

Chemical characterization and antibacterial efficacy of Saudi sesame oil against human pathogenic bacteria

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Abstract

Bacterial infection and its resistance is a major health issue that affects millions of people throughout the world. There is always a need to search forth new and safest drug from natural resources to fight these challenges. Sesame seed essential oil is a rich source of protein with high medicinal value since the ancient time peoples are using for several remedies in Saudi Arabia. Therefore, the current study is aimed to discover the potential activity of the locally available sesame oil for antibacterial action based on ethnobotanical knowledge and traditional utilization as a therapeutic agent to treat several kinds of health problem in Saudi culture. Gas chromatography / mass spectrometry (GC/MS) analysis of sesame essential oil extracted from locally available sesame seeds represented 39 different chemical compounds. Sesamin and sesamol were the principal components alongwith fatty acids and triglycerides. Results indicated that the locally available sesame oil was found rich in sesamin contents (24.45%). The spectrum of antibacterial effect of sesame seed essential oil was exhibited significantly against *Escherichia coli* followed by *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* respectively. The results indicate that sesame oil was found most effective against three bacteria i.e. *E. coli*, *S. aureus* and *S. pyogenes*.

Key words: Antibacterial activity, ethnobotanical, GC/MS analysis, Saudi sesame oil.

Introduction

Sesame oil is the edible oil obtained from the seeds of plant *Sesamumindicum* L., family Pedaliaceae. Sesame is one of the oldest crops cultivated in the tropical and subtropical region for its edible oil. In Saudi Arabia, this plant is commonly known as "semsem" and is cultivated in coastal plains and mountain regions of Jazan province (Al-Turki*et al.*, 2019).Sesame seeds are a rich source of proteins, lignans, fatty acids and various other bioactive components and have been widely used as traditional medicine for several remedies in Saudi Arabia since ancient times (Al-Attar *et al.*,2017;

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Koshak et al., 2019). Sesame oil has high nutritional value and used for cooking and salad dressings in various countries. In Saudi Arabia, it is an important part of kitchen contributing a significant role in the preparation of many varieties of sweetspies and taken as such with dates and olive oil (Özcan, and AL-Juhaimi, 2015). In many other South-East Asian societies, sesame oil is an important ingredient of various cosmetic products and body massage oils. Its commercialized products are available as an important ingredient in soap, cosmetics, lubricants, and medicines. In the pharmaceutical industry, sesame oil is used as an emollient, carrier oil for other essential oils and fatsoluble compounds. Sesame oil is reported to have significant antineoplastic, neuroprotective, antioxidant, anti-inflammatory and lipid-lowering activities (Deme et al., 2018; Narasimhulu et al.,2018; Majdalawiehet al.,2019; Mekkyet al., 2019; Ruankham et al., 2019). Recently a researcher hypothesized that adding sesame oil in the nostril can prevent the spreading of coronaviruses (Wen Fan et al., 2020). Sesame oil



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has high concentrations of unsaturated fatty acids such as linoleic and oleic acid that contribute the potential in prophylaxis and treatment against many bacterial infections (Dilika et al., 2000; Uzun et al., 2008). A bacterial infection is a serious health issue affecting millions of people worldwide every year. Increased antibiotic resistance against multiple drugs in different bacterial strains is posing a great threat worldwide thus limiting the antibiotic choice. Some studies have suggested that there is a considerable increase in the rate of antibiotic resistance in Saudi Arabia over a last few decades (Zowawi et al., 2016). Considering the role of unsaturated fatty acids against bacterial infections, Saudi sesame oil is a rich source of them and it can evaluated for its antibacterial activity against certain human pathogens.

Material and Methods

Procurement of chemicals and Sesame oil

Sesame seeds purchased from the local market of Jazan, Saudi Arabia. The standard sesame oil (SSO), media and broth purchased from Sigma Aldrich USA.

Extraction of oil from sesame seeds

Extraction of sesame oil was performed using cold press technique described by Kate *et al.* 2014. Approximately 500 g of sesame seeds were washed and dried in an oven under nitrogen at room temperature for 2 h. The dried seeds were milled, kneaded, sparkled with warm water intermittently, and pressed to remove the oil. The oil was allowed to settle for 24 h and decanted in the clean container. The resulting oil was then filtered, centrifuged (3000 rpm for 5 minutes), separated named ESO and stored at 4°C in the refrigerator for further analysis.

