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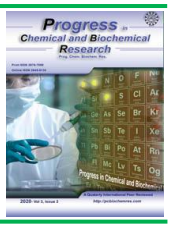

**Original Research Article**

# Phytochemical Analysis, Antibacterial and antioxidant Activities of Essential Oil from Hibiscus sabdariffa (L) Seeds, (Sudanese Karkadi)

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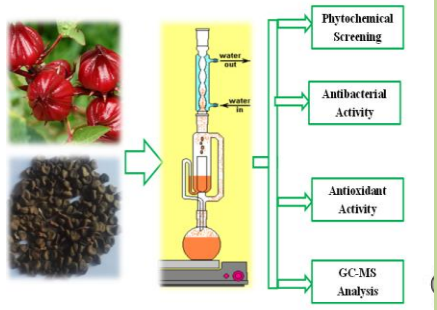
## GRAPHICAL ABSTRACT

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## ABSTRACT

In this study we investigated the chemical constituents, phytochemical screening of the essential oil from *H. sabdariffa (L*) Seeds, (Sudanese Karkadi) and evaluated its potential antibacterial and antioxidant activities, using Soxhlet method to extract the essential oil. The chemical constituents of *H. Sabdariffa (L*) Oil were identified and quantified by GC-MS, where DPPH and paper, disc diffusion assay were employed to evaluate the antioxidant and antibacterial activities respectively. Phytochemical screening showed that Alkaloids, Flavonoids, Carbohydrates, Saponins, Triterpens, Streols, Tannins and phenolic compounds are present in seeds of the *H. Sabdariffa*. Thirty eight components have been identified which classified in to four categories; 31 fatty acid ester derivatives , the majority of them are; Hexadecanoic acid, methyl ester (16.94%), 9,12- Octadecadienoic acid (Z,Z), methyl ester (21.93%), 9, -Octadecadienoic acid (Z), methyl ester (30.11%), methyl stearate(7.39%), Cyclopropaneoctanoic acid (3.17 %), Dotriacontane(2.17 %), two phenolic derivatives; 1,3-Benzodioxole, 4methoxy-6-(2-propenyl) (0.01 %) and Apiol (0.04 %), two steroidal derivatives; 17-Androstannone, 3-(3, 4-dimethylphenyl) (1.81%) and Stigmasta-4,7,22-trien-3.beta.-ol (0.41%) and three pentacyclic triterpenes derivatives; Beta.-Amyrin (3.82%), Alpha.-Amyrin (1.65 %) Urs-12-en-3-ol, acetate,(3.beta.) (1.17%). The DPPH assay, showed moderate antioxidant potential (50 ± 0.01 compared with standard 89 ± 0.01; the antibacterial showed high inhibitory effect against Bacillus subtilis (13mm). In conclusion, the study showed that the Oil of *H. sabdariffa* seed is a good source of antioxidants due to the presence of phenolic compounds, also is a potential source of natural antibacterial, and justify its uses in folkloric medicines.



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## INTRODUCTION

*Hibiscus sabdariffa* L is commonly known as Roselle (English) and Karkadeh (Arabic). It is an annual tropical short shrub and distributed in many tropical and sub-tropical regions in the world [1]. It is used traditionally for many purposes, such as hot and cold beverage, flavoring agent, food industry and traded as herbal medicine [2], It also holds a plentiful potential of phytochemical compounds and has antioxidant, hypertensive, hypocholesterolemic, immune-modulated, hepatoprotective, renoprotective, diuretic, anti-obesity, antiurolithic, antidiabetic, antimicrobial and anticancer properties without any significant genotoxic effects [3]. *H. sabdariffa* (L) is a famous public beverage in Sudan employed traditionally for the treatment of many ailments, such as respiratory tract infections, colds, fevers, hypertensions and malaria [4]. The previous study showed that it has significant antibacterial activity against Staphylococcus aureus, Staphylococcus epidermidis, Salmonella enterica, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris and Bacillus cereus [4, 5]. The aims of the present study are to evaluate the phytochemical screening, investigate the chemical constituents of the Fixed Oil from Sudanese *H. sabdariffa* seeds and to evaluate its potential Materials and methods.

