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CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF *PHASEOLUS VULGARIS* GROWN IN SUDAN

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ABSTRACT

Common bean (Phaseolus vulgaris L.) is a legume com-monly grown in sub-Saharan Africa for food, cash, ani-mals' food, and as soil improver.[1] Common bean (Phaseolus vulgaris L.) is a legume com-monly grown in sub-Saharan Africa for food, cash, ani-mals' food, and as soil improver.[1] *Phaseolus vulgaris* **L**. is a legume which is cultivated worldwide. This plant is characterized by high protein and starch content. *Phaseolus vulgaris* possesses antioxidant activity. In this study *Phaseolus vulgaris* seed oil was analyzed by GC-MS. The analysis revealed the presence of 13 components. In the well diffusion bioassay the oil showed significant activity against *Staphylococcus aureus, Pseudomonas aeroginosa* and the yeast *Candida albicans,* However, the oil did not show any inhibitory effect against *Bacillus subtilis* and *Escherichia coli.*

KEYWORDS: *Phaseolus vulgaris* L., Oil, GC-MS analysis, Antimicrobial Activity.

INTRODUCTION

Phaseolus vulgaris is a plant in the family Leguminosae (Fabaceae). This family comprises around 600 genera and about 13 000 species.[1,2] The genus *Phaseolus* has 150–200 species of plants and many of these plants are cultivated worldwide as food . *Phaseolus vulgaris* L. is extremely valued in human nutrition with world production of 26,833,394 tons in 2016.[3] *Phaseolus vulgaris* has been cultivated for thousands of years. This legume is highly consumed in Latin America, Sub-Sahara Africa.[4] It grows best in warm climate at temperature of 18 to 24^{o}C.[5,6] Most of the production of this legume takes place in developing countries. *Phaseolus vulgaris* is one of the cash legumes and it is widely cultivated in the tropics.[7] This plant is considered as a vital source of protein, carbohydrates, vitamins and minerals. *Phaseolus vulgaris* is also rich in linoleic acid, oleic acids and other unsaturated fatty acids.[8] The antioxidant activity of *Phaseolus vulgaris* has been demonstrated.[9]

MATERIALS AND METHODS

Plant material

Seeds of *Phaseolus vulgaris* were purchased from the local market, Khartoum- Suda. The plant was authenticated by The Medicinal and Aromatic Plants Research Institute-Khartoum (Sudan).

Instruments

GC-MS analysis was conducted on a Shimadzo GC-MSQP2010 Ultra instrument with a 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*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Escherichia coli* and *Candida albicans*.

Methods

Extraction of oil

Powdered plant material (300g) was exhaustively macerated with n-hexane. The solvent was removed under reduced pressure to afford the oil.

GC-MS analysis

The studied oil was analyzed by the hyphenated technique gas chromatography-mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness) was used. Helium (99% pure) was used as carrier gas. Chromatographic conditions are: Column oven temperature: **150.0 ^{o}C**; Injection temperature: **300.0^{o} C**; Injection mode: **Split;** Flow control mode: **Linear velocity;** Pressure: **139.3KPa;** Total flow: **50.0ml/ min**; Column flow: **1.54ml/sec.**; Linear velocity: **47.2cm/sec.;** Purge flow: **3.0ml/min.**; Spilt ratio : **- 1.0.**

Antimicrobial assay

Antimicrobial activity was performed by the well diffusion method.[10,11] Four strains of bacteria (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeroginosa and Escherichia coli*) and one yeast(*Candida albicans*) were used in the antimicrobial assay. The inoculum size of each test strain was standardized according to the National Committee for Clinical Laboratory Standards,[12] The bacterial and yeast strains were inoculated into Mueller Hinton broth - MH agar plates. A volume of (20 µL) of the test sample was applied into 6.0 mm diameter wells. After holding the plates at room temperature for 2 hours to allow diffusion of test sample into the agar, they were incubated at 37 °C for 24 hours. Tests were performed in duplicates. After incubation the diameters the inhibition zones were measured in millimeters (mm) and averaged as indicator of activity.

RESULTS AND DISCUSSION

Figure 1 presents the total ions chromatograms, while Table 1 displays the different constituents of the oil. *Phaseolus vulgaris* L seed oil was analyzed by GC-MS.

