

1

2 **Comparison of the Chemical Compositions of Two Different Non-polar**
3 **Extracts of *Heliotropium sudanicum* Aerial Parts from Sudan**

4

5 **Abstract**

6 **Aim:** The aim of this work is to provide answers to impending research questions on
7 compounds present in petroleum ether and n-hexane extracts of the aerial parts of
8 *H.sudanicum*.

9 **Methodology:** Phytochemical analysis of plant extracts was performed by using GC/MS
10 . Various constituents were identified after matching their mass fragmentation pattern
11 with data available in GC/MS library of National Institute of Standards Technology
12 (NIST).

13 **Results:** Petroleum ether extract of *H. sudanicum* was characterized by abundance of
14 1,6,10,14,18,22- Tetracosahexaen-3-ol , 2,6,10,15,19,23-hexamethyl-, (all-E)- (33.99%) ,
15 Squalene (20.94%) , Phytol (7.62%) and Hexatriacontane (7.49%). The constituents of
16 the extract were fatty acid derivatives (15.24%) ,hydrocarbons (8.41%) and terpenoids
17 (28.56%). *H.sudanicum* n-hexane extract was characterized by abundance of 9,12-
18 Octadecadienoic acid (Z,Z)-, methyl ester (15.14%) , 9,12,15-Octadecatrienoic acid,
19 methyl ester (14.67%) , Cholesterol (11.12%) , Fucosterol (10.30%) , . gamma.-
20 Sitosterol (9.59%) and 1,6,10,14,18,22- Tetracosahexaen-3-ol, 2,6,10,15,19,23-
21 hexamethyl-, (all-E)- (7.73%).

22 **Conclusion:** The resulted compounds in this study provide evidence for the utilization of
23 the plant as alternative natural resource of medicinal and industrial interest. To best of
24 our knowledge this is the first report that directly compares the two extraction solvents
25 for active compounds from *Heliotropium sudanicum* aerial parts shows that petroleum
26 ether should be the solvent of choice .

27 **Keywords:** *Heliotropium sudanicum*; Boraginaceae; aerial parts; chemical composition;
28 non-polar solvents; Sudan

29 **1.INTRODUCTION**

30 The genus *Heliotropium* L. (Boraginaceae) with about 300 species all over the world. Is a
31 genus of herbs rarely shrubs. It is used in folk medicine for the treatment of gout,
32 rheumatism and as antiseptic and inflammatory agent. In Sudan, the whole herb is used for
33 animal wound healing [1,2,3]. It is one of the main sources of pyrrolizidine alkaloids
34 (PAs). PAs – containing plants have been recognized responsible for poisoning of

35 livestock and other ruminants [1,4-6] . *H. sudanicum* aerial parts was found by AL-Hassan
36 et al.,[2] contains alkaloids, coumarins, flavonoids, tannins and steroids/triterpenes.

37 The *H. sudanicum* F.W.Andr. plant is a branched annual herb . Stems sulcate ,hispid .
38 Leaves alternate , petiolate .sinuate , often of unequal base ,5 cm. long. Spikees often in
39 pairs . Corolla white , twice as long as calyx, pubescent outside .Stigma conical , thickened
40 at base .Nutlets glabrous [7]. In the stem transverse section . the anatomical structure of
41 stem⁶ were shows that the upper epidermis is a single layer of barrel-shaped dense
42 cytoplasm with conspicuous nuclei, it is found as one layer. The presence of hairs and
43 other epidermal outgrowths have been noted. The hypodermis consist of 2-3 layers of
44 collenchymatous cells. The cells of general cortex consist of parenchymatous cells in 4
45 layers. While in the leaf transverse section, the anatomical structure of leaf⁷ shows that the
46 epidermal cells are rectangular and show the presence of an external cuticular layer. The
47 upper epidermal cells are somewhat larger than the lower. Unicellular epiderma; trichomes
48 have been noted on both surfaces. Stomata is of anomocytic type and are distributed on the
49 upper and lower epidermis of the leaves. Palisade layers range from 2 to 4 . The main
50 vascular bundle is 1 The lateral vascular bundle are 4 [8].

