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Research Article

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Constituents and Antimicrobial Activity of the Sudanese Material of *Lepidum sativum* L. (Brassicaceae)

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Abstract *Lepidium sativum* L. is an annual fast-growing edible plant in the family Brassicaceae. This plant is a key element in African traditional system of medicine. This herb has many health promoting properties including: hypoglycemic, antioxidant, antimicrobial, antiosteoporotic, antiasthmatic and diuretic properties [1]. The GC-MS analysis of *Lepidium sativum* oil revealed the presence of twenty four components. Main constituents are: i)-linolinic acid, 2-hydroxy-1-(hydroxymethyl) (31.88%) ii)- 11-eicosenoic acid, methyl ester (15.18%). iii)- methyl 10-trans,12-cis-octadecadienoate (13.60%), iv)-hexadecanoic acid methyl ester (9.99%) and v)-13-docosenoic acid, methyl ester (7.88%). The antibacterial activity of the oil was evaluated via the diffusion assay against five standard human pathogens (Gram positive: *Staphylococcus aureus* and *Bacillus subtitlis; Gram* negative: *Esherichia coli* and *Pseudomonasa aeruginosa* and the fungus *Candida albicans*). *Foeniculum vulgare* oil showed excellent activity against *Staphylococcus aureus* in the concentration range: 100-12.5 mg/ml. It also exhibited significant activity against the yeast *Candida albicans* at 100mg/ml. It seems that the oil is a lead for further optimization.

Keywords *Lepidium sativum*, Oil, GC-MS Analysis, Antimicrobial Activity

Introduction

Lepidium sativum L. is an annual fast-growing edible plant in the family Brassicaceae. This plant is a key element in African traditional system of medicine where it is used for a wide array of human disorders. *Lepidium sativum* is used traditionally against gastrointestinal disorders and for regulating the menstrual cycle. This herb has many health promoting properties including hypoglycemic, antioxidant, antimicrobial, antiosteoporotic, antiasthmatic and diuretic properties [1].

Seeds of *Lepidium sativum* are used for iron deficiency, rheumatism and hair loss [1]. Seeds contain alkaloids beside an ideal ratio of omega-3 and -6 fatty acids [2,3]. Seeds showed significant antimicrobial potency against a panel of human pathogens [2]. Leaves are stimulant, diuretic, and hepatoprotective [4,5]. Root is used traditionally against syphilis while fresh fruit is a natural remedy for eye diseases and wounds [6-8].

It has been shown that this plant possesses diverse pharmacological activities including: antimicrobial [9,10], antoxidant [11], cytotoxic [12], diuretic [13], hepatoprotective [14], hypoglycemic [15], antiosteoporotic [16], antiasthmatic [17], anticarcenogenic [18], cardiotonic, fracture healing [18] and anti-inflammatory [19] properties.

Materials and Methods

Plant material

The *Lepidum sativum* seeds were purchased from the local market - Omdurman, Sudan. The plant was authenticated by the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan.

Instruments

A Shimadzo GC-MS-$QP_2$2010 Ultra instrument with RTX-5MS column (30m, length; 0.25m, diameter; 0.25 mm, thickness) was used for GC-MS analysis.

Test Organism

Lepidum sativum oil was screend for antimicrobial activity using the standard microorganisms: Gram positive: *Staphylococcus aureus* and *Bacillus subtilis;* Gram negative: *Esherichia coli* and *Pseudomonasa aeruginosa* and the fungus *Candida albicans*.

Methods

Extraction of *Lepidum sativum* oil

The seeds of *Lepidum sativum*(300g) were macerated with n-hexane at room temperature for 48.h The Solvent was removed under reduced pressure to afford the oil. Esterification of the oil, for GC-MS analysis, was accomplished via methonolic solution of sodium hydroxide and methanolic suphuric acid.

GC-MS analysis

A Shimazdo ultra instrument was used for GC-MS analysis of *Lepidum sativum* oil. Analytical grade helium was used as carrier gas. Chromatographic condition are depicted in Table 1.

Table 1: Chromatographic conditions

Coloumn oven temperature	1300 ^{0}C
Injection temperature	280 ^{0}C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	93.1KPa
Total flow	50.0ml\sec
Coloumn flow	44.7cm\sec
Linear velocity	3.0ml\mint
Purge flow	-1.0
Split ratio	

Antimicrobial Activity

Preparation of bacterial suspensions

Diffusion method was used for screening the oil for antimicrobial activity. Meuller Hinton and Sabouraud dextrose agars were the media used for the growth of bacteria and fungi respectively.