### Plant material

*H. Sabdariffa* (L) seeds were purchased from a local market in Bahri city, Sudan, and it was identified at the herbarium of the Aromatic and Medicinal Plants Research Institute.

### Extraction of oil:

100 g of the seeds was grounded into a fine powder. Powdered seeds were extracted with n-hexane using Soxhlet extractor for six hours. The volume of hexane was reduced under reduced pressure. The oil of *H. sabdariffa* (L) was obtained by evaporating the reduced hexane by air drying in a steady current. The oil was kept in a refrigerator for further manipulation. The extract was screened for the presence of phenolic compounds, flavonoids, tannins, terpenoids, saponins, alkaloids and carbohydrates using standard methods [6].

### GC /MS method:

The qualitative and quantitative analysis of the sample was carried out by using GC MS technique model (GC /MS-QP2010-Ultra) from japan "Simadzu Company, with capillary column (Rtx-5ms -30 m × 0.25 mm ×0.25 µm). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60°C with rate 10°C /min to 300°C as final temperature degree, the injection port temperature was 300°C, the ion source temperature was 200°C and the interface temperature was 250°C. The sample was analyzed by using scan mode in the range of m/z 40 – 550 charge to ratio. The identification of components in the sample were achieved by comparing their retention times and mass fragmentation with those available in the library, the National Institute of Standards and Technology (NIST) and the results were recorded.

### Antimicrobial assay:

**Disc diffusion method**:

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines [7]. Bacterial suspension was diluted with sterile physiological solution to 108cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

**Antibacterial assay:**

**DPPH radical scavenging assay**:

The DPPH radical scavenging was determined according to the method of [8] with some modification. In 96 –well plate, were allowed to react with 2, 2 Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37 °C. The concentration of DPPH was kept as (300µL M). The test sample was dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using a mutilate reader spectrophotometer. Percentage radical scavenging activity of the sample was determined in comparison with DMSO treated control group all testes and analysis were run in triplicate.

**Results and Discussions:**

The preliminary phytochemical screening of *H. sabdariffa* seeds ethanolic extracts showed the presence of various secondary metabolites such as, Alkaloid, carbohydrate, flavonoid, saponins, triterpenes, sterol, and tannins which are the bioactive principles responsible for medicinal values of the respective plants were all present. These phytoconstituents were detected in varied concentrations in the methanol extracts were presented in **Table 1.**

The essential oil extracted from *Hibiscus sabdariffa* seeds was investigated by GC-MS analysis. The identification of the oil constituents was based on interpretation of the mass spectra fragmentation data, matching their mass spectra with Wiley 9.0 and NIST databases, and by comparison of the mass spectra obtained with those reference compounds published in the literature.

**Table 1:** Phytochemical screening of *Hibiscus sabdariffa* seed extract

| No | Constituents | Test | Results |
|---|---|---|---|
| **1** | Alkaloids | Mayer's,Wanger's reagent | +++ |
| **2** | Flavonoid | Alkaline reagent | +++ |
| **3** | Carbohydrate | Molish's | + |
| **4** | Saponins | Forth | + |
| **5** | Triterpen,Streol | Liberman | +++ |
| **6** | Tannins,phenolic | Ferric hloride,/Alminum chloride | +++ |

(+++)-Heavy ;( ++)-Medium; (+)-Low ;(-) -indicates absent.

