Journal of
Medical Pharmaceutical
And Allied Sciences

www.jmpas.com
ISSN NO. 2320 - 7418

RESEARCH ARTICLE

GC-MS Analysis and Antimicrobial Activity of Sudanese Zea mays subsp. Mays (Gramineae) Fixed Oil

Abdel Abdel Karim. M.1(*) , Fatima,M. 2 and Ayat, A.

1. Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry
2. El-Nelein University , Faculty of Science

Correspondence
Abdel Karim. M.
Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry

Keywords
Zea mays, Fixed oil, GC-MS, Antimicrobial activity.

Received
06 December 2016
Reviewed
15 December 2016
Accepted
28 December 2016

ABSTRACT

The present study was designed to investigate the chemical constituents of Sudanese Zea mays seed oil and to evaluate its potential antimicrobial activity. 20 components were detected by GC-MS analysis. Major constituents are: methyl 10-trans-12-cis-octadecadienoate (48.13%) , hexadecanoic acid(21.60%),9-octadecenoic acid(17.01%), methyl stearate(5.99%) and methyl 18-methylnonadecanoate(2.17%). The antimicrobial activity of the oil was evaluated via cup plate agar diffusion assay against six standard human pathogens(Gram positive: Staphylococcus aureus and Bacillus subtilis; Gram negative : Escherichia coli and Pseudomonasa aeruginosa and the fungi Candida albicans and Aspergillus niger) . Zea mays oil showed good activity against Staphylococcus aureus and the fungus Aspergillus niger.However , it was partially active against other test organisms except Bacillus subtilis.

INTRODUCTION

Zea mays subsp. Mays is a large grain plant first domesticated by indigenous peoples in Mexico[1] about 10,000 years ago. The six major types of corn are dent corn, flint corn, pod corn, popcorn, flour corn, and sweet corn.

Due to the effectiveness in treating various ailments, Zea mays hair is frequently worldwidely used as an old folk therapeutic agent. Zea mays belongs to family Gramineae. It can be found in tropical regions and various parts of the world including Sudan. Zea mays hair is found inside the husks of corn. It hardly shows themselves until the emergence of the pale yellow silks from the end of the husks. The silk are elongated stigmas that resemble bunch of hair [2]. Zea mays hair contains various bioactive constituents including proteins, vitamins, minerals , salts , flavonoids, steroids , carbohydrates and volatile components[3]. Phytochemicals present in this species showed potential hypoglycemic activity [4]. On the other hand, Zea mays hair extract has been reported to increase insulin level together with healing of injured β-cell. Zea mays hair is claimed to treat hypersensitivity related to type I allergy disease [5,6]. Besides that, Zea mays hair has been documented to exhibit anti-proliferative effect on cancer cell line[7].Zea mays hair has been claimed to have effect more particularly on renal diseases including chronic nephritis, gout and cystitis [8] .It helps to pass stones from kidney and urinary tract and prevents inflammatory effect. Zea mays hair has anti-prostatitis and anti-spasmodic activities [9] . Recently, Zea mays hair has been reported to have anti-fatigue activity. Flavonoid compounds isolated from hair increased the hepatic glycogen and consequently, increased the exercise tolerance[10].The hair has antioxidative properties, it protects cells from damages due to oxidation process in the body triggered by free radicals [11].

Constituents of the dichloromethane extract of Zea mays grown in Egypt were quantified and identified by GC-MS . Major constituents were: cis-α-terpineol (24.22%), 6,11-oxidoacor-4-ene (18.06%), citronellol (16.18%), trans-pinocamphone (5.86%), eugenol (4.37%), neo-iso-3-thujanol (2.59%), and cis-sabinene hydrate (2.28%).Different extracts of the plant exhibited clear antioxidant activities at levels of 50–400 µg/mL in the 2,2-diphenyl-1-picrylhydrazyl (DPPH)/linoleic acid bioassay [12].

Materials and Methods

Plant material

Zea mays seeds were collected from Gezira state , Sudan in September-2016.The plant was authenticated by direct comparison with a herbarium sample.