Aliquots of 24 hours broth culture of the test microorganism were aseptically distributed onto agar slopes and incubated at 37 ^{0}C for 24 hours. Bacterial growth was harvested and washed off with sterile normal saline, then it was suspended in (100ml) of normal saline. Average number of viable organism per ml of the stock suspension was determined by the means of surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline. (0.02ml) of the appropriate dilutions were transferred onto the surface of dried agar plates. The plates were allowed to stand for two hours at room temperature and then incubated at 37^o C for 24 hours.

Fungal culture were maintained on Sabouraud dextrose agar incubated at 25^o C for 72h. The fungal growth was harvested and washed with sterile normal saline, and suspension was stored in the refrigerator until used.

Testing for antimicrobial activity

(2ml) of standardized bacterial stock suspension were mixed with (200ml) of sterile molten nutrient agar which was maintained at 45^{o} C. (20ml) aliquots of the incubated agar were distributed into sterile Petri dishes. The agar was left to settle. Each plate were then divided into two halves. In each half two cups (6mm in diameater) were cut using sterile cork borer (No 4). Each half was designed for a test solution.

Agar discs were removed, alternate cups were filled with (0.1ml) sample of each test solution and allowed to diffuse at room temperature for two hour. The plates were then incubated at 37^{o} C for 24 hours. After incubation, the diameters of resultant growth inhibition zone were measured as an average of two replicates.

Result and Discussion

GC-MS analysis of *Lepidum sativum* oil

GC-MS analysis of *Lepidum sativum* oil was conducted. The MS library (NIST) was checked for identification of constituents (a 90-95% match was observed). Furthermore, the observed fragmentation pattern was interpreted. The GS-MS spectrum of the studied oil revealed the presence of 24 components (Table 2). Major constituents of the oil are:

i) Linolinic acid, 2-hydroxy-1-(hydroxymethyl) (31.88%)

ii) 11-Eicosenoicacid, methyl ester (15.18%).

iii) Methyl 10-trans, 12-cis-octadecadienoate (13.60%).

iv) Hexadecanoic acid methyl ester (9.99%).

v) 13-Docosenoic acid,methyl ester (7.88**%**).

Fig. 2 shows the EI mass spectrum of linolinic acid, 2-hydroxy-1-(hydroxymethyl ethyl ester). The peak at m/z 352, which appeared at R.T. 17.581 in total ion chromatogram, corresponds: M^{+} $[C_{21}H_{36}O4]^{+}$. The mass spectrum of 11-eicosenoic acid, methyl ester is displayed in the Fig. 3. The peak at m/z 324 (R.T. 19.320) corresponds: $M^{+}[C_{21}H_{40}O_2]^{+}$. Fig. 4 shows the mass spectrum of methyl 10-trans, 12-cis-octadecadienoate.

Table 2: Constituents of the oil

No.	Name	RT.	Area%
1	D-Limonine	4.835	0.2
2	Estragole	7.205	0.05
3	Butylated hydroxyltoluene	11.368	0.16
4	Methyl tetradecanoate	13.720	0.24
5	5-Octadecenoic acid methyl ester	14.532	0.03
6	4-Octadecenoic acid methyl ester	14.635	0.01
7	Pentadecanoic acid methyl ester	14.797	0.05
8	7,10-Hexadecadienoic acid methyl ester	15.529	0.03
9	6-Octadecenoic acid methyl ester	15.589	0.17
10	9-Hexadecenoic acid methyl ester	15.631	0.34
11	Hexadecanoic acid methyl ester	15.834	9.99
12	Hexadecanoic acid , 14-methyl methyl ester	16.532	0.03
13	Cis-1-Heptadecenoic acid methyl ester	16.595	0.09
14	Heptadecanoic acid methyl ester	16.802	0.10
15	Methyl 10-trans, 12-cis-octadecadienoate	17.496	13.60
16	Linolenic acid, 2-hydroxy-1(hydroxymethyl ethyl ester	16.581	31.88
17	Methyl stearate	17.745	4.91
18	11-Eicosenoic acid methyl ester	19.320	15.18
19	8,11,14-Docosatrienoic acid methyl ester	19.354	2.43
20	Methyl 18-methylnonadecanoate	19.501	6.25
21	13-Docosenoic acid methyl ester	20.951	7.88
22	Methyl 20-methyl-heneicosanoatee	21.118	2.80
23	15-Tetracosenoic acid methyl ester	22.468	2.40
24	Tetracosanoic acid methyl ester	22.620	1.36