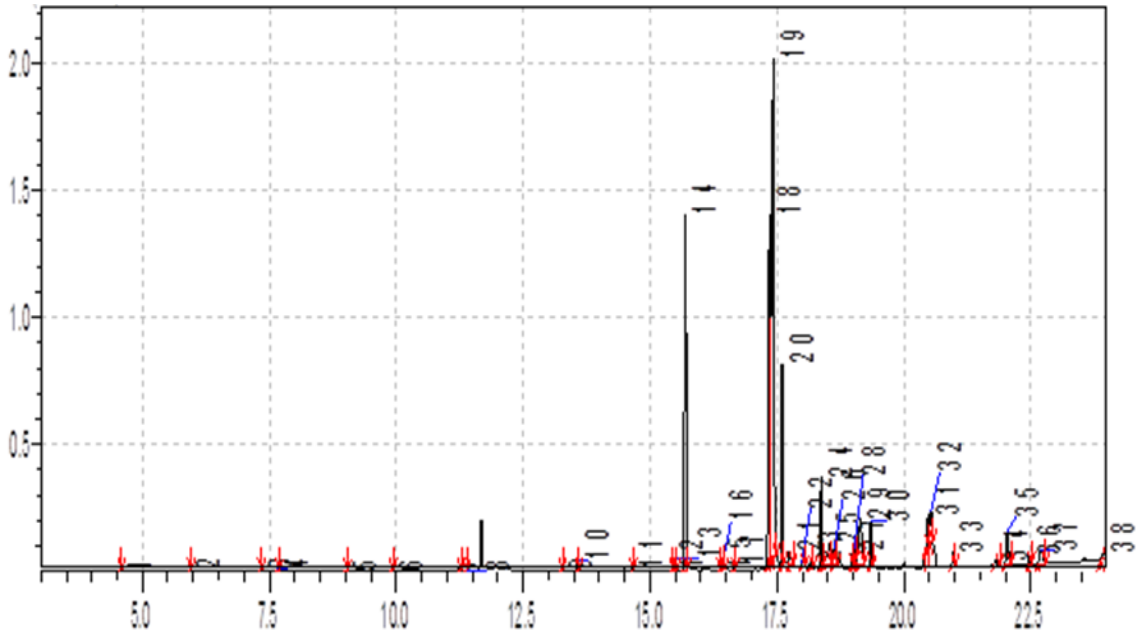


**Fig. 1.** The typical GC chromatogram of Hibiscus sabdariffa seeds oil

The typical GC chromatogram was presented in **Fig. 1,** which revealed the presence of thirty eight components which were presented in **Table. 2.**

The identified compounds were classified into four categories; 31 fatty acid ester derivatives**,** six of them were found to be Major, numbered as compound 14,1 8, 19, 20, 24 and 35 with peak area 16.94%, 21.93%, 30.11%, 7.39%, 3.17 % and 2.17 % respectively. Compound 14 with the retention time of 15.699 min, the spectrum of fragmentation ion mass spectra m/z at 270 $[M]^+$ in its mass spectra corresponded to an elemental composition $C_{17}H_{34}O_2$. Molecular ion of $C_{17}H_{34}O_2$ experiences fragmentation by releasing $C_3H_7$ radical and produces fragments by m/z 227 originating from $C_{14}H_{27}O_2$, while the bottom peak lies in m/z 74 originating from $C_3H_6O_2$. This compound was identified as Hexadecanoic acid, methyl ester; by comparing it MS data with reference [9] which were in full agreement, it is wide-spread in plants and humans. This acid finds applications in soap and cosmetics industries. It is also used in food industry [10]. Compound 18, showed molecular ion m/z at 294 $[M]^+$ corresponded to molecular formula $C_{19}H_{34}O_2$ and 263 [M-31], also showed a rising peak at *m/z* 220 $[M-74]^+$, by direct comparison with 9, 12-Octadecadienoic acid (Z,Z)-, methyl ester reported in [9, 11] was full agreement.

This fatty acid ester is implicated in human physiology and pathology, deficiency of linolate caused hair loss and poor wound healing in model animals [11, 12]. The results of GC-MS analysis of Compound 19 indicate that the retention time required is 17.428 min. The mass spectrum of compound 19 is written by m/z 296 originating from the molecular ion of $C_{19}H_{36}O_2$.

