**International Journal of Research in Pharmacy and Pharmaceutical Sciences**
**www.pharmacyjournal.in**
**ISSN: 2455-698X**
**Received: 25-03-2022, Accepted: 11-04-2022, Published: 26-04-2022**
**Volume 7, Issue 2, 2022, Page No. 15-19**

# Chemical composition and antimicrobial activity of oil from *Gardenia ternifolia* Schum & Thonn grown in Sudan

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**Abstract**
This study was carried out to investigate the constituents and antimicrobial activity of *Gardenia ternifolia*.. *Gardenia ternifolia* Schum. & Thonn. is a small tree or shrub in the family Rubiaceae..GC- MS analysis of *Gardenia ternifolia* oil revealed the presence of 14 constituents dominated by i) 9,12-octadecadienoic acid (Z,Z), methyl ester (17.87 % ) ii) stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)- (12.66%) iii)oleic acid(8.50%) iv) hexadecanoic acid methyl ester (7.37%) and v)n-hexadecanoic acid(7.33%). The oil was screened for antimicrobial activity against five standard human pathogens: *Bacillus subtilis* (G+ve), *Staphylococcus aureus(*G+ve*), Escherichia coli*(G-ve), *Pseudomonas aeroginosa*(G-ve) and the fungal species *Candida albicans. Gardenia ternifolia* oil showed significant activity against *Pseudomonas aeroginosa*, *Staphylococcus aureus* and *Escherichia coli.*

**Keywords:** *Gardenia ternifolia*, oil, constituents, antimicrobial activity

## Introduction
The genus *Gardenia* comprises around 140 species distributed in Asia, Africa, Hawaiian Islands and western Pacific region [1]. *Gardenia ternifolia* Schum. & Thonn. Is a small tree or shrub in the family Rubiaceae.Three infra specific taxa of *Gardenia ternifolia* are known [2]. The bark of *Gardenia ternifolia* is yellow-grey; leaves are in whorls of three, clustered near the ends of branches [3]; flowers are white-yellow and fruits are oval [4].Young leaves are taken by some African communities as a leafy vegetable , while branches are used as toothbrush. Roots of *Gardenia ternifolia* are aphrodisiac and anticancer. They are used traditionally against infertility, headache, hernia, menstrual pain, jaundice, ulcerative lymphangiectasis and snake bite [3, 5-9]. Stem is a natural remedy for measles [3, 5, 9]. Bark is prescribed by herbalists for epilepsy and convulsions. Bark is said to boost the immune system [10, 11]. Leaves are used in phytotherapy against fever, gastrointestinal disorders, skin infections, diabetes, syphilis, asthma and tuberculosis [5, 6, 12-14]. Fruits are used traditionally against diarrhea dysentery, haemorrhoids, eye diseases, toothache and trypanosomiasis [13, 14]. It has been reported that *Gardenia ternifolia* possesses antibacterial [13], antiviral [15], antioxidant [16, 17], hepatoprotective [18], antiplasmodial [19], larvidical [9] and cytotoxic [10] activities.

## Materials and Methods
### Materials
### Plant material
Seeds of *Gardenia ternifolia* were collected from a forest reserve around Damazin -Sudan. The plant was authenticated by the Department of Phytochemistry and Taxonomy, Medicinal and Aromatic Plants Research Institute, Khartoum-Sudan.

### Instruments
GC-MS analysis was conducted on a Shimadzo GC-MS-QP2010 Ultra instrument with RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μm, thickness)**.**

### Test organisms
The studied oil was screened for antibacterial and antifungal activities using the standard microorganisms: *Bacillus subtilis* (G+ve), *Staphylococcus aureus (*G+ve*), Escherichia coli* (G-ve), *Pseudomonas aeroginosa*(G-ve) and the fungal species *Candida albicans.*

### Methods
### Extraction of oil
Powdered seeds of studied plant (400g) were exhaustively extracted with n-hexane by maceration.The solvent was removed under reduced pressure and the oil was kept in the fridge at 4ºC for further use.