51 The aim of the research is to compare chemical analysis of petroleum ether and n-hexane
52 extracts from *H.sudanicum* aerial parts through gas chromatography mass spectrometer
53 (GC/MS).

54

55 **2.MATERIALS AND METHODS**

56 **2.1 Plant Materials:**

57 Aerial parts from Heliotropium sudanicum were collected in April 2017 from Shambat
58 area (Khartoum State, Central Sudan). The studied species was taxonomically identified
59 by Prof. M. Kordofani (Department of Botany, Khartoum University) and Ustaz. H.Tag
60 EL-Sir (Department of Agricultural Botany, Khartoum University).

61

62 **2.2 Sample preparation :**

63 Each plant sample was carefully dried in the shade at room temperature. The aerial parts
64 were then ground to fine powder with a mechanical crusher and the powder was kept in
65 glass container at room temperature.

66 **2.3 Extraction:**

67 Petroleum ether and n-hexane were used as solvents of extraction. The powder was
68 extracted separately with 250 ml petroleum ether and n-hexane at room temperature for

69 24 hours. After extraction it was filtered and then the removal of solvent was done by
70 distillation device. Then the (70ml) extract was kept in glass container.

71 **2.4 Gas Chromatography Mass Spectrometer (GC/MS) :**

72 GC was equipped with MS (Shimatzo QP 2010 GC/MS instrument equipped with
73 reference libraries).packed material for column were 50% phenyl and 50% methyl
74 polysiloscane , column length 30 meter , diameter 0.025 mm, the flow rate of helium as
75 carrying gas was 1 ml/min .the temperature of program consisted of 60-270 C ,at rate of 4
76 C/min .MS were taken at ionization voltage 70 EV. Library search was carried out using
77 Wiley GC/MS library.

78 **2.4.1 Identification of components :**

79 Interpretation on mass spectrum GC-MS was conducted using the database of National
80 Institute Standard and Technology (NIST). The spectrum of the unknown component was
81 compared with the spectrum of the known components stored in the NIST library. The
82 name, formula, molecular weight and area % of the components of the test materials were
83 ascertained.

84 **3. RESULTS AND DISCUSSION**

85 **3.1 GC/MS Analysis of *H. sudanicum* Petroleum Ether extract:**

86 A total of 15 compounds representing 100% of the Petroleum Ether extract were
87 identified and is given in the Table 1. Quantitatively, the extract was characterized by
88 abundance of 1,6,10,14,18,22- Tetracosahexaen-3-ol , 2,6,10,15,19,23-hexamethyl-, (all-
89 E)- (33.99%) , Squalene (20.94%) , Phytol (7.62%) and Hexatriacontane (7.49%).

90 The identified compounds comprised many secondary metabolites as hydrocarbons ,
91 terpenes, phytosterol, Phenolic compounds and the majority is the fatty acids derivatives.
92 The major component was identified as a 1,6,10,14,18,22- Tetracosahexaen-3-ol,
93 2,6,10,15,19,23-hexamethyl-, (all-E)-(33.99%). Many methyl esters of fatty acids are
94 identified including Hexadecanoic acid,methyl ester, 9,12,15-Octadecatrienoic acid,
95 methyl ester, 9-Octadecenoic acid (Z)-,methyl ester , Methyl stearate.

96 The fatty acid methyl ester 9-Octadecenoic acid (Z)-,methyl ester (5.42%) known also as
97 Methyl oleate is used in Industry as Intermediates , lubricants and lubricant additives,
98 Solvents (which become part of product formulation or mixture). and the consumer use it
99 in ink, toner, colorant products lubricants , greases and water treatment products [9].

100 Many hydrocarbons are identified such as Heneicosane , Hexatriacontane and they are
101 acyclic alkanes (aliphatic hydrocarbon). The detected Phenolic compound was Phenol,
102 2,4-bis(1,1-dimethylethyl)- (0.16%) it is very toxic to aquatic organisms, and uses as a

103 chemical intermediate for the synthesis of UV stabilizers or antioxidants and a chemical
 104 intermediate for the synthesis of other chemical intermediates [10]. And also the
 105 triterpenoid, Squalene was detected .

