World Journal of Pharmaceutical and Life Sciences
WJPLS

www.wjpls.org

SJIF Impact Factor: 3.347

GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *CUCURBITA PEPO* (CUCURBITACEAE) FIXED OIL

Abdel Karim M.[1*], Balsam, O.[2] and Inas,O.[3]

[1]Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.

[2]Bakt-Elrida University, Facuty of Education.

[3]University of Bahri, College of Applied and Industrial Sciences.

Article Received on 15/11/2016 Article Revised on 04/12/2016 Article Accepted on 25/12/2016

***Corresponding Author**
Prof. Mohamed Abdel Karim
Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.

ABSTRACT

Information on the constituents of medicinal plants used in Sudanese ethnomedicine is very scarce. Hence, this study was aimed to investigate the chemical constituents of the medicinally important *Cucurbita pepo* seed oil and to evaluate its antimicrobial activity. 26 components were detected by GC-MS analysis. Major constituents are: 9,12-octadecadienoic acid(47.17%),hexadecanoic acid(17.16%), 9-octadecenoic acid(16.49%) and methyl stereate(12.57%). The antimicrobial activity of the oil was evaluated via cup plate agar diffusion bioassay against six standard human pathogens(Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseudomonasa aeruginos*a and the fungi *Candida albicans* and *Aspergillus niger*). The oil showed strong activity against *Escherichia coli*. However, it was inactive against other test organisms. It seems that the oil is a lead for further optimization.

KEYWORDS: *Cucurbita pepo*, Fixed oil, GC-MS, Antimicrobial activity.

INTRODUCTION

The family Cucurbitaceae is a large family including about 95 genera and 950-980 species, many are crop plants being cultivated worldwide. Many plants belonging to this family are known for their medicinal and nutritional benefits.

Cucurbita pepo (Pumpkin) is indigenous to warm and temperate regions of central and north America. Seed oil and seeds have long been used for enlarged prostate gland and micturition problems associated with overactive bladder.[1] The plant is traditionally used as anti-inflammatory, antiviral, antiulcer, antidiabetic and analgesic.[2,3] Seeds contain about 50% fatty oil being dominated by linoleic acid , oleic acid and tocopherols.[4] They contain the bioactive constituents: avenasterol, spinasterol(Δ^7-sterols), sitosteol and stigmasterol(Δ^5-sterols).[1] Seeds are used traditionally for renal infections, hypertension, kidney stones, enlargement of prostate gland and skin infections.[5-13] Seeds also contain vitamins, carotenoids, tocopherols[14] beside fatty acids.[15] Tocopherols are natural antioxidants. The antioxidant capacity of *Cucurbita pepo seeds was* evaluated against stable DPPH radical and soybean lipoxygenase.[16] Cellular damage due to oxidatvie stress seems to be the fundamental mechanism undrlaying a wide array of human disorders including allergy, inflammation and cancer.[17] The effect of a seed protein on CCl_4 –induced injury was studied in model animals and significant increase in antioxidant enzymes including superoxide dismutase was observed.[18] This study demonstrated the ability of seeds to reduce several parameters associated with liver injury.Other species belonging to Cucurbiteae family like *Cucurbita ficifolia* demonstrated hypoglycemic properties and increased serum insulin levels and regenerated β- cells.[19,20] Tocopherol fraction of seed oil induced a decrease in glycemic levels in model animals.[21] A protein isolated from seeds showed significant hepatoprotective properties in acetaminophen-induced liver damage.[22]

The triterpene – squalene which has a positive effect on treatment of certain types of cancer-has been isolated from seeds.[23] Some acylated phenolics known as cucuritosides were reported from seeds.[24]

Fruit has a food value of: 80Kcal/100g. It contains: 2-3%(fats), 3%(proteins), 66%(carbohydrates) and 11% (fibre).[13] Mineral analysis gave (mg / Kg): Ca(179), Ni(0-5), P(11-38)Pb(0-29),Cu(3-9),Fe(1-37),Mn(0-5),Mg190), Na(159) and K(160).[25]

MATERIALS AND METHODS

Plant material

Fruits of *Cucurbita pepo* were purchased from the local market, Khartoum, Sudan. The plant was kindly authenticated by Institute of Aromatic and Medicinal Plants- Khartoum, Sudan.

Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness) was used for GC-MS analysis.

Test organisms

Cucurbita pepo oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in Table(1).

Table 1: Test organisms.

Ser. No	Microorganism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
5	*Aspergillus niger*	fungi
6	*Candida albicans*	fungi

METHODS

Extraction of oil from *Cucurbita pepo*

Powdered shade-dried seeds of *Cucurbita pepo* (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 shaked 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 shaked 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

Cucurbita pepo 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 2, while other chromatographic conditions are depicted in Table 3.

Table 2: Oven temperature program.

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

Table 3: 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

Preparation of bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°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 a suspension containing about 108-109 colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock 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 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.

Preparation of fungal suspensions

Fungal cultures were maintained on dextrose agar incubated at 25°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.

Testing for antibacterial activity

The cup-plate agar diffusion method was adopted, with some minor modifications, to assess the antibacterial activity. (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 (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the test solutions. Separate Petri dishes were designed for standard antibacterial chemotherapeutics (ampicillin and gentamycin).The agar discs were removed, alternate cups were filled with(0.1 ml) samples of each test solution 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°C for 24 hours.

The above procedure was repeated for different concentrations of the test solutions and the standard chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.

RESULTS AND DISCUSSION

GC-MS analysis of *Cucurbita pepo* fixed oil

GC-MS analysis of *Cucurbita pepo* 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

GC-MS analysis of the studied oil showed the presence of 26 components(Table 4).The typical total ion chromatograms (TIC) is displayed in Fig.1.

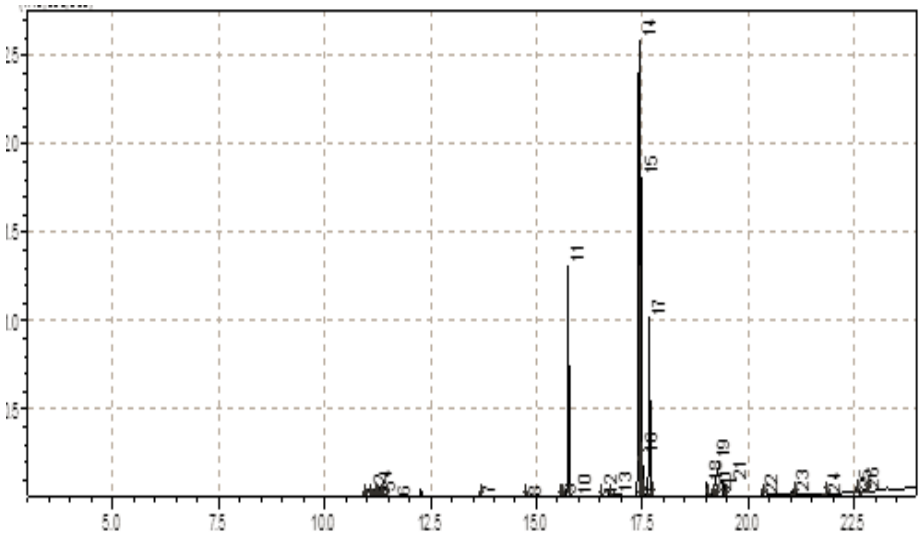

Fig 1: Typical total ion chromatograms (TIC)

Table 4: Contituents of *Cucurbita pepo* oil.