**Table 2.** Chemical constituents of *Hibiscus sabdariffa* (L) Seeds Oil

| **Peak report TIC** | | | | | |
|---|---|---|---|---|---|
| **Compound** | RT | M+ | BP | Key fragment ions | Name of compound |
| **1** | 4.567 | 284 | 74 | 241 183 143 129 87 55 | Heptanoic acid ,methyl ester |
| **2** | 5.931 | 158 | 74 | 127 115 101 87 57 43 41 | Octanoic acid ,methyl ester |
| **3** | 7.326 | 172 | 74 | 141 129 101 59 41 27 | Nonanoic acid ,methyl ester |
| **4** | 7.652 | 168 | 74 | 128 114 108 94 67 43 41 | 8-Nonynoic acid ,methyl ester |
| **5** | 9.014 | 196 | 74 | 122 107 95 67 55 41 | 10-Undecenoic acid ,methyl ester |
| **6** | 9.911 | 198 | 74 | 124 98 87 69 55 41 | 10-Undecynoic acid ,methyl ester |
| **7** | 11.253 | 214 | 74 | 214 143 87 59 50 | Dodecanoic acid ,methyl ester |
| **8** | 11.369 | 192 | 192 | 161 147 131 119 103 91 65 | 1,3-Benzodioxole,4methoxy-6-(2-propenyl)- |
| **9** | 13.245 | 222 | 222 | 207 191 177 149 121 91 77 | Apiol |
| **10** | 13.570 | 242 | 74 | 199 143 129 87 55 | Methyl tetradecanoate |
| **11** | 14.644 | 256 | 74 | 213 143 87 57 41 | Pentadecanoic acid , methyl ester |
| **12** | 15.436 | 268 | 55 | 236 194 152 123 96 74 69 | Methyl hexadec-9-enoate |
| **13** | 15.479 | 268 | 55 | 236 194 152 123 96 74 69 41 | 9- Hexadecanoic acid, methyl ester, (Z)- |
| **14** | 15.699 | 270 | 74 | 227 199 143 129 87 57 43 | Hexadecanoic acid, methyl ester |
| **15** | 16.375 | 280 | 67 | 248 150 136 109 95 81 55 | Methyl 9,12-heptadecadienoate |
| **16** | 16.428 | 282 | 55 | 208 166 152 97 74 69 41 | Cis-10- heptadecenoic acid, methyl ester |
| **17** | 16.651 | 144 | 74 | 113 87 59 43 41 27 | Heptadecanoic acid , methyl ester |
| **18** | 18.358 | 294 | 67 | 150 136 123 109 95 81 55 41 | 9, 12-Octadecadienoic acid (Z,Z)-, methyl ester |
| **19** | 17.428 | 296 | 55 | 264 222 207 180 166 111 55 | 9, -Octadecenoic acid (Z) -, methyl ester |
| **20** | 17.601 | 298 | 74 | 225 199 143 129 115 87 55 43 | Methyl stearate |
| **21** | 17.744 | 282 | 55 | 264 222 151 97 83 69 42 | Oleic acid |
| **22** | 17.989 | 292 | 79 | 236 149 135 108 95 67 55 41 | 9,12,15-Octadecatrienoic acid ,methyl ester |
| **23** | 18.159 | 294 | 67 | 263 109 95 81 55 | Methyl 10-trans,12-cis-octadecadienoate |
| **24** | 18.367 | 310 | 55 | 278 226 194 139 123 97 74 69 | Cyclopropaneoctanoic acid |
| **25** | 18.534 | 392 | 392 | 377 359 187 159 146 133 119 | 17-Androstannone, 3-(3, 4-dimethylphenyl) |
| **26** | 18.624 | 292 | 79 | 261 163 150 135 107 93 67 | Methyl 9.cis. 11. Trans.t,13.trans. octadecatrienoate |
| **27** | 18.991 | 366 | 99 | 280 247 193 151 136 96 81 | PGH1,methyl ester |
| **28** | 19.023 | 292 | 79 | 194 175 150 121 107 93 67 | gamma,-Lionlenic acid, methyl ester |
| **29** | 19.143 | 426 | 218 | 365 203 189 175 135 95 69 | Beta.-Amyrin (3.82%) |
| **30** | 19.344 | 326 | 74 | 295 283 143 87 57 43 41 | Eicosanic acid methyl ester |
| **31** | 20.475 | 426 | 218 | 203 135 107 95 81 69 55 41 | Alpha.-Amyrin |
| **32** | 20.534 | 424 | 218 | 405 203 189 135 122 109 95 | 4,4,6a,6b,8a,11,12,14b-Octamethyl-1,4,4a. |
| **33** | 20.963 | 354 | 74 | 255 199 143 87 57 43 41 | Docosanoic acid , methyl ester |
| **34** | 21.815 | 410 | 392 | 392 377 253 157 143 95 8355 | Stigmasta-4,7,22-trien-3.beta.-ol |
| **35** | 22.034 | 450 | 57 | 127 113 99 85 71 43 41 | Dotriacontane |
| **36** | 22.466 | 382 | 74 | 339 283 143 87 55 41 18 | Tetracosanoic acid, methyl ester |
| **37** | 22.704 | 468 | 218 | 453 203 189 135 120 93 69 43 | Urs-12-en-3-ol, acetate,(3.beta.) |
| **38** | 23.923 | 466 | 225 | 243 155 141 97 71 57 43 | Hexadecane ,1,1-oxybis- |