Physiochemical properties

The extracted sesame oil (ESO) was evaluated for its physicochemical properties and compared with standard sesame oil (SSO). The specific gravity, acid value, iodine value, saponification value, solidification temperature of fatty acids and the unsaponifiable matter were determined by reported methods described by Olaleye *et al.*,2018 and USP general chapters 841 and 401 (US pharmacopeial convention 2015)

GC/MS Analysis

GCMS analysis of ESO and SSO was performed using Thermo Scientific GC/MS equipped with AS 3000 autosampler, trace ultra GC and ISQ^{TM} detector. The separation was achieved using capillary TR-5MS column (30 m \times 0.25 mm ID \times 0.25 µm) and helium carrier gas at a flow rate of 1.2 ml/min. The sesame oil was diluted 10 times v/v with n-hexane, filtered through 0.45µm PTFE filter and injected in splitless mode (2 µL). The injection port was set at 320°C and temperature of the oven was initially set at 70°C with holding time for 5 minutes. The oven temperature was subsequently ramped to 205°C at a rate of 5°C/min for 5 minutes, 280°C at a rate of 5°C/min for 5 minutes, 290°C at a rate of 5°C/min for 5 minutes, and finally to 300°C at a rate of 5°C/min for 5 minutes. For GC-MS detection, an electron ionization system with ionization energy 70 eV was used. Ion source and MS line transfer temperature were set at 320 °C and 340°C respectively with 0.6s scan time. The mass range of 60-900 amu was selected using positive ion mode and spectra was recorded solvent delay time of 5 minutes to avoid initial solvent peaks. The mass spectra were interpreted using Xcalibur software and the fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using the NIST, MAINLIB and REPLIB built-in libraries. The relative percentage amount of each component was calculated by comparing its average peak area to the total area.

Compound identification

identified based The ESO was on its physicochemical characteristics and compared with SSO. The experimentally determined values were also compared with those of standardpharmacopoeial values. The components of ESO and SSO were identified by using match factor (SI) and reverse match factor (RSI) thresholds of 900 and above between measured spectrum and standard library spectrum. Percentage areas of each component were obtained by using Xcalibur software without the use of any internal standard and are uncorrected.

Antibacterial Analysis

Standardization of bacterial cultures

Six bacterial strains namely *Staphylococcus aureus* (S. aureus), Streptococcus pyogenes (S. pyogenes), Bacillus subtilis (B. subtilis), Escherichia coli (E.



coli), Pseudomonas aeruginosa (P. aeruginosa), and Klebsiella pneumoniae (K.pneumoniae) were used for this study was taken from the department of microbiology general hospital, Jazan. Briefly, 24 h culture was prepared and standardized by gradient dilution from 10^{-1} to 10^{-7} with nutrient broth. The viability of bacterial culture was identified by assessing colony forming unit in 1 ml (CFU/ml) as described Cappauccino and Sherman, 2014.

Antibacterial spectrum analysis

The antibacterial susceptibility test was done as established by Alam et al., 2016 and Moni et al., 2018. Briefly, Muller Hinton (MH) agar plates were prepared for performing the antibacterial study (Valgas, et. al., 2007).Bacterial subcultures were prepared from the stock culture and after 24 h incubation; the culture was subjected to antibacterial studies. Agar well diffusion technique was performed for sesame oil (ESO) and disc diffusion was utilized for ciprofloxacin disc (5 mcg/disc) as standard. The inoculation was done by dipping a sterile cotton swab into the standardized (CFU/ml) culture individually with various organisms and streaked on the MH agar plate by rotating the petri dish in order to distribute the culture evenly. The plates were allowed dried for about 10 minutes before administration of sesame oil (ESO). The agar well diffusion technique was performed by punching holes on the inoculated MH agar plates using standard sterile stainless-steel borer. The agar plates were incubated at 37°C for 24 h and the antibacterial spectrum was assessed by the development of inhibitory zones.