**GC/MS Conditions**

The qualitative and quantitative analysis of the sample was carried out by using GM/MS technique model (GC/MS-QP2010-Ultra) from japan 'Shimadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm).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 10cm/min to 300° C as final temperature with 3 minutes hold time , 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 40-500 charges to ratio and the total run time was 26 minutes. Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST)..

**Antimicrobial activity**

Bacterial growth was maintained on nutrient agar.The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about $10^8$-$10^9$colony forming units per ml. The average number of viable organism per ml of the stock suspension was determined. Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours. Fungal cultures were maintained on Sabouraud dextrose agar incubated at 25°C for 72h.The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

**Testing for antimicrobial activity**

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antimicrobi activity of the oil. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes, the agar was left to settle and in each of these plates - which were divided into two halves - two cups in each half (6 mm in diameter) were cut using sterile Cork borer (No 4), each one of the halves was designed for a test sample. Separate Petri dishes were designed for the positive control (ampicillin and gentamicin). The agar discs were removed, alternate cup were filled with 0.1 ml of test sample and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured as an average of two replicates. The same procedure was adopted for antifungal activity using Sabouraud dextrose agar.

**Results and Discussion**

**GC-MS analysis**

GC/MS was conductud for *Gardenia ternifolia* oil. The analysis revealed the presence of 14 components - Table (1).The total ions chromatogram is presented in Fig.1.

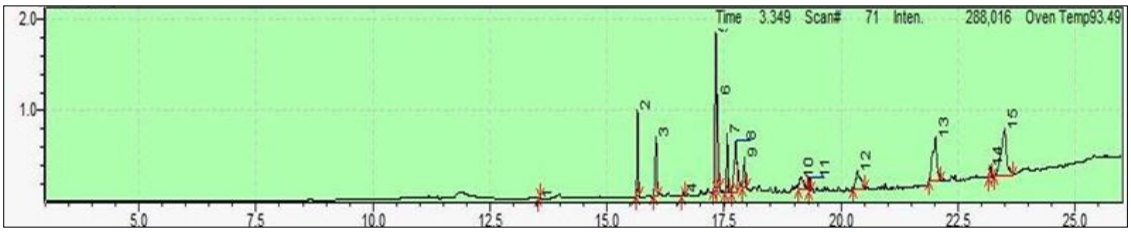


**Fig 1:** Total ions chromatogram

The following compounds were detected in the chromatograms as major constituents:

1. 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (17.87 % )
2. Stigmast-7-en-3-ol, (3. beta., 5. alpha., 24S)- (12.66%)
3. Oleic acid (8.50%)
4. Hexadecanoic acid methyl ester (7.37%)
5. Hexadecanoic acid (7.33%).

The GC-MS analysis showed a mass spectrum (Fig.2) identical with 9, 12-octadecadienoic acid methyl ester. The peak at m/z 294 (RT. 18.164) corresponds $M^+$ $[C_{19}H_{34}O_2]$ $^+$. It also showed a mass spectrum (Fig.3.) characteristic of stigmast-7-en-3-ol, (3. beta., 5. alpha., 24S)-.The signal at m/z454 (RT.22.022) accounts for the molecular ion: $M^+[C_{31}H_{50}O_2]$. The analysis revealed a mass spectrum (Fig.4) identical with the spectrum of oleic acid.The peak at m.z282 is due to:$M^+[C_{18}H_{34}O_2]^+$.The mass spectrum of hexadecanoic acid methyl ester(Fig.5) showed a peak at m/z 270(RT. 16.416) accounting for: $M^+$ $[C_{17}H_{34}O_2]^+$.