106 The constituents of the extract were fatty acid derivatives (15.24%) ,hydrocarbons
 107 (8.41%) and terpenoids (28.56%).

108 Table 1. chemical compounds from *H. sudanicum* petroleum ether extract

| Sr. NO. | R.time | Name of the Compound | Other Name | Molecular Formula | Molecular weight | Area % |
|---------|--------|--|--|---|------------------|--------|
| 1 | 3.184 | Hexane,2-nitro | - | C ₆ H ₁₃ NO ₂ | 131.1729 | 3.81 |
| 2 | 11.194 | Phenol, 2,4-bis(1,1-dimethylethyl)- | 2,4-di-tert-butylphenol | C ₁₄ H ₂₂ O | 206.33 | 0.16 |
| 3 | 14.865 | 2-pentadecanone, 6,10,14-trimethyl- | Perhydrofarnesyl acetone | C ₁₈ H ₃₆ O | 268.4778 | 2.08 |
| 4 | 14.902 | 1-Hexadecanol,3,7,11,15-tetramethyl- | Dihydrophytol | C ₂₀ H ₄₂ O | 298.5469 | 1.72 |
| 5 | 15.672 | Hexadecanoic acid,methyl ester | Methyl palmitate | C ₁₇ H ₃₄ O ₂ | 270.4507 | 2.69 |
| 6 | 17.324 | 9-Octadecenoic acid (Z)-,methyl ester | oleic acid ester | C ₁₉ H ₃₆ O ₂ | 296.4879 | 5.42 |
| 7 | 17.380 | 9-Octadecenoic acid (Z)-,methyl ester | oleic acid ester | C ₁₉ H ₃₆ O ₂ | 296.4879 | 1.87 |
| 8 | 17.394 | 9,12,15-Octadecatrienoic acid,methyl ester | methyl octadeca-9,12,15-trienoate | C ₁₉ H ₃₂ O ₂ | 292.4562 | 4.45 |
| 9 | 17.493 | Phytol | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | C ₂₀ H ₄₀ O | 296.5310 | 7.62 |
| 10 | 19.072 | Methyl stearate | methyl octadecanoate | C ₁₉ H ₃₈ O ₂ | 298.511 | 0.81 |
| 11 | 19.072 | Heneicosane | - | C ₂₁ H ₄₄ | 296.583 | 0.92 |
| 12 | 20.684 | Tetratriacontyl heptafluorobutyrate | - | C ₃₈ H ₆₉ F ₇ O ₂ | 690.9421 | 5.95 |
| 13 | 23.207 | Squalene | - | C ₃₀ H ₅₀ | 410.73 | 20.94 |
| 14 | 23.612 | Hexatriacontane | - | C ₃₆ H ₇₄ | 506.988 | 7.49 |
| 15 | 23.953 | 1,6,10,14,18,22-Tetracosahexaen-3-ol, | (6E,10E,14E,18E)-2,6,10,15,19,23- | C ₃₀ H ₅₀ O | 426.729 | 33.99 |

| | | | | | | |
|--|--|--|--|--|--|--------|
| | | 2,6,10,15,19,23-hexamethyl-, (all-E)- | hexamethyltetracos -1,6,10,14,18,22- hexaen-3-ol | | | |
| | | | | | | 100.00 |

109

110 **3.2 GC/MS Analysis of *H. sudanicum* n-hexane extract:**

111 A total of 20 compounds representing 100% of the Petroleum Ether extract were
 112 identified and is given in the Table 2. Quantitatively, the extract was characterized by
 113 abundance of 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (15.14%) , 9,12,15-
 114 Octadecatrienoic acid, methyl ester (14.67%) , Cholesterol (11.12%) , Fucosterol
 115 (10.30%) , . gamma.-Sitosterol (9.59%) , 1,6,10,14,18,22- Tetracosahexaen-3-ol,
 116 2,6,10,15,19,23-hexamethyl-, (all-E)- (7.73%) , .beta.- Sitosterol (6.66%) and
 117 Hexadecanoic acid,methyl ester (5.05%).