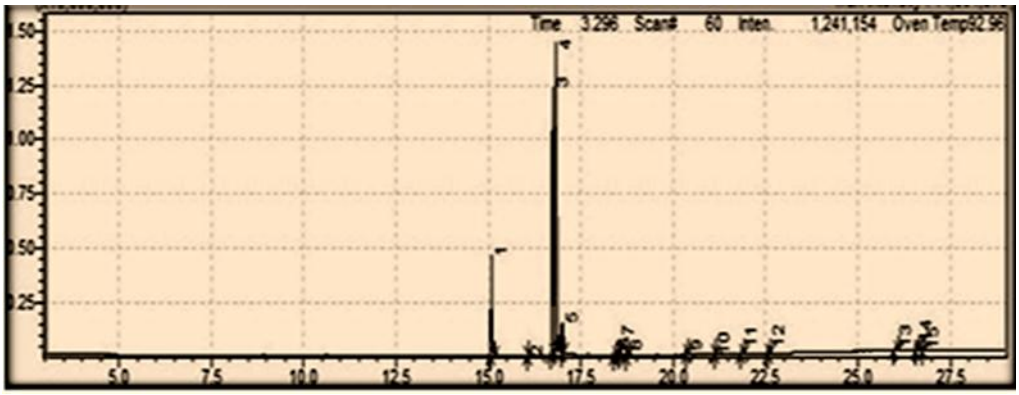

Fig. 1: Total ions chromatograms.

Table 1: Constituents of the oil.

No.	Name	Ret.Time	Area%
1.	Hexadecanoic acid, methyl ester	15.086	10.10
2.	Heptadecanoic acid, methyl ester	16.070	0.05
3.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.748	33.16
4.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	16.833	47.63
5.	Methyl stearate	16.998	2.87
6.	11,14,17-Eicosatrienoic acid, methyl ester	18.416	1.19
7.	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	18.543	0.09
8.	Eicosanoic acid, methyl ester	18.749	0.47
9.	Docosanoic acid, methyl ester	20.366	0.45
10.	Heptacosanoic acid, methyl ester	21.133	0.11
11.	Tetracosanoic acid, methyl ester	21.866	0.78
12.	Squalene	22.583	0.79
13.	Stigmasterol	26.025	0.67

The following compounds were detected as major constituents of the oil:

i) 9,12,15-Octadecatrienoic acid, methyl ester(47.63%).
ii) 9,12-octadecadienoic acid, methyl ester(33.16%)
iii) Hexdecanoic acid methyl ester(10.10%)

Fig. 2 shows the mass spectrum of 9,12,15-octadecatrienoic acid, methyl ester. The peak at m/z292(RT. 16.833) corresponds the molecular ion $M^+[C_{19}H_{32}O_2]^+$. The signal at m/z 261 is due to loss of a methoxyl. The mass spectrum of 9,12-octadecadienoic acid, methyl ester is presented in Fig.3. The peak at m/z294(RT.16.748) corresponds the molecular ion $M^+[C_{19}H_{34}O_2]^+$. The signal at m/z 263 accounts for loss of a methoxyl. The mass spectrum of hexdecanoic acid methyl ester is presented in Fig.4. The peak at m/z 270 (RT.15.086) corresponds the molecular ion $M^+[C_{17}H_{34}O_2]^+$.

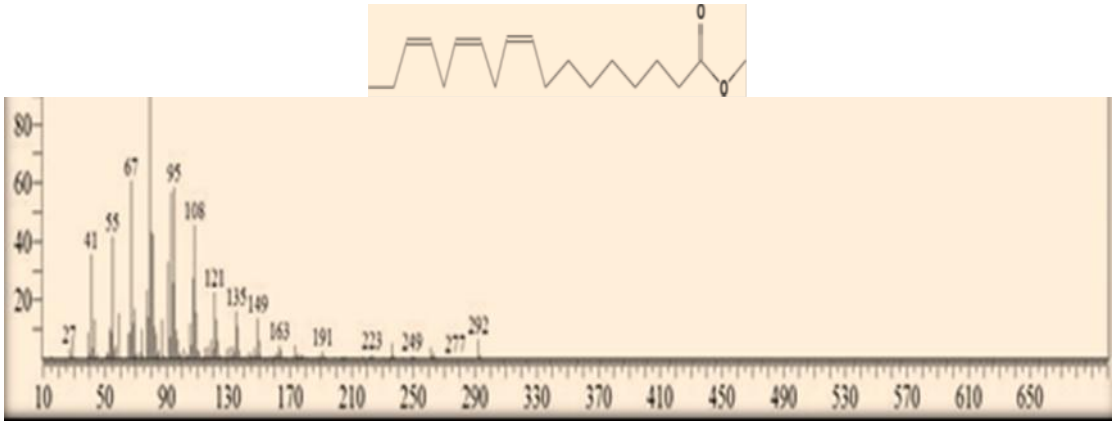

Fig. 2: Mass spectrum of 9,12,15-octadecatrienoic acid, methyl ester.