Methods

Phytochemical screening

Zea mays (hair) was screened for major secondary metabolites according to the method described by Harborne[13]. *Zea mays* hair was extracted with 80% aqueous methanol (soxhlet) until exhaustion. This prepared extract(PE) was used for phytochemical screening.

Test for unsaturated sterols and triterpenes

10 ml of the (PE) were evaporated to dryness on a water bath, and the cooled residue was stirred with petroleum ether to remove most of the coloring materials. The residue was then extracted with 10 ml chloroform. The chlorform solution was dehydrated over sodium sulphite anhydrous. 5 ml portion of the solution was mixed with 0.5 ml of acetic anhydride, followed by two drops of concentrated sulphuric acid. Two separate layers (green, red) were observed.

Test for flavonoids

(20 ml) of the (PE) were evaporated to dryness on water bath. The cooled residue was defatted with

petroleum ether and then dissolved in 30 ml of 30% aqueous methanol and filtered. The filtrate was used for the following tests:

- To 3 ml. of filtrate a fragment of magnesium ribbon was added, shaken and then few drops of concentrated hydrochloric acid were added. Red color was observed.
- To 3 ml. of the filtrate few drops of aluminium chloride solution were added. A dark yellow color was formed.
- To 3 ml. of the filtrate few drops of potassium hydroxide solution were added. A dark yellow color was observed.

Test for alkaloids

(10 ml) of the (PE) were evaporated to dryness on water bath and 5 ml of 0.2N hydrochloric acid were added and the solution was heated with stirring for 10 minutes, then cooled and divided into two portions:

To one portion a few drops of Mayer reagent were added. A white precipitated appeared, to the other portion few drops of Wagner reagent were added. A brown precipitate appeared.

Test for tannins

(10 ml) of (PE) were evaporated to dryness and the residue was extracted with n-hexane and then filtrated. The insoluble residue was stirred with n-hexane and 10 ml of hot saline (0.9% w/v of sodium chloride and freshly prepared distilled water) were added. The mixture was cooled , filtrated and the volume adjusted to 10 ml. with more saline solution. 5 ml of this solution was treated with few drops of ferric chloride solution. A dark blue color was observed.

Test for saponins

(1 g)of dried powdered plant material was placed in a test tube. 10 ml of distilled water were added and the tube was stoppered and vigorously shaken for about 30 seconds, and allowed to stand. Honey comb was formed.

Extraction of oil from *Zea mays* seeds

Dry powdered seeds of *Zea mays* (300g) were macerated with n-hexane at room temperature for 48h..The solvent was removed under reduced pressure and the oil was kept in the fridge at 4°C for further manipulation.

Esterification of oil

A Methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in 100ml methanol. A stock solution of methanolic sulphuric acid was prepared by mixing (1ml)of concentrated sulphuric acid with (99ml) methanol.

The oil(2ml) was placed in a test tube and (7ml) of alcoholic sodium hydroxide were added followed by (7ml) of alcoholic sulphuric acid.The tube was stoppered and shaken vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added, then (2ml) of n-hexane were added and the tube was vigorously shaken for five minutes .The hexane layer was then separated.(5µl) of the hexane extract were mixed with 5ml diethyl ether . The solution was filtered and the filtrate(1µl) was injected in the GC-MS vial.

GC-MS analysis

Zea mays fixed oil was analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 μm, thickness)was used. Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program is given in Table 1, while other chromatographic conditions are depicted in Table 2.

Table 1: Oven temperature program

Rate	Temperature(C)	Hold time ($mim.^{-1}$)
-	60.0	0.00
10.00	300.0	0.00

Table 2: Chromatographic conditions

Column 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/ min
Column flow	1.50ml/sec
Linear velocity.	44.7cm/sec
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial assay

Zea mays oil was screened for antimicrobial activity against six standard human pathogens (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger*) using the cup plate agar method with some minor modifications.

Preparation of bacterial suspensions

One ml. aliquots of 24 hours broth culture of the test organisms were distributed onto agar slopes and incubated at 37^{o} C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce suspension containing about 10^{8}. 10^{4} colony forming units per ml. The suspension was stored in refrigerator at 4^{o}C until used. The average number of viable organism per ml of the saline suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volume (0.2 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 to dry, and then incubated at 37^{o} C for 24 hours.