118 The constituents of the extract were fatty acid derivatives (42.04%), phytosterols
 119 (37.67%) and terpenoids (5.52%).The identified compounds comprised many secondary
 120 metabolites as hydrocarbons , terpenes, phytosterol, vitamin and the majority is the fatty
 121 acids and their derivatives. The major component was identified as 9,12-Octadecadienoic
 122 acid (Z,Z)-, methyl ester (15.14%) the second major component is 9,12,15-
 123 Octadecatrienoic acid, methyl ester and they are fatty acid methyl ester.

124 The identified methyl esters of fatty acids are the same as that detected in the petroleum
 125 ether extract beside three different methyl esters , they are Methyl tetradecanoate,
 126 Eicosanoic acid, methyl ester, and Docosanoic acid, methyl ester.

127 Many phytosterols (plant sterols) were detected and they are .gamma.-Sitosterol, .beta.-
 128 Sitosterol, Cholesterol, Fucosterol. .beta.-Sitosterol and these may have the potential for
 129 prevention and therapy for human cancer, used to make medicine, used for heart disease
 130 and high cholesterol and the common cold and flu (influenza). Cholesterol it is the major
 131 precursor for the synthesis of vitamin D of the various steroid hormones. One vitamin
 132 was detected it is Vitamin E (alpha tocopherol), and it's has antioxidant activity, It may
 133 also have anti-atherogenic, antithrombotic, anticoagulant, neuroprotective, antiviral,
 134 immunomodulatory, cell membrane-stabilizing and antiproliferative actions.

135

136

137 Table 2. chemical compounds from *H.sudanicum* n-hexane extract :

| Sr. NO. | R.time | Name of the Compound | Other Name | Molecular Formula | MW | Area % |
|---------|--------|--|--|--|----------|--------|
| 1 | 13.571 | Methyl tetradecanoate | Methyl myristate | C ₁₅ H ₃₀ O ₂ | 242.403 | 0.22 |
| 2 | 14.864 | 2-pentadecanone, 6,10,14-trimethyl- | Perhydrofarnesyl acetone | C ₁₈ H ₃₆ O | 268.4778 | 0.47 |
| 3 | 14.900 | 6,10,14- Trimethyl-pentadecan-2-ol | - | C ₁₈ H ₃₈ O | 270.501 | 0.33 |
| 4 | 15.671 | Hexadecanoic acid,methyl ester | Methyl palmitate | C ₁₇ H ₃₄ O ₂ | 270.4507 | 5.05 |
| 5 | 17.325 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | Methyl linolate | C ₁₉ H ₃₄ O ₂ | 294.4721 | 15.14 |
| 6 | 17.380 | 9-Octadecenoic acid (Z)-,methyl ester | oleic acid ester | C ₁₉ H ₃₆ O ₂ | 296.4879 | 4.04 |
| 7 | 17.397 | 9,12,15-Octadecatrienoic acid, methyl ester | methyl octadeca-9,12,15-trienoate | C ₁₉ H ₃₂ O ₂ | 292.4562 | 14.67 |
| 8 | 17.491 | Phytol | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | C ₂₀ H ₄₀ O | 296.5310 | 1.20 |
| 9 | 17.584 | Methyl stearate | methyl octadecanoate | C ₁₉ H ₃₈ O ₂ | 298.511 | 2.04 |
| 10 | 18.731 | .gamma.-Sitosterol | Clionasterol | C ₂₉ H ₅₀ O | 414.7067 | 9.59 |
| 11 | 18.886 | .beta.- Sitosterol | - | C ₂₉ H ₅₀ O | 414.7067 | 6.66 |
| 12 | 19.003 | Cholesterol | - | C ₂₇ H ₄₆ O | 386.664 | 11.12 |
| 13 | 19.341 | Eicosanoic acid,methyl ester | Methyl eicosanoate | C ₂₁ H ₄₂ O ₂ | 326.5570 | 0,45 |
| 14 | 19.939 | Fucosterol | - | C ₂₉ H ₄₈ O | 412.702 | 10.30 |
| 15 | 20.963 | Docosanoic acid,methyl ester | Methyl Behenate | C ₂₃ H ₄₆ O ₂ | 354.6101 | 0.88 |
| 16 | 21.087 | Stigmast-5-en-3-ol,oleate | | C ₄₇ H ₈₂ O ₂ | 679.171 | 1.98 |
| 17 | 21.764 | Vitamin E | alpha tocopherol | C ₂₉ H ₅₀ O ₂ | 430.717 | 2.51 |
| 18 | 23.202 | Squalene | - | C ₃₀ H ₅₀ | 410.73 | 4.36 |
| 19 | 23.607 | Hexatriacontane | - | C ₃₆ H ₇₄ | 506.988 | 1.27 |
| 20 | 23.949 | 1,6,10,14,18,22-Tetracosahexaen-3-ol, | (6E,10E,14E,18E)-2,6,10,15,19,23- | C ₃₀ H ₅₀ O | 426.729 | 7.73 |