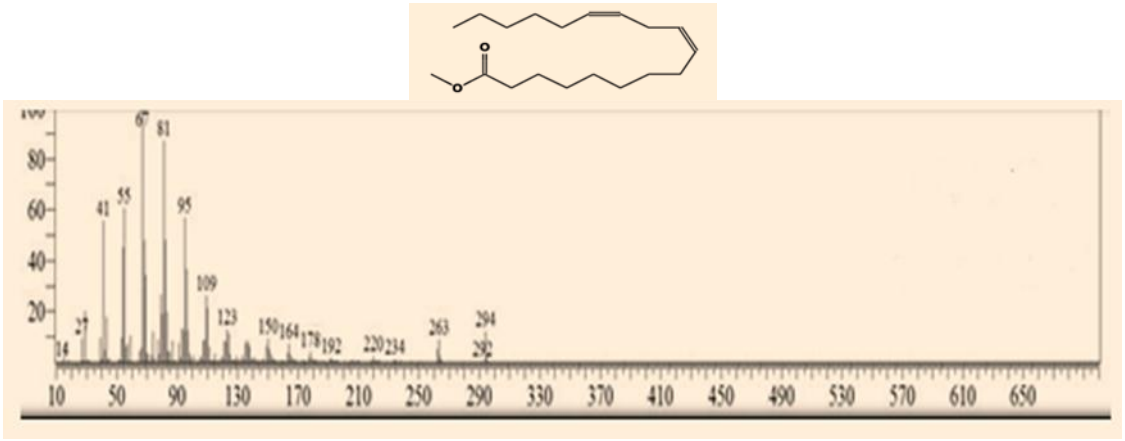

Fig. 3: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester.

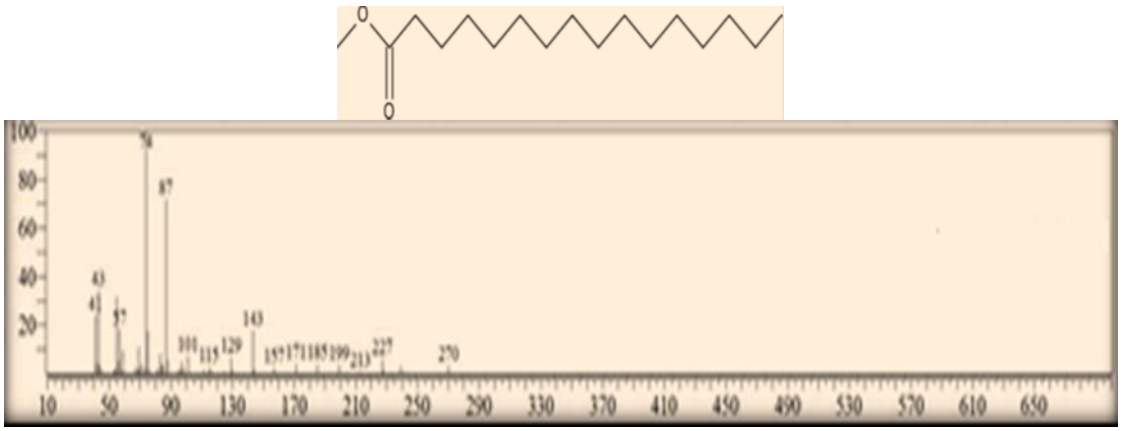

Fig. 4: Mass spectrum of Hexdecanoic acid methyl ester.

Antimicrobial activity

Phaseolus vulgaris L oil was screened for antimicrobial activity against five standard human pathogens. The inhibition zones are displayed in Table 2. The oil showed significant anticandidal activity. It also showed good activity against *Pseudomonas aeroginosa.* and *Staphylococcus aureus.* However, the oil did not show any inhibitory effect against *Bacillus subtilis* and *Escherichia coli*.

Table 2: Inhibtion zones of *Phaseolus vulgaris* oil.

Sample	Sa	Bs	Ec	Pa	Ca
Oil 100mg/ml	14	---	---	15	20

Sa.: Staphylococcus aureus.
Bs.: Bacillus subtilis.
Ec.: Escherichia coli.

Pa.: Pseudomonas aeroginosa.
Ca.: Candida albicans.

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