Peak#	R.Time	Area	Area%	Name
1	10.915	126010	0.09	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-met
2	11.065	111337	0.08	.beta.-curcumene
3	11.169	35892	0.02	.alpha.-Farnesene
4	11.231	41392	0.03	Isocaryophillene
5	11.290	407768	0.28	Butylated Hydroxytoluene
6	11.435	73470	0.05	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-
7	13.644	169016	0.11	Methyl tetradecanoate
8	14.720	90008	0.06	Pentadecanoic acid, methyl ester
9	15.554	255797	0.17	9-Hexadecenoic acid, methyl ester, (Z)-
10	15.593	45876	0.03	9,12-Hexadecanoic acid,methyl ester
11	15.752	25284010	17.16	Hexadecanoic acid, methyl ester
12	16.514	107735	0.07	cis-13,16-Docasadienoic acid, methyl ester
13	16.723	294089	0.20	Heptadecanoic acid, methyl ester
14	17.428	69502143	47.17	9,12-Octadecadienoic acid (Z,Z)-, methyl e
15	17.463	24294856	16.49	9-Octadecenoic acid (Z)-, methyl ester
16	17.495	2513315	1.71	9-Octadecenoic acid, methyl ester, (E)-
17	17.664	18524456	12.57	Methyl stearate
18	19.015	1522380	1.03	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethyl
19	19.162	268453	0.18	9-Octadecenoic acid, 12-hydroxy-, methyl
20	19.217	372000	0.25	cis-11-Eicosenoic acid, methyl ester
21	19.414	1483228	1.01	Methyl 18-methylnonadecanoate
22	20.325	138823	0.09	Phenol, 2,2'-methylenebis[6-(1,1-dimethyle
23	21.035	345424	0.23	Methyl 20-methyl-heneicosanoate
24	21.799	74959	0.05	Tricosanoic acid, methyl ester
25	22.539	320786	0.22	Tetracosanoic acid, methyl ester
26	22.718	954286	0.65	.beta.-Sitosterol
		147357509	100.00	

Some important constituents are discussed below.

9,12-Octadecadienoic acid methyl ester (47.17%)

Fig. 2 shows the EI mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z294, which appeared at R.T. 17.42 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.

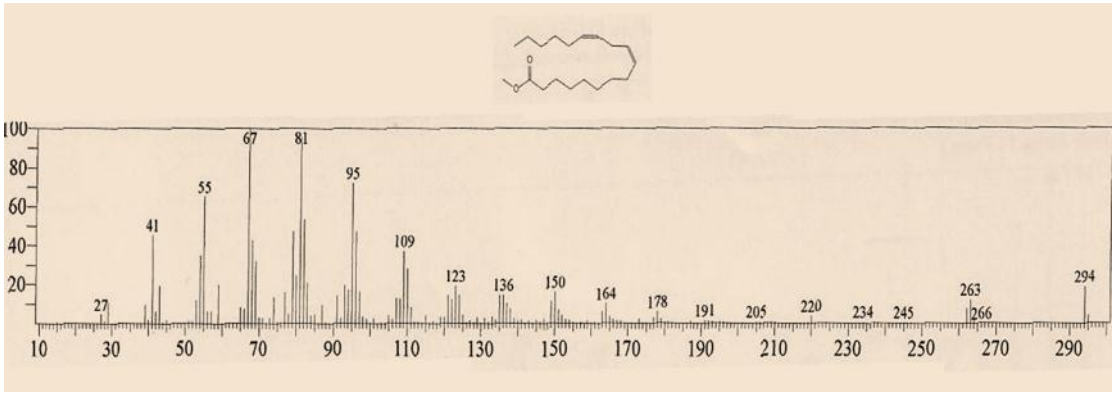

Fig. 2: Mass spectrum of 9, 12-octadecadienoic acid methyl ester.