The molecular ion of $C_{19}H_{36}O_2$ releases $C_3H_6O_2$ radical and produces fragments by m/z 222 originating from $C_{16}H_{30}$ which then releases $CH_3$ radical which forms the peak of m/z 207 originating from $C_{15}H_{27}$. The peak of m/z 180 originating from $C_{13}H_{24}$ which undergoes fragmentation by releasing $CH_2$ radical produces peak by m/z 166. Molecular ion of $C_{19}H_{36}O_2$ can also experience fragmentation by

releasing $CH_3OH$ and it produces the peak within m/z 264 originating from $C_{18}H_{32}O$. The peak with m/z 111 originating from $C_7H_{11}O$ releases $CH_2$ radical and produces m/z 97 originating from $C_6H_9O$; while the bottom peak originates from $C_4H_7^+$ with m/ z 55. According to the above justification this compound identified as methyl 9-octadecanoate [9]. It is a common monounsaturated fat in the human diet. It may be responsible for the hypotensive potential of olive oil [13]. It has some applications in soap industry and it is used in small amounts as excipient in pharmaceutical industries, also used as soldering flux in stained glass work and employed as emollient [14]. Compound 20, appeared at 17.601 min. It mass spectrum of was written by m/z 298 originating from the molecular ion of $C_{19}H_{38}O_2$, this molecular ion experiences radical fragmentation by releasing $C_3H_7$ radical and produces fragments by m/z 225 originating from $C_{16}H_{31}O_2$. The peak of m/z 129 originating from $C_7H_{13}O_2$ þ resulted from $C_{16}H_{31}O_2$ þ which releases $C_5H_{10}$. Molecular ion of $C_7H_{13}O_2$ þ experiences McLafferty reorganization by releasing $C_3H_6O_2$ and it produces the peak within m/z 55 originating from $C_4H_7$, while the peak of m/z 87 comes from $C_4H_7O_2$ originating from the fragment of $C_{19}H_{38}O_2$ þ releasing $C_{15}H_{31}$ radical. The bottom peak lies in m/z 74 originating from $C_3H_6O_2$ takes its form since it undergoes McLafferty reorganization. This fatty acid ester was identified as methyl stearate (methyl octadecanoate) by comparing it MS data with reference [15] which were in full agreement. Compound 24, appeared at 18.367 min was produced molecular ion m/z at 310 $[M]^+$ in its mass spectra corresponded to an elemental composition $C_{20}H_{38}O_2$, by direct comparison with Cyclopropaneoctanoic acid reported in [16] was full agreement. Compound 35, appeared at 18.367 min, has Ms Ions *m/z* 450 [M] + correspond to formula $C_{32}H_{66}$, by direct comparison with a NIST spectrum of n-dotriacontane was full agreement