Results and Discussion

The sesame oil extracted from its seed by cold press technique (Kate et al., 2014) appeared as a light yellow color with a characteristic fragrant odor. The ESO was evaluated for its physicochemical properties and compared with SSO and standard codex values depicted in Table.1 (US pharmacopeial convention 2015).The results suggested that ESO and SSO conform to the specific tests listed in USP. Both ESO and SSO showed no reddish color on boiling with equal volumes of amyl alcohol and sulphur in carbondisulphide solution indicating the absence of the cottonseed oil (US pharmacopeial convention 2015). The GC-MS analysis of ESO and SSO

revealed the presence of lignans, fatty acids, triglycerides, phytosterols, vitamins, triterpenes, steroids, hydrocarbons, fatty alcohols, and phenolic compounds. In ESO, a total of 35 compounds have been identified with the mass balance of 90.70% of total peak area (Figure 1) whereas SSO revealed a total of 38 compounds with the mass of 97.04% (Figure 2). The compounds with less than 900 SI and RSI are not reported. The details of all identified compounds with their retention time, molecular weight, molecular formula, and % peak area etc. are represented in Table 2. The comparison of compounds between ESO and SSO showed that except for variation in the content of sesamin and some additional triglycerides observed in SSO, all other major components were observed at a similar level. Triglyceride, triolein was also not observed in SSO.(+)-Sesamin, a benzodioxole and a furofuranlignan, was found to be the predominant component (24.45%) alongwith sesamol(17.91%) in ESO. Both these components were observed in SSO with variation in the content of sesamin (28.24%). They had been reported to have excellent antioxidant and the anti-bacterial activities thereby preventing the spoilage of food (Mahendra et al., 2015). The obtained quantities were similar to those reported in the literature with only slight variations. The quantity of these natural antioxidants showed variations with the country of origin but have similar chemical compositions indicating the role of environment or genetic conditions of the seeds (Carlsson et al., 2008). Linoleic acid (4%) was found to be the main polyunsaturated fatty acid in ESO followed by oleic acid (3%) (mono unsaturated fatty acid). The contents of saturated fatty acids, stearic acid, and palmitic acid were comparatively lesser than unsaturated fatty acid and were mainly observed as esters. These results are in good agreement with a high iodine value (106) of sesame oil suggesting more degree unsaturation in the ESO and SSO. The contents of saturated and unsaturated fatty acids obtained in ESO and SSO differed considerably from the literature (Were et al., 2006b; Uzun et al., 2008). The variations obtained could be attributed to extraction procedure employed by the author, use of FID detectors and derivatizing techniques for sample analysis. Triglycerides such as triolein (0.64%), 2oleoglycerol (1.38%), glycerol-1-palmitate 3-oleate (1.5%) and 1,2-Dipalmitoyl-rac-



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Parameters	Extracted	Standard Sesame	United State Pharmacopeia (USP)
	Sesame oil (ESO)	Oil (SSO)	Value
Yield	0.67 %		
Specific gravity	0.912	0.912	0.912
Color	Light Yellow	Light Yellow	Light Yellow
Acid Value (For free fatty	1.9 mL	1.9 mL	NMT 2.0 mL of 0.020 N NaOH
acid)			required for neutralization
Iodine value	110	112	103-116
Saponification value	190	191	188-195
Solidification temperature	23°C	23°C	20-25°C
for fatty acids			
Unsaponifiable matter	1.3 %	1.3 %	NMT 1.5 %
Cotton seed oil	Not present	Not present	

Table 1. Comparison of physiochemical properties of extracted sesame oil (ESO), standard sesame oil (SSO) and United State Pharmacopeia (USP) value

Table 2. Chemical compounds of Sesame oil sample (ESO) with their retention time, molecular weight, molecular formula, and % peak area using GC/MS