Linoleic acid (9,12-octadecadienoic acid) can not be synthesized by humans and is available through diet.[26] It belongs to one of the two families of essential fatty acids. It exists in lipids of cell membrane and is used in the biosynthesis of arachidonic acid. It is converted enzymatically into mono-hydroxy products which are subsequently oxidized by some enzymes to keto metabolites. These metabolites are implicated in human physiology and pathology. Deficiency of linolate caused hair loss and poor wound healing in model animals.[27,28]

Hexadecanoic acid methyl ester(17.16%)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig. 3.The peak at m/z 270, which appeared at R.T. 15.752 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m/z239 is attributed to loss of a methoxyl function.

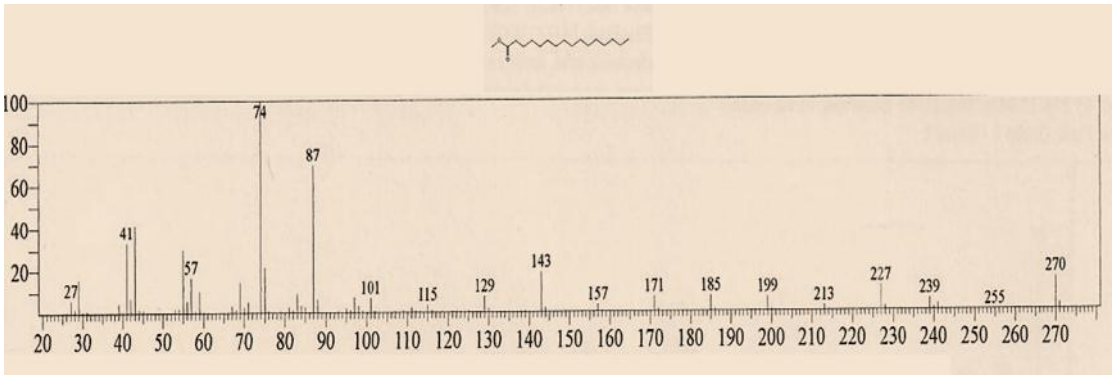

Fig. 3: Mass spectrum of hexadecanoic acid methyl ester.

Hexadecanoic acid (palmitic acid) is a saturated fatty acid .It is wide-spread in plants and humans. This acid is produced first during the synthesis of fatty acids[29] and is considered as precursor of long-chain fatty acids. Palmitic acid is a major lipid component of human breast

milk.[30,31] The acid finds applications in soaps and cosmetics industries. It is also used in food industry.

9-Octadecenoic acid methyl ester(16.49%)

Fig. 4 shows the EI mass spectrum of 9-octadecenoic acid methyl ester. The peak at m/z 296, which appeared at R.T. 17.46 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$, while the peak at m/z266 accounts for loss of a methoxyl function.

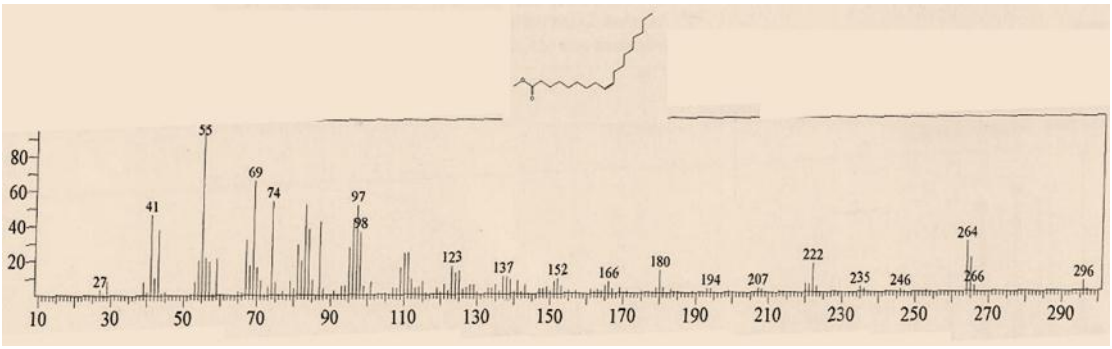

Fig. 4: Mass spectrum of 9-octadecenoic acid methyl ester.