The peak at m/z 294, which appeared at R.T.17.496 in total ion chromatogram, corresponds: $M^+[C_{19}H_{34}O_2]^+$. Fig. 5 illustrates the mass spectrum of hexadecanoic acid methyl ester. The signal at m/z270 (RT.15.834) corresponds the molecular ion: The mass spectrum of 13-docosenoic acid, methyl ester is shown in Fig.6. The molecular ion: $M^+[C_{23}H_{44}O_2]^+$ appeared at m/z 352 (RT.20.951).

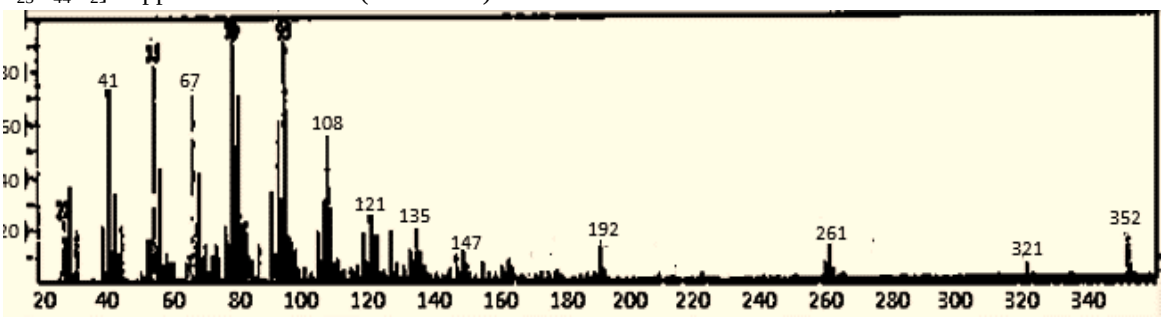

Figure 2: Mass spectrum of linolinic acid, 2-hydroxy-1-(hydroxymethyl ethyl ether)

