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GC.MS Analysis of Pumpkin Seeds (*Cucurbita maxima*, *Cucurbitaceae*)

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ABSTRACT

The present study was conducted to confirm the traditional uses of pumpkin plant in the treatment of many ailments on scientific basis. Pumpkin seeds, also known as pepitas , are small. Flat. whitish green in colour. Most pumpkin seeds are encased in tan or soft white testa. Seeds were purchaced from Khartoum local market, then dried in shade and powdered. Active constituents were extracted in soxhlet-s apparatus using petroleum ether ,chloroform and ethanol in successive manner. Each extract was dried reserved in refrigerator for further investigations.. GC-Ms analysis for pumpkin seeds extract, and the structure activity relationship and correlation between the detected active constituents were don e Phytochemical screening was carried out for all extracts which revealed the presence of tannins and glycosides.

Key words: pumpkin seeds , phytochemical groups and GC-MS analytical compound.

INTRODUCTION

The pumpkin is a gourd of the genus *Cucurbita* and the family Cucurbitaceae, it is refers to cultivars of any one of the species *Cucurbita pepo* or *Cucurbita maxima* (Cred, 2008).

Pumpkin seeds, also known as pepitas are small, flat, green, edible seeds; Most pumpkin seeds are encased in tan or soft white husk (testa) (Watson, 2012).

Pumpin seeds

Pumpkin in Quran & Sunnah:-

Pumpkin mentioned in Quran Allah says :((and we caused by a tree of gourd to grow over him)) (Al-saaffat, 146), as the wisdom of the god in the pumpkin to germinate pumpkin plant on the prophet Yunus for food and healing.

The Mufassirin (commentators) said: The gourd (al-Yaqtin) is a kind of pumpkin. Some of them described the benefits of the pumpkin, such as: it grows quickly, it provides shade, it has large, smooth leaves, it keeps flies away and its fruit provides good nourishment: it can be eaten raw or cooked. It is known that the Messenger of Allah (peace and blessings be upon him) liked this kind of pumpkin and used to look for it on the plate of food (Tafsir Ibn Kathir).

Ibin Al-Jawziyyah for pumpkin fruit :(it cuts thirst, relief headache, laxative, and is one of the nicer foods, the seeds of pumpkin expel tape worm from intestine, and good for patients with kidney problems, bowel, arthritis and rheumatism). The prophet Mohamed (ppbuh) said :(it is strengthens the mind and the brain) (Albukhary, 5379).

Current uses of pumpkin seeds:-

Popularity of pumpkin seeds in various systems of traditional medicine for several uses as diuretic, anthelminth (for tapeworm). Because of their zinc content and anti mitotic effect, seeds are used to arrest enlargement of prostate gland. Also used in cystitis and minor kidney dysfunction. (Khare, 2007; Chonoko&Rufai., 2011).The medicinal uses of pumpkin seeds were adopted by oriental healer in the seventeenth century which includes:

- Pumpkin seeds may promote prostate health (Raver, Anne, Oct 2007).
- Anti` inflammatory benefits to arthritis (Hyun T, Barrette E- sep 2004)
- The pumpkin seeds and fruit increase power of intelligence and mental vitality (Carbin BE-1990).
- Address depression (Jennifer Murray, 2012).
- Prevention of Kidneys stones (Suphakarn VS-1987).
- Anticancer agent and Anti-oxidant activities (Liu et al., 2008).
- Anti-diabetic agent (Quanhong et al., 2005).
- Antihypertensive agent (decrease elevated blood pressure) (Zhang X-1994).
- Antimicrobial agent and for intestinal inflammation (Cowan, 1999).
- A Rich Source of Healthful Minerals and Protein (Credo-2008).
- Lower cholesterol blood level (Phillips KM, 2005).

MATERIALS AND METHODOLOGY

Materials:

Plant:

A pumpkin seeds was purchased from the local market of Khartoum city in February 2012; authenticated, dried at shade and reduced to fine powder using pestle and mortar in the laboratory as described by (Bean, A.R, 2006).The powder was stored dry, and used as the stock sample for further analyses.