Oleic acid (9-octadecenoic acid) is a common monounsaturated fat in human diet. It may be responsible for the hypotensive potential of olive oil.[32] Oleic acid finds some applications in soap industry and it is used in small amounts as excipient in pharmaceutical industries. It is also used as soldening flux in stained glass work. Oleic acid is employed as emollient.[33] The consumption of oleate in olive oil has been associated with decreased risk of breast cancer.[34]

Methyl stearate(12.57%)

Mass spectrum of methyl stearate is shown in Fig. 5.The peak at m/z 298, which appeared at R.T. 17.66 corresponds to $M^+[C_{19}H_{38}O_2]^+$ .The peak at m/z267 corresponds to loss of a methoxyl function.

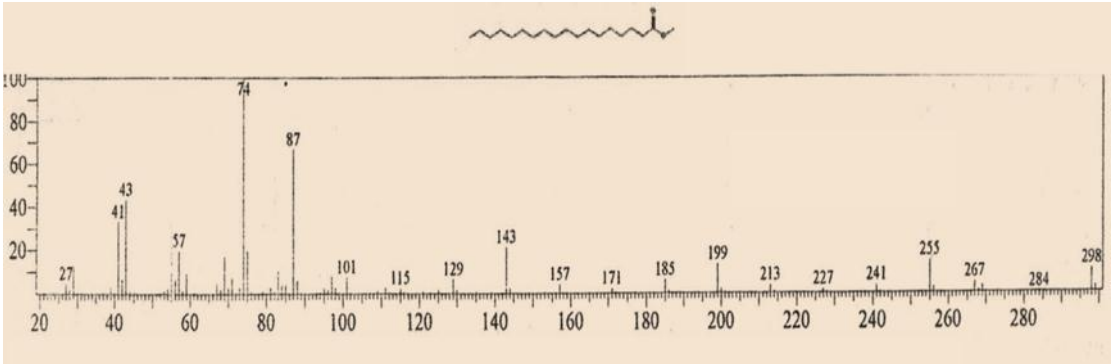

Fig. 5: Mass spectrum of methyl stearate.

Antimicrobial assay

In cup plate agar diffusion assay ,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 *Cucurbita pepo* oil.

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca	An
Oil	100	-	-	20	-	-	-

Table 6: Antibacterial activity of standard chemotherapeutic agents.

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	14	15	12

Table 7: Antifungal activity of standard chemotherapeutic agent.

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

Sa.: *Staphylococcus aureus*

Ec.: *Escherichia coli*

Pa.: *Pseudomonas aeruginosa*

An.: *Aspergillus niger*

Ca.: *Candida albicans*

Bs.: *Bacillus subtilis*

The oil showed strong activity against *Escherichia coli*. However, it was inactive against other test organisms(Table 5).