Preparation of fungal suspensions

Fungal cultures were maintained on potato dextrose agar incubated at 25^{o}C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Antimicrobial assay

The cup plate agar diffusion method was adopted with some minor modification, to assess the antimicrobial activity of the *Zea mays* oil .Two ml of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45^{o} C in water bath.

(20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes and the agar was left to settle . In each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No. 4). Each of the halves was designed for one of the test samples.

The agar discs were removed and cups were filled with(0.1) ml of test sample using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ^{o} C for 24 hours.After incubation the diameters of the resultant growth inhibition zones were measured in duplicates and averaged.

Testing for antifungal activity

The above mentioned method was adopted for antifungal activity, but instead of nutrient agar potato dextrose agar was used. Samples were used here by the same concentrations mentioned above.

Results and Discussion

Phytochemical screening

In the present study, Sudanese *Zea mays* hair extracts were screened for the occurrence of bioactive compounds. The results(Table3) positively showed the presence of flavonoids, saponins, tannins and alkaloids in both aqueous and methanolic extract of *Zea mays* hair.These findings suggested that phytochemicals present in *Zea mays* hair are potentially beneficial as therapeutic and antioxidative agents in pharmaceuticals, food and other related industries.

Table 3:Phytochemical screening of *Zea mays* hair

Species	Flavonoids	Tannins	Alkaloids	Saponins
Zea mays	+ve	+ve	+ve	+ve

GC-MS analysis of *Zea mays* oil

GC-MS analysis of *Zea mays* oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern. Comparison of the mass spectra with the database on MS library revealed about 90-95% match.

Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 20 components(Table 4).The typical total ion chromatogram(TIC) is shown in Fig.1.

Table 4: Constituents of *Zea mays* oil

Peak#	R.Time	Area	Area%	Name
1	11.374	360116	0.10	Butylated Hydroxytoluene
2	11.403	35664	0.01	Dodecanoic acid, methyl ester
3	13.727	684536	0.19	Methyl tetradecanoate
4	14.537	182667	0.05	cis-5-Dodecenoic acid, methyl ester
5	14.803	211541	0.06	Pentadecanoic acid, methyl ester
6	15.537	107903	0.03	7,10-Hexadecadienoic acid, methyl ester
7	15.596	753989	0.21	Methyl hexadec-9-enoate
8	15.639	1862677	0.52	9-Hexadecenoic acid, methyl ester, (Z)-
9	15.873	78092578	21.60	Hexadecanoic acid, methyl ester
10	16.602	1067559	0.30	cis-10-Heptadecenoic acid, methyl ester
11	16.811	1842150	0.51	Heptadecanoic acid, methyl ester
12	17.589	173993704	48.13	Methyl 10-trans,12-cis-octadecadienoate
13	17.638	61484370	17.01	9-Octadecenoic acid, methyl ester, (E)-
14	17.775	21668086	5.99	Methyl stearate
15	19.142	530827	0.15	Methyl 5,13-docosadienoate
16	19.305	5567552	1.54	11-Eicosenoic acid, methyl ester
17	19.505	7834412	2.17	Methyl 18-methylnonadecanoate
18	21.122	2022392	0.56	Methyl 20-methyl-henicosanoate
19	22.625	2503223	0.69	Tetracosanoic acid, methyl ester
20	23.372	702708	0.19	Squalene
		361508654	100.00	

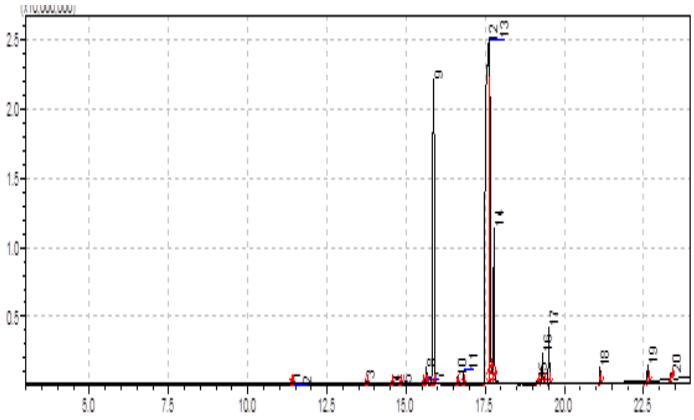


Fig.1:Cromatograms of *Zea mays* oil

The following major constituents were detected in the chromatograms:

Methyl-10-trans-12-cis-octadecadienoate (48.13%)

The EI mass spectrum of methyl 10-trans-12-cis-octadecadienoate is shown in Fig.2.The peak at m/z 294, which appeared at R.T. 17,589 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$ .The peak at m/z263 corresponds to loss of a methoxyl function.