INSTRUMENTS AND CHEMICALS

A wide range of instruments and chemical were used during the course of this study.

Method:

In this study all experiments were divided into four main parts:

1- Extraction of all active constituents in pumpkin seeds using ethanol as solvent.
2- Phytochemical screening test and antimicrobial activity tests.
3- GC-Ms analysis for pumpkin seeds extract.
4- The structure activity relationship and correlation between the detected active constituents and the traditionally reported uses of the pumpkin seeds.

GENERAL, GC ANALYSIS CONDITIONS:

We were likely need to optimize GC conditions depending on their analytical needs, equipment, and sample type. In general, a typical gas chromatography method will include:

- Column: HP-5 MS 30m length, inner diameter 0.25mm, 0.25mm film thickness.
- Temperature program: 110°C to 280°C at a rate of 10°C/min.
- Injector temperature 250°C.
- Injection volume: 2 µl.
- Carrier gas**:** helium at a constant flow rate of 1 ml/min.

RESULTS:

Phytochemical and antimicrobial result of pumpkin seeds:

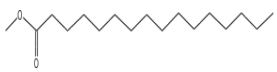
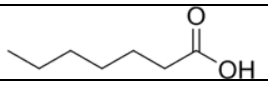
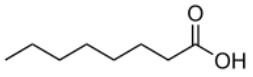
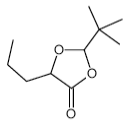
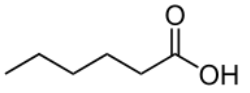
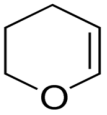
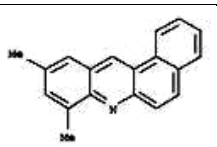
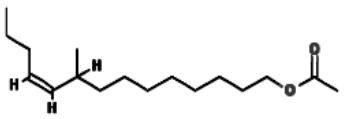
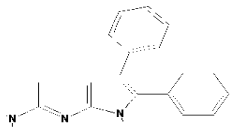
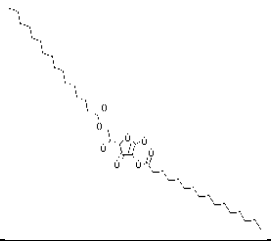
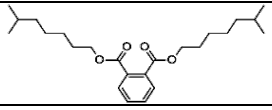
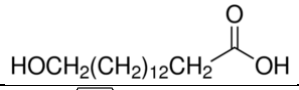
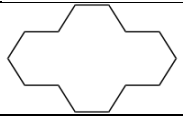
Alkaloids and glycosides, coumarins and flavonoids were found to be present in the ethanolic extract of pumpkin seeds.

