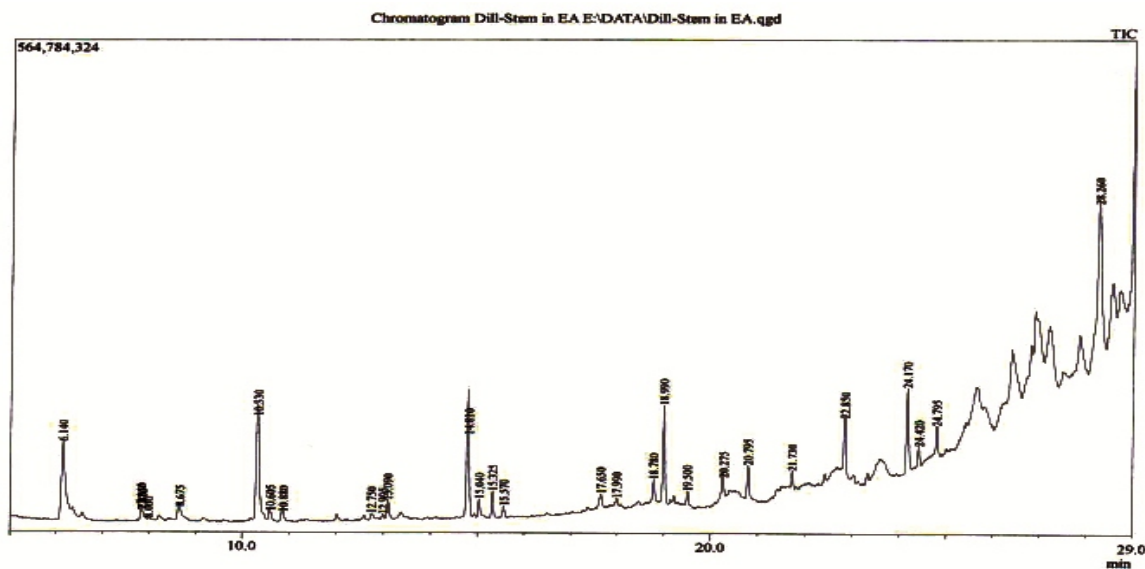


Figure 1. Chromatogram (GC/MS) of the ethyl acetate extract of stem of *A. sowa*.

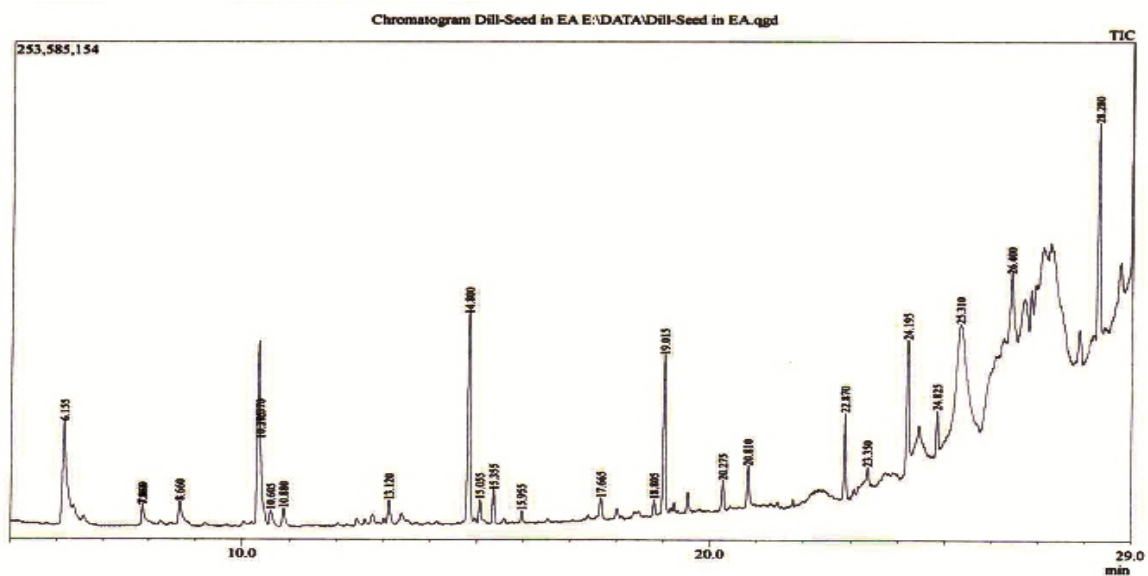


Figure 2. Chromatogram (GC/MS) of the ethyl acetate extract of seed of *A. sowa*.