Hexadecanoic acid methyl ester(21.60%)

The EI mass spectrum of hexadecanoic acid methyl ester is shown in Fig.3.The peak at m/z 270, which appeared at R.T. 15,873 in total ion chromatogram, corresponds to $M^+[C_{17}H_{34}O_2]^+$ .The peak at m/z239 corresponds to loss of a methoxyl function.

9-Octadecenoic acid methyl ester(17.01%)

The EI mass spectrum of 9-octadecenoic acid methyl ester is shown in Fig. 4.The peak at m/z 296, which appeared at R.T. 17,638 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$ .The peak at m/z266 corresponds to loss of a methoxyl function.

Methyl stearate(5.99%)

The EI mass spectrum of methyl stearate is shown in Fig.5.The peak at m/z 298, which appeared at R.T. 17,775 in total ion chromatogram, corresponds to $M^+[C_{19}H_{38}O_2]^+$ .The peak at m/z284 corresponds to loss of a methyl function.

Methyl 18-methylnonadecanoate(2.17%)

The EI mass spectrum of methyl 18-methylnonadecanoate is shown in Fig. 6.The peak at m/z 326, which appeared at R.T. 19,505 in total ion chromatogram, corresponds to $M^+[C_{21}H_{42}O_2]^+$ .The peak at m/z311 corresponds to loss of a methyl function.