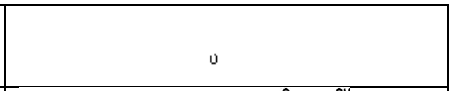
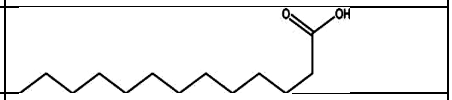
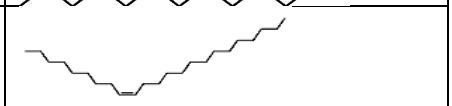
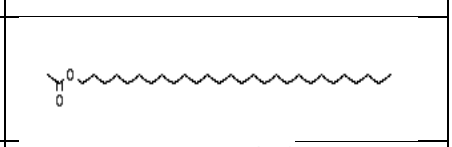
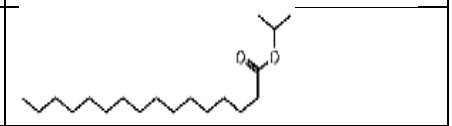
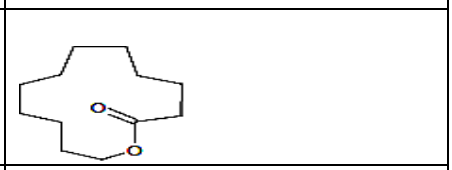
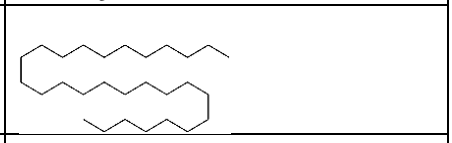
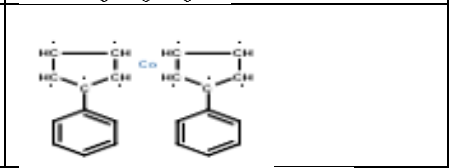
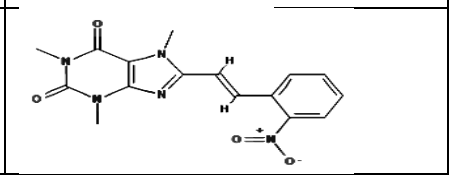
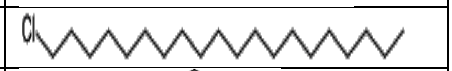
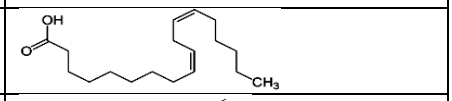
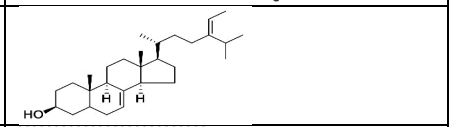
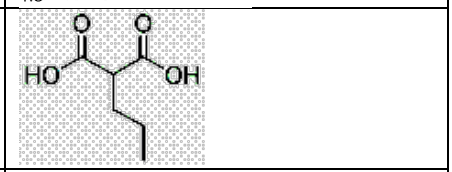
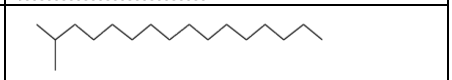
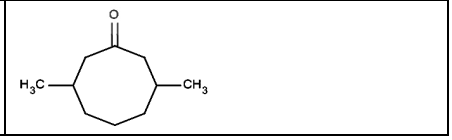
Ethanol extract of pumpkin seeds has a high activity against selected gram positives (***Staphyloccocus aereus*** and ***Bacillus subtilis)*** **and** gram negatives bacteria (***Pseudomonas aeruginosa*** and ***Escherichia coli).***

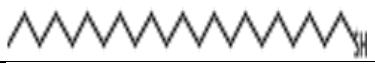
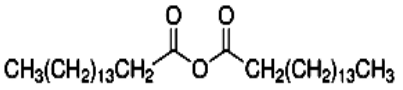
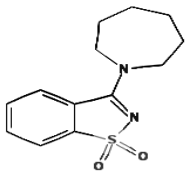
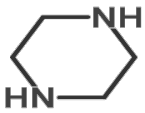
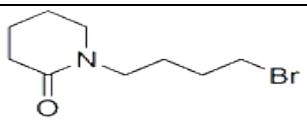
GC-MS Analysis Result of Pumpkin seeds:

This investigation was carried out to determine the possible chemical components from pumpkin seeds by GC-MS. The results pertaining to the GC-MS analysis of pumpkin seeds were given in table and figure bellow:

Table 1: The Name, Formula and Structures of phyto-chemical components detected in Pumpkin seeds by GC-MS:

№	Name	Formula	Structure
1	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	
2	Heptanoic acid	$C_7H_{14}O_2$	
3	Octanoic acid	$C_8H_{16}O_2$	
4	2-t-Butyl-5-propyl-[1,3]Dioxolan-4-one	$C_{10}H_8O_3$	
5	Hexanoic acid	$C_6H_{12}O_2$	
6	2H-pyran,3,4-dihydro-	C_5H_8O	
7	Benz[c]acridine,5,10-dimethyl-	$C_{19}H_{15}N$	
8	9-methyl-z-10-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	
9	1-Hexadecanethiol	$C_{16}H_{34}S$	
10	6-Amino-2,3diphenyl-1H-pyrrolo[2,3- b] pyridine	$C_{19}H_{15}N_3$	
11	1-(+)-Ascorbic acid-2,6-dihexadecanoate	$C_{38}H_{68}O_8$	
12	Heptadecane	$C_{17}H_{36}$	
13	1,2-Bezenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	
14	15-Hydroxy pentadecanoic acid	$C_{15}H_{30}O_3$	
15	Cyclotetradecane	$C_{14}H_{28}$	

16	Dodecane,1,1-oxybis	$C_{24}H_{50}O$	
17	Tridecanoic acid	$C_{13}H_{26}O_2$	
18	9-Tricosene,(z)-	$C_{23}H_{46}$	
19	Octadecane,1-iodo-	$C_{18}H_{37}I$	
20	Octacosyl acetate	$C_{30}H_{60}O_2$	
21	I-propyl hexadecanoate	$C_{19}H_{38}O_2$	
22	Oxacyclotridecan-2-one	$C_{12}H_{22}O_2$	
23	Octacosane	$C_{28}H_{58}$	
24	Cobaltocene,1,1'-diphenyl-	$C_{22}H_{18}Co^{-}{}_{6}$	
25	Purin-2,6-dione,1,3,9-trimethyl-8-[2-nitrophenethenyl]-	$C_{16}H_{15}N_5O_4$	
26	1- Chloroeicosane	$C_{29}H_{41}cl$	
27	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	
28	Delta-7-avenasterol	$C_{29}H_{48}O$	
29	Propanedioic acid, propyl	$C_6H_{10}0_4$	
30	Hexadecane, 2-methyl	$C_{17}H_{36}$	
31	Cyclooctanone,3,7-dimthyl	$C_{10}H_{18}O$	

32	1-Docosanethiol	$C_{22}H_{46}S$	
33	Palmitic anhydride	$C_{32}H_{62}O_3$	
34	1,2-Benisothiazole, 3-(hexahydro-1H-azepin-1-yl)-,1,1-dioxide	$C_{13}H_{16}N_2O_2S$	
35	Diethylenediamine	$C_4H_{10}N_2$	
36	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	

DISCUSSION

Miracles from Quran represent a real challenge in discovering of chemical ingredients and its relationship with remedies. Selection of pumpkin within billion of plants did not come by chance but it was an indirect message from God to search in-depth in the mysteries and secrets of this great plant and through this work, thirty six natural chemical compounds have been detected in the investigated part of pumpkin (seeds), they need more efforts and deep analysis and interpretation to elucidate them in the plant. Through the presence of thirty six of natural chemical compounds in pumpkin seeds can clearly see the extent of role of pumpkin seeds in the therapy.

ACTIVITY OF THE IDENTIFIED PHYTO-COMPONENTS IN SEEDS PUMPKIN

(1). **Hexadecanoic acid, methyl ester**:
Is a saturated fatty acid ester which has been reported to show antioxidant, hypocholesterolemic and nematocide activities (Sermalkani M et al, 2012).

(2). **Heptanoic acid**:
Also called Enanthic acid, it is an oily liquid organic compound used to esterify steroids in the preparation of drugs such as testosterone (Merck index, 11th edition.4581).

(3). **Octanoic acid:**
This saturated fatty acid, it can even help balance your blood sugar, improve digestion, balance your hormones, and keep your heart healthy, in one early experiment some evidence showed that medium-chain triglycerides (MCT), like caprylic acid, can help to treat children with epilepsy and can control seizures, However, there have been no follow-ups to confirm these findings (Physicians Desk Reference; 2001).There is also some evidence to suggest that caprylic acid can delay the progression of Alzheimer's disease, according to the Alzheimer's Association of America, but larger trials are needed to confirm this. It is also used as disinfectant in health care facilities, schools/colleges, animal care/veterinary facilities (P.J.; Mallard, W.G).