SN	RT	Name of Compound	Molecular	Molecular	% Peak	% Peak
T •			Formula	Weight	Area (ESO)	Area (SSO)
Lignans (1) Secondary (2011) 90(254) 24.45						28.02
1	58.03	(+)-Sesamin	C20H18O6	354	24.45	28.92
2	59.16	Sesamol	C/H6O3	138	17.91	17.98
Fatty	Fatty acids and triglycerides				4.01	4.00
3	37.33	Linoleic acid	C18H32O2	280	4.01	4.02
4	37.00	Oleic acid	C18H34O2	282	3.01	3.01
5	40.10	Stearyloleate (Oleic acid	C36H70O2	534	1.51	1.52
		octadecyl ester)				
6	44.26	3-Hydroxypropyl oleate	C21H40O3	340	0.57	0.56
7	45.21	2-Monoolein; 2-Oleoylglycerol	C21H40O4	356	1.38	1.38
		(Oleic acid 2-hydroxy-1-				
		(hydroxymethyl)ethyl ester)				
8	62.09	Eicosyloleate	C38H74O2	562	0.15	0.14
9	63.56	Glycerol 1-palmitate 3-oleate	C37H70O5	594	1.51	1.49
10	41.36	1,2-Dipalmitoyl-rac-glycerol	C35H68O5	568	1.62	1.59
11	68.17	Triolein	C57H104O6	885	0.64	NF
12	68.12	1,2-dilinoleoyl-3-oleoyl-rac- glycerol	C57H100O6	881	NF	0.32
13	65.81	1,2-dioleoyl-3-linoleoyl-rac- glycerol	C57H102O6	883	NF	0.42
14	67.89	Trilinolein	C57H98O6	879	NF	0.18
15	65.12	1,2-dioleoyl-3-palmitoyl-rac-	C55H102O6	859	NF	0.21
		glycerol				
Phyt	Phytosterols					
16	58.58	Campesterol	C28H48O	400	4.10	4.09
17	60.46	β-Sitosterol	C29H50O	414	16.13	16.12
Trite	erpene					
18	50.74	Squalene	C30H50	410	0.23	0.24
Phen	olic vola	tile oil				
19	18.49	Eugenol	C10H12O2	164	0.12	0.13

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Vitamin E						
20	54.75	Gamma-Tocopherol	C28H48O2	416	3.46	4.48
Aliphatic hydrocarbons						
21	19.20	1-Hexadecene	C16H32	224	0.12	0.13
22	32.76	1-Nonadecene	С19Н38	266	0.34	0.35
23	43.49	Heptacosane	C27H56	380	0.70	0.69
24	47.31	Heneicosane	C21H44	296	1.07	1.09
25	50.55	Pentacosane	C25H52	352	1.23	1.24
26	52.95	Tetratetracontane	C44H90	618	0.20	0.20
Alip	hatic alco	ohols				
27	24.20	1-Hexadecanol	C16H34O	242	0.35	0.35
28	28.66	1-Hexadecenol,	C16H32O	240	0.37	0.58
29	38.40	1-Eicosanol	C20H42O	298	0.32	0.33
Aroi	natic hyd	lrocarbons				
30	7.29	Trimethyl benzene (mixture)	C9H12	120	1.86	1.87
31	10.02	4-Ethyl-1,2-dimethylbenzene	C10H14	134	0.15	0.15
32	21.26	2-Methoxynaphthalene	C11H10O	158	0.14	0.15
33	22.38	2,4-bis(1,1-dimethylethyl)-Phenol	C14H22O	206	0.36	0.36
34	49.79	Di-(2-ethylhexyl)-tetraphthalate	C24H38O4	390	0.38	0.40
Ster	oids					
35	53.83	2-(3-acetoxy-4,4,14- trimethylandrost-8-en-17-yl) propanoate	C27H42O4	430	0.37	0.36
36	60.75	24-propylidene-(3β)-Cholest-5- en-3-ol	C30H50O	426	1.11	1.12
37	61.01	4-Pregnen-6β,17β-diol-3,20-dione	C21H30O4	346	0.26	0.25
38	61.30	(3β)- Stigmasta-5,24(28)-dien-3- ol,	C29H48O	412	0.32	0.31
39	63.79	21-hydroxy-Pregn-4-ene-3,11,20- trione	C21H28O4	344	0.31	0.31

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 Table 3. Chemical structures of main components present in extracted sesame oil (ESO) using gas chromatography mass spectrometry (GCMS)

Name	Structure
(+)-Sesamin	
Sesamol	HOOO
Linoleic acid	







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Table 4. Antibacterial effects of sesame oil (ESO) against human pathogenic bacteria

Organisms	Concentration of 24 h culture	Zone Of Inhibition in (mm) (Mean ± S.D)*			
	(cfu /ml)	Local Sesame Oil	Ciprofloxacin (5 µg/disc)		
Bacillus subtilis	2×10^{-5}	18.7 ± 1.2	27.2 ± 1.2		
Staphylococcus aureus	3×10^{-4}	25 ± 1.7	26.6 ± 1.3		
Streptococcus pyogenes	3×10^{-4}	22.3 ± 2.5	24.16 ± 0.4		
Escherichia coli	2×10^{-5}	27.3 ± 1.5	28 ± 2.1		
Pseudomonas aeruginosa	2×10^{-4}	12.7 ± 1.15	26.6 ± 0.6		
Klebsiella pneumoniae	2×10^{-4}	14.6 ± 2.11	28.8 ± 1.3		