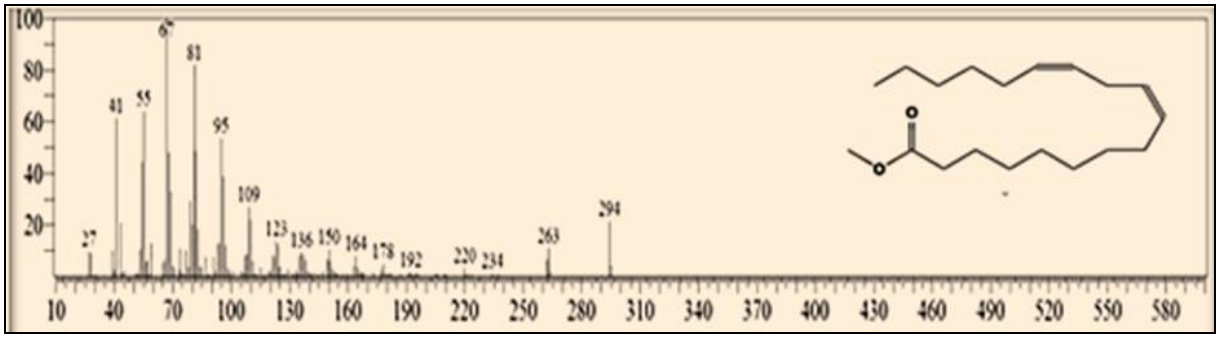

**Fig 2:** mass spectrum of 9.12-octadecadienoic acid (Z, Z)-, methyl ester

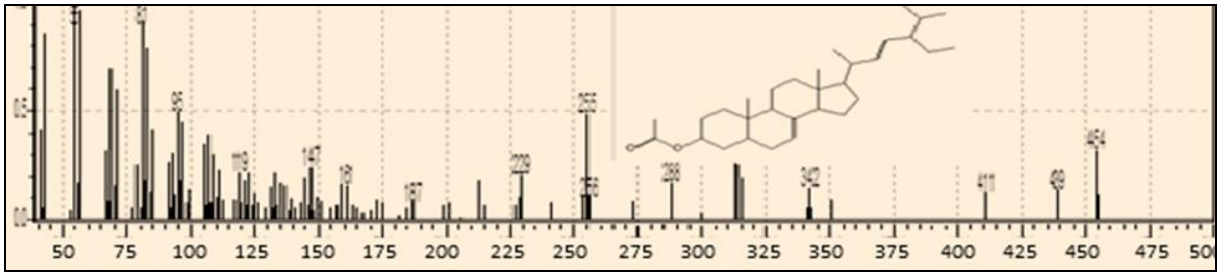

**Fig 3:** Mass spectrum of Stigmast-7-en-3-ol, (3.beta, 5. alpha., 24S)

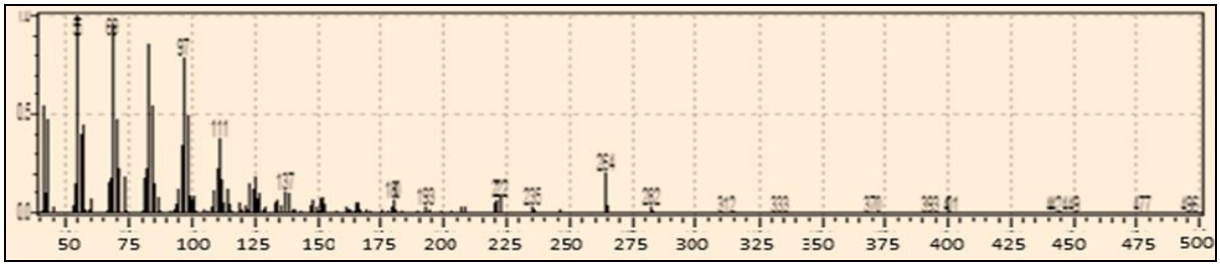

**Fig 4:** Mass spectrum of oleic acid

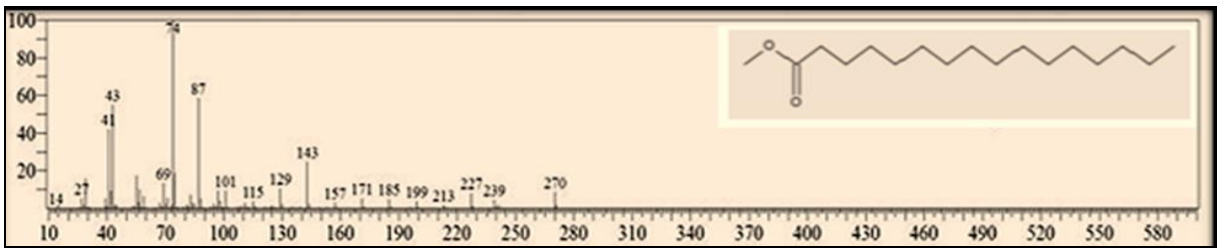

**Fig 5:** mass spectrum of hexadecanoic acid, methylester

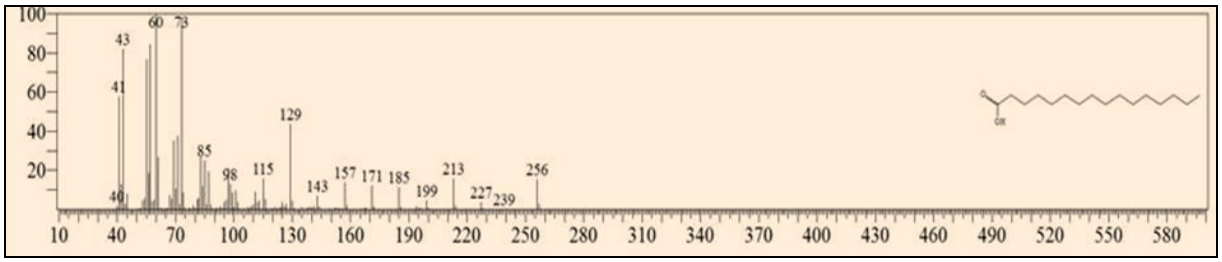

**Fig 6:** Mass spectrum of hexadecanoic acid

**Table 1:** Constituents of *Gardenia ternifolia*oil

| No. | Name | Ret. Time | Area% |
|---|---|---|---|
| 1 | Methyl tetradecanoate | 13.550 | 0.10 |
| 2 | Hexadecanoic acid, methyl ester | 15.655 | 7.37 |
| 3 | n-Hexadecanoic acid | 16.051 | 7.33 |
| 4 | Hexadecanoic acid, 14-methyl-, methyl ester | 16.631 | 0.27 |
| 5 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 17.327 | 17.87 |

| | | | |
|---|---|---|---|
| 6 | 9-Octadecenoic acid (Z)-, methyl ester | 17.363 | 7.74 |
| 7 | Methyl stearate | 17.570 | 5.04 |
| 8 | Oleic Acid | 17.755 | 8.50 |