(4). **2-t-Butyl-5-propyl-[1,3]Dioxolan-4-one**:
Is a new drug with potent spasmolytic activity (Morsdorf K, et al. Pharmacology, 1970).

(5). **Hexanoic acid**:
Studies have also shown that hexanoic acid is useful for neurodegenerative disorders like Alzheimer's disease, Parkinson's disease and epilepsy, and it is an important ingredient in parenteral nutritional emulsions for the treatment of a variety of malabsorption condition (Journal of lipid research sept, 1967).

(6). **2H-pyran,3,4-dihydro-**:
Also known as dihydropyran, it is a heterocyclic compound. Has especially high cytotoxicity against tumour cells with less toxic effect on human normal non cancer cells, also it is antioxidant in vitro (Journal of bioactive and compatible compounds Oct, 1990). It is also antiviral agent and useful in the treatment of influenza (Abbott Lab, 1999).

(9 -32). **1- Hexadecanethiol & 1- Docosanethiol**:
Is an organosulfur compounds. There is evidence that plant containing organosulfur compounds has a protective against cancer in humans (Fukushima S, et al. J, 1997).

(10). **6-Amino-2,3diphenyl-1H-pyrrolo[2,3- b] pyridine**:
Is an indole compound, it exhibit anti-inflammatory, analgesic and antipyretic activities (Weiss E, Winey M, 1996).

(12). **Heptadecane**:
It exhibit potent anti-oxidative effect (ameliorate several oxidative stress related diseases) and anti-inflammatory agent (Kim DH, Park MH, 2013).

(13). **1,2-Bezenedicarboxylic acid, diisooctyl ester**:
Show potent antifungal activity against six pathogenic fungi (Asian Journal of microbiology, 2006). Also it has antibacterial effect (Rao P, PS, Karmarkan, s.m, 1986). This compound was identified as inhibiting melanogenesis, compound with a hypo pigmenting capability used in cosmetics (DT Nguyen, DH Nguyen, HL Lyun, 2007). Diisooctyl phthalate also showed significant activity against Salmonella (Gibbons JA, Alexander M, 1989).

(15). **Cyclotetradecane**:
Is a new potential copper chelating agent for neuroprotective in Alzheimer's disease which is associated with copper metabolism in adrenal cortex (Mort V, et al, 2006).

(17). **Tridecanoic acid**:
Has the highest antioxidant and anti-inflammatory activities (J. Agric, 2002).

(21). **I-propyl hexadecanoate:**
It is an emollient, moisturizer and thickening agent (JE Ramirez, M, 2015). Also has antimicrobial activity (Cardoso, V.M, 2006).

(22). **Oxacyclotridecan-2-one**:
Is cyclolactone compound; 30 years ago, research revealed that cyclic lactones' compounds have anthelmintics (especially control nematodes), antileishmaniasis and antibacterial activity (Brown DD, et al. Vet, 2012).

(23). **Octacosane**:
This saturated hydrocarbon with branched chain structure has mosquitocidal activity (J. Ethnopharmacol, 2004).

(24). **Cobaltoecne,1,1'-diphenyl-**:
Known as phenylcyclopentadienyl-cobalt, is an organometallic compounds used as a radiopharmaceutical to treat small cancers also in vitro it has protozoocidal activity (Loiseau PM, et al, 1988).

(25). **Purin-2,6-dione,1,3,9-trimethyl-8-[2-nitrophenethenyl]-**:
Known as antidepressant and anxiolytic agent (Chlon-Rzepa G, t al. Pol J, 2001).

(27). **9, 12-Octadecadienoic acid**:
It reduces skin scaling, prevents hair loss and stimulates wounds healing (Ruthing DJ, Meckling KA, 1999). Also has antibacterial (especially gram positive) and antioxidant activities (F Dilika, P. D Bremner, 2000).