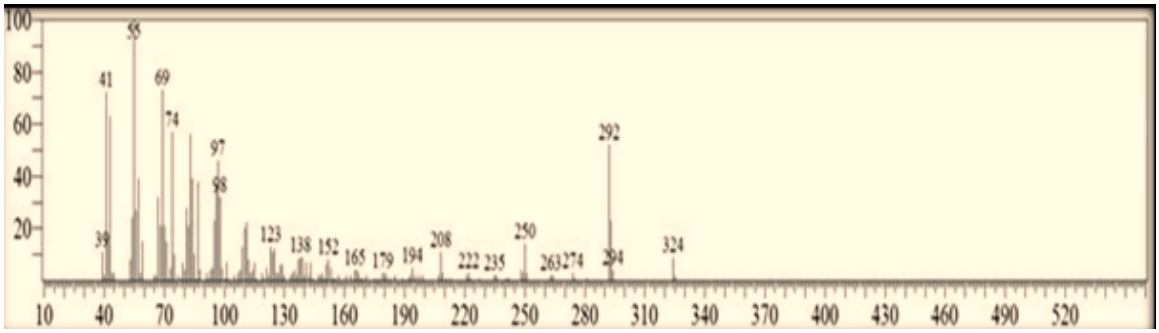

Figure 3: Mass spectrum of 11-eicosenoicacid, methyl ester

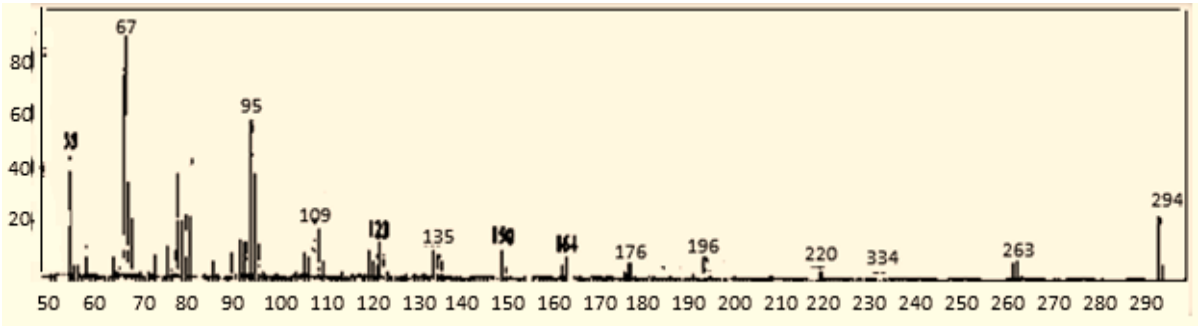

Figure 4: Mass spectrum of methyl 10-trans, 12-cis-octadecadienoate

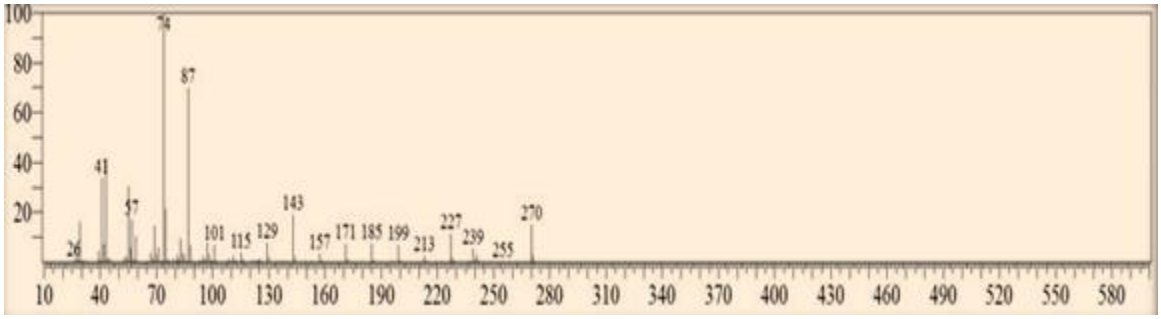

Figure 5: Mass spectrum of hexadecanoic acid methyl ester

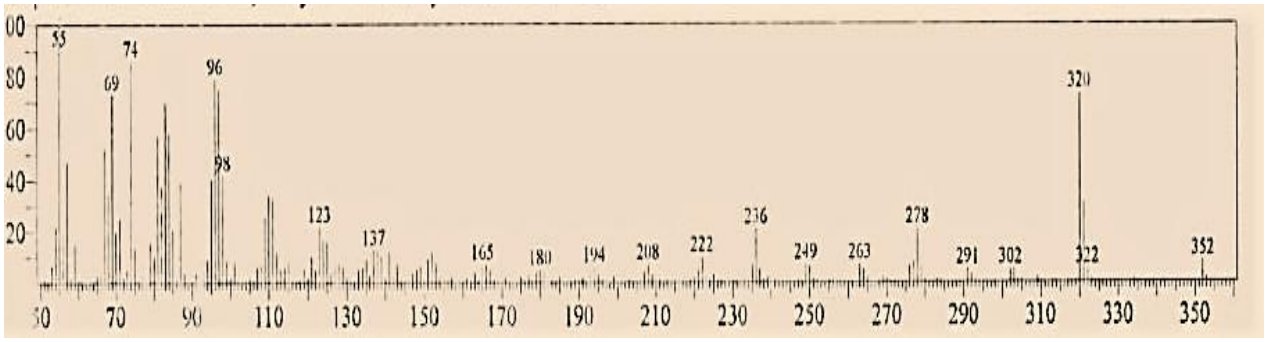

Figure 5: Mass spectrum of 13-docosenoic acid, methyl ester

Antimicrobial Activity

Lepidum sativum oil was screened for antimicrobial activity against five standard pathogenic microbes. The diameters of the growth of inhibition zones are shown in Table (3). Conventional terms were used for interpretation of the results: (<9mm: inactive; 9-12mm: partially active; 13-18mm: active; 13-18mm: active; very active). Tables (4) and (5) represent the antimicrobial activity of standard drugs. *Lepidum sativum* oil showed significant activity against *Staphylococcus aureus* in the concentration range: 50-12.5mg/ml. It also exhibited significant anticandidal activity at 50mg/ml.

Table 3: Inhibition zones (mm/mg sample) of oil

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	50	18	-	16	16	17
	25	17	-	14	15	15
	12.5	16	-	-	14	13
	6.25	12	-	-	-	12

Table 4: Inhibition zones (mm/mg sample) of standard drugs

Drug	Conc (mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	40	15	30		
	20	14	25		
	10	11	15		
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	15	15	12

Table 5: Inhibition zones (mm/mg sample) of standard antifungal

Drug	Conc.(mg/ml)	Ca
Clotramizole	30	38
	15	31
	7.5	29

References

[1]. Jabeen A, Rani S, Ibrahim M, Mohammad AS. A review on Lepidium sativum. Indo Am J Pharm Sci., 2017; 4: 2223-7.