| 9 | Octadecanoic acid | 17.939 | 3.54 |
| 10 | Stigmasta-7,22-dien-3-ol, acetate, (3.beta.,5.alpha.,22E)- | 19.137 | 3.11 |
| 11 | Eicosanoic acid, methyl ester | 19.323 | 0.97 |
| 12 | Stigmasterol | 20.353 | 5.88 |
| 13 | Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)- | 22.022 | 12.88 |
| 14 | Squalene | 23.182 | 0.91 |
| 1. | .beta.-Estradiol 17-valerate, methyl ether | 23.496 | 18.49 |

**Antimicrobial activity of *Gardenia ternifolia* oil**

*Gardenia ternifolia* oil was screened for antimicrobial activity against five standard human pathogens. The inhibition zones are presented in Table 2. The oil showed significant activity against *Pseudomonas aeroginosa*, *Staphylococcus aureus* and *Escherichia coli.*

**Table 2:** Inhibtion zones (mm) of *Gardenia ternifolia*oil

| Sample | Sa | Bs | Ec | Pa | Ca |
|---|---|---|---|---|---|
| Oil 100mg/ml | 27 | -- | 18 | 19 | 13 |

*Sa.: Staphylococcus aureus.*
*Bs.: Bacillus subtilis.*
*Ec.: Escherichia coli.*
*Pa.: Pseudomonas aeroginosa.*
*Ca.: Candida albicans.*

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