The chromatogram also showed three phenolic derivatives numbered as 8, 9 and 25, with peak area (0.01 %), (0.04 %) and (1.81%) respectively. Compound 8 appeared at 11.369 min was produced molecular ion m/z at m/z 192 M+ $[C_{11}H_{12}O_3]^+$. The signal at m/z 177 is due to the loss of a methyl function. While compound 9 appeared at R.T. 13.428 min in total ion chromatogram, mass spectrum showed peak at m/z 222 which corresponds $M^+$ $[C_{12}H_{14}O_4]^+$. The peak at m/z 207 corresponds to loss of a methyl function and the peak at m/z 191 corresponds to loss of a methoxyl function. These two compounds compared their spectra, respectively with 1,3 Benzodioxole, 4methoxy-6-(2-propenyl and Apiol reported in reference [17] were well matched. Two compounds 25 and 34 were appeared in 18.534 and 21.185min which showed intense fragment ions at *m/z* 392, *m/z*410 in their mass spectrometry respectively, similar to those characteristic of the Steroidal of the 17-Androstannone, 3-(3, 4-dimethylphenyl) and Stigmasta-4, 7,22-Turin-3. Beta -ol. The GC-Ms chromatogram showed three pentacyclic triterpenes derivatives numbered as 29, 31 and 37, with the peak area with area (3.82%), (1.65%) and (0.01 %) respectively, they were appearing in 19.143, 20.475 and 20.963. The mass spectrum of compound 13, 16, 19 and 21 are seem to be similar, with the $M^+$ at *m/z* 426, 426 and 468, corresponded to molecular formula $[C_{30}H_{50}O]^+$, $[C_{30}H_{50}O]^+$, and $[C_{32}H_{52}O_2]^+$ respectively, the differentiation between oleanane- and ursane characterized by a base peaks at *m/z* 189, 203 and 218 can be related to the typical

fragmentation pathway of oleanane (β-Amyrine) and ursine (α-Amyrine) -type molecules with a double bond in position 12, as reported in [18], their mass spectra are also good fits with those reported in [19]. According to the above justification these compounds identified as β.-Amyrin, α-amyrin and Ursa-12-en-3β-yl acetate.

The Oil of Hibiscus sabdariffa (L) showed a high inhibitory effect (13mm) against Bacillus subitus compared with standard Ampicillin. The observation results were recorded in **Table 3**. According to DPPH scavenging activity, the seeds oil showed moderately antioxidant potential 50 ± 0.01 µg/ml activities comparable to that of Propyl Gallate (Standard) 89 ± 0.01 µg /mL against DPPH. **Table 4.**

**Table 3.** Antibacterial activity of *Hibiscus sabdariffa* (L) seeds oil

| **Sample conc. 100 µl/ml** | **Zone of inhibition (mm)** | | | | |
|---|---|---|---|---|---|
| | **Bs.** | **Ps.** | **Ba.** | **Ec.** | **Ca** |
| ***Hibiscus sabdariffa* seeds oil** | - | - | 13 | - | - |
| **Ampicillin** | 16 | 15 | - | 13 | - |

**Table 4.** The antioxidant activity of *Hibiscus sabdariffa (L)* seeds oil

| **Sample Code** | **%RSA±SD (DPPH) µg/ml** |
|---|---|
| ***Hibiscus sabdariffa* seeds oil** | 50 ± 0.01 |
| **Propyl Gallate (Standard )** | 89 ± 0.01 |

## CONCLUSION

The results showed that the essential oil extracted from *Hibiscus sabdariffa* (L) seeds rich with various fatty acids derivatives, phenolic compounds, Steroidal derivatives and pentacyclic triterpenes. The existence of these bioactive chemical compounds proved the use of this plant for various ailments by traditional medical practitioners. The H. *Sabdariffa* (L) seeds essential oil has significant antibacterial and antioxidant capacity, suggesting to use in flavoring agent, food industry and medicinal purposes.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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