[2]. Mennicke WH, Görler K, Krumbiegel G, Lorenz D, Rittmann N. Studies on the metabolism and excretion of benzyl isothiocyanate in man. Xenobiotica 1988; 18: 441-7.

[3]. Mirza M, Navaei MN. Essential oil composition of Lepidium sativum L. Iran J Med Aromat Plants, 2006; 21: 481-8.

[4]. Maghrani M, Zeggwagh NA, Michel JB, Eddouks M. Antihypertensive effect of Lepidium sativum L. In spontaneously hypertensive rats. J Ethnopharmacol., 2005; 100: 193-7.

[5]. Wright CI, Van-Buren L, Kroner CI, Koning MM. Herbal medicines as diuretics: A review of the scientific evidence. J Ethnopharmacol. 2006, 102; 98-104.
[6]. Gokavi SS, Malleshi NG, Guo M. Chemical composition of garden cress (Lepidium sativum) seeds and its fractions and use of bran as a functional ingredient. Plant Foods Hum Nutr., 2004; 59: 105-11.
[7]. Kirthikar KR, Basu BD. Indian Medicinal Plants. Dehradun: Popular Publications; 1999. p. 1224-7.
[8]. Bigoniya P, Singh CS, Shukla A. Pharmacognostical and physicochemical standardization of ethnopharmacologically important seeds of Lepidium sativum Linn. and Wrightiatinctoria R. Br. Indian J Natl Prod Resour., 2011; 2: 464-71.
[9]. Gupta PC, Pant D, Joshi P, Lohar DR. Evaluation of antibacterial activity of Lepidium sativum Linn. Seeds against food-borne pathogens. Int J Chem Anal Sci., 2010; 1: 74-5.
[10]. George RE, Thomas SK, Kunjumon M, Thankamani V. Analysis of phytoconstituents and in vitro antifungal evaluation of methanolic extract of Lepidium sativum Linn. Seeds. Int J Pharm Bio Sci., 2015; 6: 490-7.
[11]. Malara J, Chairmanb K, Singh AR, Vanmathid JS, Balasubramaniana A, Vasanthie K. Antioxidative activity of different parts of the plant Lepidiumsativum Linn. Biotechnol Rep., 2014; 3: 95-8.
[12]. Ait-Yahia O, Bouzroura SA, Belkebir A, Kaci S, Aouichat AB. Cytotoxic activity of flavonoid extracts from Lepidium sativum (Brassicaceae) seeds and leaves. Int J PharmacognPhytochem Res 2015; 7: 1231-5.
[13]. Patel U, Kulkarni M, Undale V, Bhosale A. Evaluation of diuretic activity of aqueous and methanol extracts of Lepidium sativum garden cress (Cruciferae) in rats. Trop J Pharm Res, 2009; 8: 215-9.
[14]. Raish M, Ahmad A, Alkharfy KM, Ahamad SR, Mohsin K, Al-Jenoobi FI, et al. Hepatoprotective activity of Lepidium sativum seeds against D-galactosamine/lipopolysaccharide- induced hepatotoxicity in animal model. BMC Complement Altern Med., 2016; 16: 501.
[15]. Elshal MF, Almalki AL, Hussein HK, Khan JA. Synergistic antiosteoporotic effect of Lepidium sativum and alendronate in glucocorticoid-induced osteoporosis in Wistar rats. Afr J Tradit Complement Altern Med., 2013;10:267-73.
[16]. Paranjape AN, Mehta AA. A study on clinical efficacy of Lepidium sativum seeds in treatment of bronchial asthma. Iran J Pharmacol Ther., 2006; 5: 55-9.
[17]. Al Obaidi LA. Study the anticancer effect of Lepidium sativum leaves extract on squamous cell carcinoma (CAL-27) cell lines. J Nat Sci Res., 2014; 417: 48-52.
[18]. Atasan SK. Studies on some herbal drugs used in fracture healing. Int J Crude Drug Res., 1989; 27: 235-9.
[19]. Reddy SK, Rao VV, Fathima B, Kapila M, Srilakshmi M. In-vitro antiinflammatory activity of ultra sonic bath assisted, methanol extract of Lepidium sativum Linn. Seeds. Int J Pharm Dev Technol. , 2013;3:63-5