(28). **Delta-7-avenasterol**:
Is unsaturated plant sterol, chemically similar to animal cholesterol then it has hypocholesterolemic effect. Research has indicated that delta -7-sterol competes with dihydrotestosterone DHT at the receptor sites in prostate, DHT is strongly responsible for enlarging the prostate and causing benign prostatic hyperplasia BPH but when this compound is present in diet it seems to help minimize the harmful effects of DHT on prostate i.e prevent BPH and prostate cancer (Health & Energy Food.com, 2015).

(31). **Cyclooctanone,3,7-dimthyl**:
Cycloalkanone like Cyclooctanone,3,7-dimthyl significantly lower cholesterol, triglycerides levels (G.S. Abermethyl, C, Piantados, 1974).

(34). **1,2-Benisothiazole, 3-(hexahydro-1H-azepin-1-yl)-,1,1-dioxide**:
In vitro, all benzisothiazole derivatives proved to have antipyretic and analgesic activities (Wang Z-Y, 2015), also it is known to have fungicidal and bactericidal effects (JP, 1973).
Benzisothiazole derivatives are cholinesterase inhibitors and are useful in enhancing memory in patients suffering from dementia and Alzheimer's disease (conditions of degenerative cholinergic neurons) (Becker et al, 1988).

(35). **Diethylenediamine**:
Known as **Piperazine**, is a cheap and readily available anthelmintic agent with very wide therapeutic index. It is used in the therapy of ascariasis (round worm) and oxyuriasis (thread worm) infestations. Also it was recently approved as an oral anticancer drug for clinical use in Japan and it has shown potent antiproliferative activity against colon, prostate, breast, lung and leukaemia tumours(Yarim et al., 2012).

(36). **2-Piperidinone, N-[4-bromo-n-butyl]-**:
N- Substituted lactam ring compounds as 2-Piperidinone, N-[4-bromo-n-butyl] - have anticonvulsant and neuro-protective activity (Wayne J. Brouillette and Garyl. Grunewald, 1983).

CONCLUSION

As a consequence of all these bioactive twenty two compounds and their important biological activities, Allah Almighty had chosen the pumpkin tree for the prophet Yunus (pbuh) because of its benefits and usefulness in the large scale in recovering health and strength, and because of the presence of several beneficial active ingredients. However, isolation of individual phytochemicals and subjecting it to biological activity will definitely give fruitful results. The

study revealed that pumpkin fruit studied was a potential food and has a multiple components medicine of benefits to the weak patients.

الملخص العربى:

هذه الدراسة اجريت لتأكيد او دحض الاستعمال التقليدى ولبيان المعجزة الربانيه من انبات نبات القرع علي سيدنا يونس عليه السلام من خلال:.

- اجراء المسح الكيميائى النباتى للمستخلص الكحولي العام لثمار القرع , حيث اثبتت الدراسة وجود مواد اساسيه مثل الاستيرويد, الفلافونيات, الصابونيات والقلايكوسيدات.

- اخضع المستحلص الايثانولى العام للثمار للتحليل الالى الغاز كروماتوغرافى لمعرفة المركبات الموجوده فيها , حيث تم التعرف علي اثنان وعشرون مركب.

- تم البحث عن الاثار العلاجيه والحيويه لكل المركبات الاثنان وعشرون التى تم التعرف عليها فى ثمار القرع , حيث اثبتت الدراسه فوائد الثمار من الناحية العلاجية، ، وفي موضوعنا هذا تبين لنا أن اليقطين يتميز بصفات وخصائص تجعله من أولويات النباتات التي تصلح لأن تكون مجاورةً ليونس عليه السلام .

التوصيات : نوصى باجرا دراسات متقدمه وضرورة عمل الرنين المغنطيسى وتحليل بالاشعه تحت الحمراء وكذلك لابد من دراسه اكلينيكيه لتوضيح العلاقه بين المكونات الكيمائيه وخواصها الدوائية

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