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 ₈ H 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 ₇ H ₈ O 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 ₉ H ₁₄ | 27572684 | 8.302 |
| 10 | Cyclonon-4-ynone | 15.032 | 136 | C ₉ H ₁₂ O | 9330595 | 2.809 |
| 11 | 3-Pyridinamine, N-methyl-2-nitro | 15.325 | 153 | C ₆ H ₇ N ₃ O 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 ₉ H ₁₂ O ₂ | 354536 | 0.107 |
| 14 | 7,12-Dihydro-6,7-bis(4-hydroxyphenyl)-6H-[1,2,4] triazolo [1,5,1,2] pyrimido [5,4-c] chromen-2 ol | 17.991 | 426 | C ₂₄ H ₁₈ N ₄ O 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 ₉ H 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 ₉ H ₇ NO | 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 ₉ H ₁₄ O ₂ S | 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*:

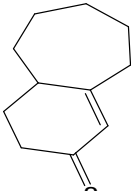
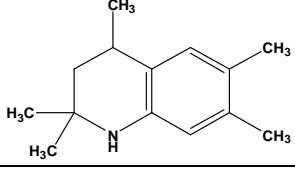
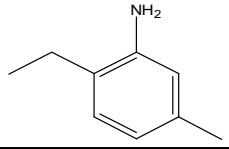
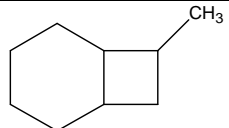
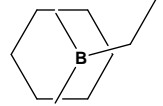
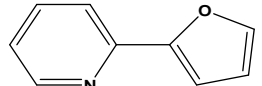
| Name of compound | Chemical structure of the compound |
|--|--|
| 3,4,4a,5,6,7,8,9-Octahydro 2H-benzocyclohepten-2-one |  |
| 2,2,4,6,7-Pentamethyl-1,2,3,4-tetrahydro quinoline |  |
| 5-Ethyl-2-methyl-pyridin-4-amine |  |
| 7-Methylenebicyclo-[4.2.0]-octane |  |
| 9-Ethyl-9-borabicyclo-[3.3.1]-nonane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

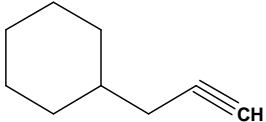
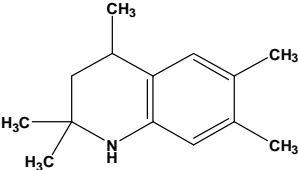
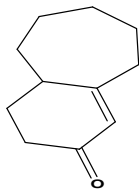
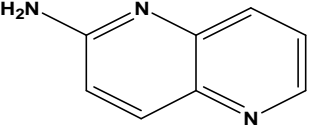
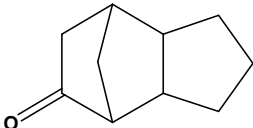
Table 4. Chemical constituents present in the ethyl acetate extract of seed using GC-MS analysis.

| No | Name of the Compound | R. time | Molecular weight | Molecular formula | Peak area | Peak area (%) |
|----|--|---------|------------------|---|-----------|---------------|
| 1 | 3-Cyclopentyl-1-propyne | 6.149 | 108 | C ₈ H ₁₂ | 45044690 | 14.137 |
| 2 | 6-(2-Ethoxy-phenyl)-5 nitro-piperidin-2-one | 7.863 | 264 | C ₁₃ H ₁₆ N ₂ O 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 ₈ H ₁₂ N ₂ | 4790942 | 1.504 |
| 5 | 3-Pyridinamine, N-methyl-2-nitro | 10.605 | 153 | C ₆ H ₇ N ₃ O ₂ | 1902397 | 0.597 |
| 6 | 5-Ethyl-2-methyl-pyridin-4-amine | 10.880 | 136 | C ₈ H 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 ₆ H ₇ N ₃ O 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 ₇ H 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 ₉ H ₁₆ O | 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 ₈ H ₇ N ₃ | 24220032 | 7.601 |
| 20 | 2-Furanacetaldehyde, alpha-isopropylidene | 24.825 | 150 | C ₉ H ₁₀ O ₂ | 9834222 | 3.086 |
| 21 | 4,7-Methano-5H-inden-5-one, octahydro | 25.296 | 150 | C ₁₀ H ₁₄ O | 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.

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