REFERENCES

1. Eurobean Medicines Agency, Committee on Herbal medicinal Products(HMPC)-1136022/2010.
2. Smith, B.D., *Sci.*, 1997; 276: 932.
3. Wang, X.L., Liu, J., Chen, Z.H., Gao, F., J. *Trad. Chin. Vet. Med*., 2001; 20: 6.
4. Rosa, M., *Medicinal Chemistry.*, 2016; 6(1): 12.
5. Dhaman, K. Gupta, A., Shama, D.K., Gill, N.S., Goyal,S.,*Asian J. Clin. Nutr*., 2012; 4: 16.
6. Yang, B.M., Yang, S.T., Chen, S.N., *Spec. Econ. Amin Plant.*, 2000; 3: 28.
7. Kessler, O.J., Keisari, Y., Servadio, C., Abramovici, A., *J. Urol*., 1998; 1049.
8. Madus, G., Lehrbuch der biologischen, Band III, Hildesheim Georg Olms Verlag., 1979; 2384.
9. State Administration of Traditional Chine Medicine, Zhong –hua –ben-cao, Shangahai Science Technology Press, Shanghai, 1999; P4600.
10. Bombardelli, E., Morazoni, P., *Fitoterapia.*, 1997; 68: 291.
11. Jiang, Z., Du, Q., *Biorganic Med. Chem. Letters*, 2011; 21: 1001.
12. Dabagham, F.H., Hamalineejad, M., Shojaei, A.M., *J. database Endocrinol*., 2012; 3: 70.
13. McGinley, M., The Encyclopedia of Earth Medicinal and Non-Medicinal Plants, Vol.2, Fruits, Springer, Neitherland.
14. Stevenson, D.G., Eller, F.G., Jane, J.L., Wang, T., *J. Agric. Food Chem.*, 2007; 55: 4005.
15. Sadutak, M., *Chem. Nat. Compounds.*, 2007; 43: 465.
16. Xanthopoulou, M.N., Nomikos, T., Fragopoulo, E., Antonopoulou, S., *Food Res. Intern*., 2009; 142: 641.
17. Masuda, T., Inaba, Y., Takeda, Y., Yamaguchi, H., *J. Agriculture and Food Chem*., 2003; 51: 1831.
18. Nkosi, c.Z., Opoku, A.R., *Phytother. Res*., 2006; 29: 935.
19. Alarcon-Agular, F.J., Hamandez-Galicia, E., Campos-Sepulveda, A.E., Xolapa-Molina, S., Tivas-Vichis, J.F., *J. Ethnopharmacol*., 2002; 82: 185.
20. Hang, Y., Chen,P., Jin,H., Zhu,L., *J. Biol. Macromol*., 2013; 62: 574.
21. Bharti, S.K., Kumar, A., Shama, N.K., Prakash, B.O., Jaiswal, S.K., *J.Formosan Med. Assoc*., 2013; 112: 676.
22. Nkosi, C.Z., Opoku, A. R., Terbianche, S.E., *Phytother. Res*., 2008; 20: 935.
23. Matus, Z., Molnar, P., Szabo, L.G., Acta *Pharm. Hung*., 1993; 63: 246.
24. Li,W., Koika, K., Tatsuzaki, M., Koide,A., Nikaido, T., *J. Nat. Prod*., 2005; 68: 1754.

25. Carvalho, L.M., Smiderie, L.A., Carvalho, J.L., Cardoso, F.S., Koblitz, M.G., *Food and Sci Technol*., 2014; 34.
26. Burr, G.O., Burr, M., Miller, E, *J.Biol.Chem*., 1930; 86: 587.
27. Cunnane, S., Anderson, M, *J.Lipid Res*., 1997; 38: 805.
28. Ruthig, D.J., Meckling-Gill, K.A. *Journal of Nutrition*, 1999; 129(10): 179.
29. Gunstone, F.D., John, L., Albert, J. "The Lipid Handbook", 3rd ed., Boca Raton, CRC Press., 2007; 30.
30. Kingsbury, K.J., Paul, S., Crossley, A., Morgan, D., *Biochemical Journal*, 1961; 78: 541.
31. Jensen, R.G., Hagerty, M.M. and Mc-Mahon, K.E., *Am. J. Clin. Nut*., 1978; 31(6): 990.
32. Terese, S., Barcelo, G., Benet, M., Alvarez, R., Bressani, R., Halver, J.E., Escriba, P.V. In: Proceedings of the Natural Academy of Science, 2008; 105(37): 13811.
33. Carresco. F., "Ingradients de Cosmeticos", Dictionario De Ingredientes,4th ed., 2002; P428: ISBN 978-84-613-4979-1.
34. Martin, M., Jose, M., Lydia, B., Jose, R., Rodinguez, A., F., Fermandez, R., Juan, C., Maisonneuve, P., *Intenational Journal of Cancer*, 1994; 58(6): 774.