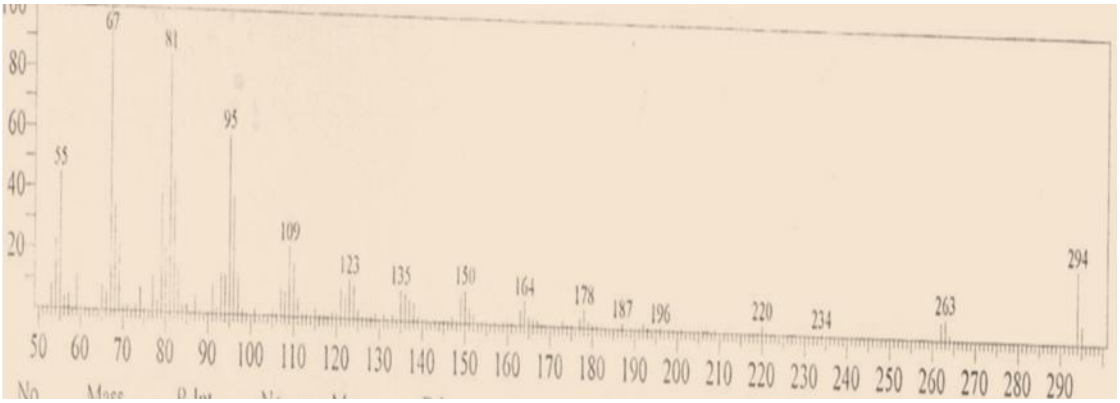


Fig. 2: Mass spectrum of methyl 10-trans-12-cis-octadecadienoate

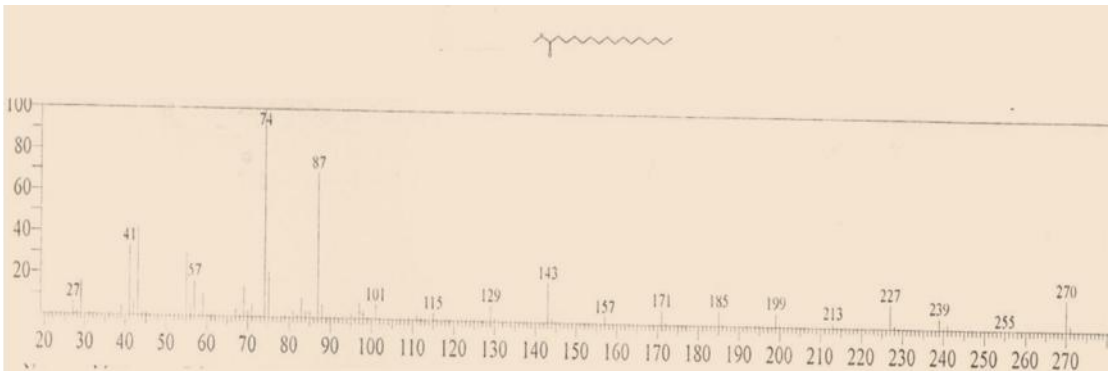


Fig. 3: Mass spectrum of hexadecanoic acid methyl ester

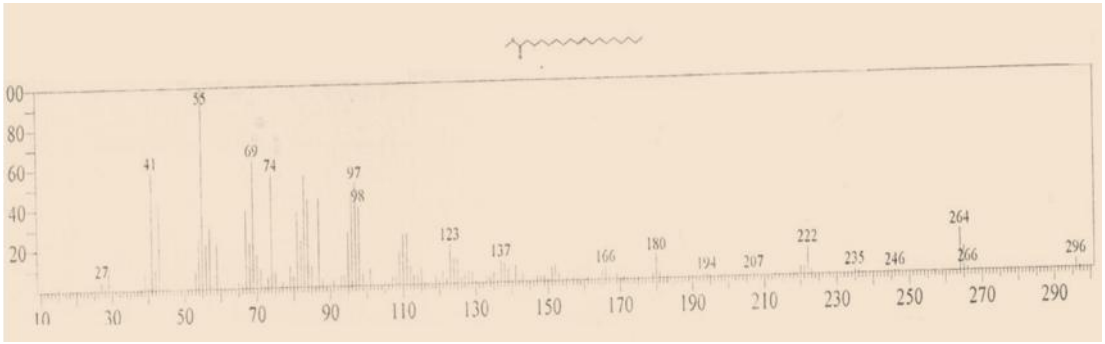
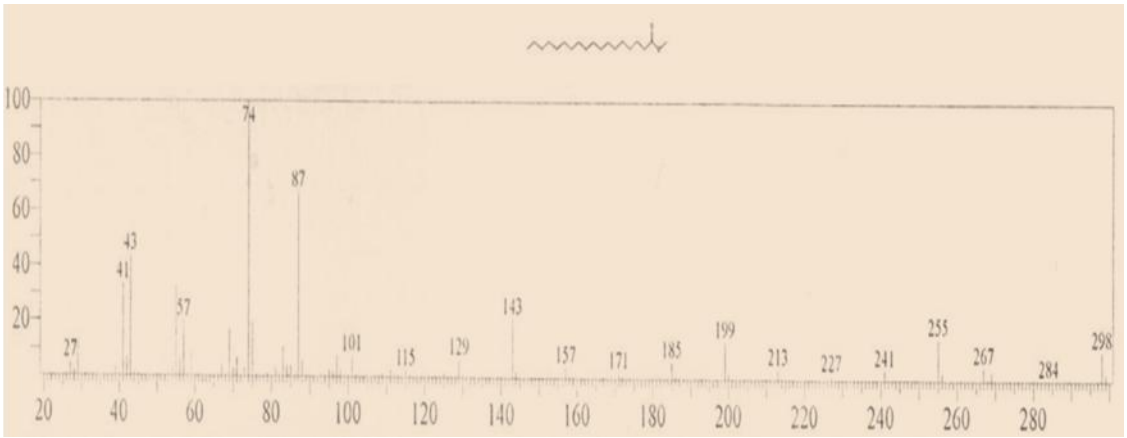
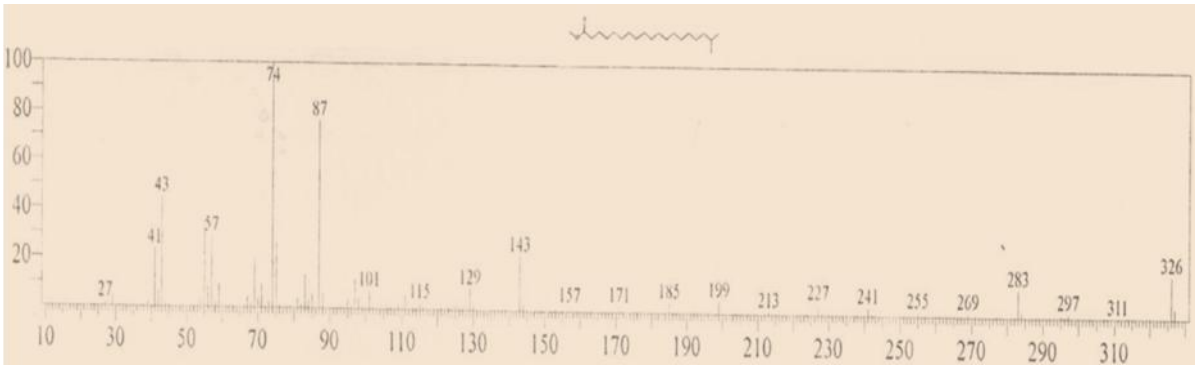