* Each value is the mean of six batches with standard deviation.

glycerol (1.6%) were also present in considerable quantities in ESO.Interestingly, some additional triglycerides such as 1,2-dilinoleoyl-3-oleoyl-racglycerol (0.32%), 1,2-dioleoyl-3-linoleoyl-racglycerol (0.42%), trilinolein (0.18%) and 1,2dioleoyl-3-palmitoyl-rac-glycerol (0.21%) were also observed in SSO. Amongst phytosterols, betasitosterol (16.13%) and campesterol (4.10%) were present in good quantities indicating the role of sesame oil in alteration of lipid metabolism and exhibiting more potent cholesterol-reducing effect (Deme *et al.*, 2018). The antioxidant, vitamin E (3.46%) (gamma-tocopherol) was also present

there by making the sesame oil as a good carrier for other essential oils. The terpenes, such as squalene (0.23%) and eugenol (0.12%) were also present in small quantities, responsible for aroma and flavor of sesame oil. Other bioactive components such as substituted xanthines, phenols and naphthols, steroids, fatty alcohols and hydrocarbons were also present.The structures of major components observed in ESO are depicted in Table 3.

Bacterial resistance is the major threat against various antibiotics and antibacterial drug of the current therapeutic era (Cohen, 1992; Nascimento, *et al.*, 2000).Therefore, developing a novel



antibacterial or the antibiotic molecule is very important in the current therapeutic world. Unfortunately, there is no assurance that new molecules never develop resistance and become inactive. In this connection, bioactive substances from the herbal origin are an alternative source for developing antibacterial which has been focused by various researchers across the world for the past three decades. In this study, the effectiveness of cold press extract of sesame oil was screened against certain human pathogenic bacteria for demonstrating the potentiality. The results are summarized in Table.4 demonstrating the potential efficacy of extracted sesame oil against grampositive bacteria including S. aureus, S. pvogenes, B. subtilis, and Gram-negative bacteria includes E. coli, K. pneumoniae, P. aeruginosa. The spectrum of activity was directly proportional to the diameter of the zones of inhibition. The study demonstrates

that the extracted sesame oil exhibited a wide spectrum of activity against Gram-positive and Gram-negative bacteria but predominant activity was observed against E. coli, S. aureus, and S. pyogenes. Bawazir et al., (2018) reported the antibacterial potential of Yamni sesame oil against pathogenic bacteria such as Escherichia coli, Staphylococcus aureus, Salmonella typhi, Candida albicans, and fungi strains such as Aspergillus niger and Aspergillus flavus. Furthermore, their report indicated that Semsumsanani and Semsumdwaini showed better antifungal activity against Aspergillus niger and Aspergillus flavus when compared to bacterial strains. Interestingly, the oil of Semsumshahri and Semsumhadedi did not show any potential activity against bacteria or fungi (Bawazir et al., 2018). The Saudi sesame oil results are more prompt and effective against certain



Figure 1. Chemical compounds of Extracted Sesame Oil (ESO) using gas chromatography mass spectrometry (GCMS)





Figure 2. Chemical compounds of Standard Sesame oil (SSO) using gas chromatography mass spectrometry (GCMS).

pathogenic bacteria. Some studies have shown that relationship exists between the chemical structures of the most abundant compounds in the tested extracts or essential oils and the antimicrobial activity (Farag et al., 1989). Shitu et al., 2007 reported the antimicrobial activity of sesame oil against skin pathogen such as staphylococcus and streptococcus as well as some fungus (Shitu et al., 2007a). Some other researchers also reported the comparative studies of the crude extracts of sesame leaves against common pathogenic bacteria and found a high level of inhibition for most of the microorganism except S. aureus (Ahmad et al., 2009). Thus, sesame oil from Saudi Arabia showed the spectrum of activity against certain human pathogenic bacteria that had been screened in this study.

Conclusions

The present findings indicated that sesame essential oils are effective against certain selected pathogenic bacteria as it may be a component of antibacterial formulation that will help to minimize the bacterial infection problem in Saudi Arabia.

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Disclosure statement

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