| | | | | | | |
|--|--|--|--|--|--|--------|
| | | 2,6,10,15,19,23-hexamethyl-, (all-E)- | hexamethyltetracos -1,6,10,14,18,22- hexaen-3-ol | | | |
| | | | | | | 100.00 |

138

139 CONCLUSION

140 To best of our knowledge this is the first report that directly compares the two extraction
 141 solvents for active compounds from *Heliotropium sudanicum* aerial parts shows that
 142 petroleum ether should be the solvent of choice . GC-MS analysis result of large
 143 Quantities of 1,6,10,14,18,22-tetracosahexaen-3-ol,2,6,15,19,23-hexamethyl-,(all-E),
 144 squalene, phytol and hexatriacontane were extracted by petroleum ether and weren't
 145 extracted by n-hexane. 9,12,15-octacatrienoic acid,methyl ester and hexadecanoic acid,
 146 methyl ester were found in n-hexane extract in a large quantity compared by the
 147 extraction quantity in petroleum ether extract.

148 **CONFLICTS OF INTEREST:** Authors have declared that no competing interests
 149 exist.

150 References:

- 151 1. Goyal N,Sharma SK. (2014) . Bioactive phytoconstituents and plant extracts from
 152 genus *Heliotropium* . Int J green pharm 2014;8(4)217-225
- 153 2. AL-Hassan M.S., Koko W.S., Khalid H.S. (2018). Evaluation of antimicrobial ,
 154 antioxidant and phytochemical screening of some Sudanese medicinal plants.
 155 International Journal of Multidisciplinary Research and Development. 5(3): 35-39.
- 156 3. Abdalla W.E., Abdalla E.M. (2016). Promising Sudanese Medicinal Plants with
 157 antibacterial acitivity - a Review Article. Biological Forum- An International Journal
 158 8(1): 299-323.

- 159 4. El-Shazly A ,Wink M . diversity of pyrrolizidine alkaloids in Boraginaceae
160 structures, distribution and biological properties . Diversity.2014;6(2):188-282
- 161 5. Fu PP Xia Q ,Lin G, Chou MW. Genotoxic pyrrolizidine alkaloids-mechanisms
162 leading to DNA adduct formation and tumorigenicity . Int J Mol Sci 2002;3(9):948-
163 964.
- 164 6. Wiedenfeld H, Edgar J. Toxicity of pyrrolizidine alkaloidsto humans and ruminants .
165 Phytochem Rev 2011;10(1):137-151.
- 166 7. Tawfig A.A (2018). Comparison of the chemical constituents of two species of
167 Heliotropium (Family : Boraginaceae) from Sudan. M.Sc. Thesis. Omdurman Islamic
168 University.
- 169 8. Ahmed H.O., Kordofani M.A. (2012). Leaf and stem anatomy of five species from
170 the genus Heliotropium L. (Boraginaceae) in Sudan. Journal of Chemical and
171 Pharmaceutical Research, 4(10): 4575-4581.
- 172 9. <https://pubchem.ncbi.nlm.nih.gov/compound/9-Octadecanoic> acid (Z),methyl ester/
173 10. <https://pubchem.ncbi.nlm.nih.gov/compound/Phenol> 2,4-bis(1,1-dimethylethyl)-/