Fig. 4: Mass spectrum of 9-octadecenoic acid methyl ester



Fg. 5: Mass spectrum of methyl stearate



Fg. 6: Mass spectrum of methyl 18-methylnonadecanoate

Antimicrobial assay

In cup plate agar diffusion bioassay ,the oil was screened for antimicrobial activity against six standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table (5) .The results were interpreted in terms of the commonly used terms (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (6) and (7) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 5 : Antibacterial activity of *Zea mays* oil :M.D.I.Z (mm)

Drug	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca	An
Oil	100	11	12	15	7	11	15

Table 6 : Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

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

Table 7 : Antifungal activity of standard chemotherapeutic agents against standard fungi

Drug	Conc. mg/ml	An.	Ca.
Clotrimazole	30 15 7.5	22 17 16	38 31 29

- Sa.*: Staphylococcus aureus*
- Ec.*: Escherichia coli*
- Pa.: *Pseudomonas aeruginosa*
- An.: *Aspergillus niger*
- Ca.: *Candida albicans*
- *Bs.: Bacillus subtilis*

In cup plate agar diffusion method *Zea mays* oil showed good activity against *Staphylococcus aureus* and the fungus

Aspergillus niger.However , it was partially active against other test organisms , but inactive against *Bacillus subtilis* (Table 5).

References

1. "Oxford English Dictionary" , online edition. 2012.

2. Solihah, M.A., Wan Rosli, W.I. and Nurhanan, A.R., *International Food Research Journal* **,19(**4),2012, 1533.

3. Zeringue, H. J., *Journal of Agricultural and Food Chemistry* ,**48**,2000, 921.

4. Guo, J., Liu, T., Han, L. and Liu, Y.,*Nutrition and Metabolism*, **6**, 2009, 47.

5. Namba, T., Xu, H., Kadota, S., Hattori, M. Takahashi, T. and Kojima, Y. , *Phytotherapy Research* ,**7** (3**),**1993, 227.

6. Kim, K. A., Choi, S. K. and Choi, H. S. , *Experimental and Molecular Medicine* ,**36**(6),2004, 545.

7. Habtemariam, S. , *Planta Medicine*, **64**(4),1998, 314.

8. Tahraoui, A., El Hilaly, J., Israili, Z.H. and Lyoussi, B. ,*Journal of Ethnopharmacology*, **110**,2007, 105.

9. Buhner, S. H. " The Natural Testosterone Plan; for Sexual Health and Energy", p. 77-78, Healing Arts Press, Vermt(2007).

10. Hu, Q. L. and Deng, Z. H. ,*African Journal of Biotechnology*, **10** (16),2011, 3163.

11. Eman, A. A. , *Journal of American Science* ,**7** (4**),**2011, 726.

12-Ahmed ,E., Hamdy,A., Khaled,F.,Tokayuki,S.,J. *Agric. Food Chem*.,**56**(13),2008,5021.

13-Harborne,J.B., " Phytochemical Methods" , John Willey and Sons ,New York, 1976.