Physio-Chemical Properties and Anti-Microbial Activities of Date Seed Oil's in Saudi Arabia

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(Received on 4th September 2023, accepted in revised form 1st February 2024)

Summary: The preparation of soap from extracted date seed oil along with anti-microbial activities, Gas Chromatography-Mass Spectrometry (GC-MS), and physio-chemical properties comprise the areas examined by the methodology implemented in this research. Ascertaining the fatty acid content in such date seed oil embodies the research objective of this investigation. Color Criteria should be within the range of 2.8 to 13.5 (red-yellow),acid value 1.42 Mg KOH/g oil, flash point 245C°, a refractive index of 1.468, a Kinetic viscosity of 20cp, and a density of 0.9188g cm^{-3} comprised the physical assessment of the date oil. On the other hand, a peroxide value of 7.4 mg of peroxide/kg oil, a saponification value of 0.279 mg g-1, an acid value of 2.67, and an iodine value of 74.31 gm I2/100 gm fat comprised the chemical assessment. GC-MS was utilized to ascertain the quantity of fatty acid content in the date seed oil. Oleic acid (Omega-9) and Linoleic acid (Omega-6) comprised the unsaturated fatty acid content in such oils producing values of 38.25% and 2.33% respectively, where the percentage represents the total fatty acid components. Palmitic acid and lauric acid encapsulated the saturated fatty acid content producing values of 0.42% and 35.76%, respectively. The elemental constituents found in the date oil include calcium (29 ppm), magnesium (0.359 ppm), iron (1.27 ppm), and sodium (70 ppm).

The date seed oil shown demonstrated anti-bacterial activity high effect 30mm against *Escherichia coli* (Gram negative) and moderated effect 20mm against *Staphylococcus aureus*, (Gram positive) respectively compared to standard drug, Cephradine. Which may be due to bioactive compounds including plant-derived phenolic molecules (589mg/100g). In this way, the date seed oil had moderate inhibitory effect 40mm against *Candida albicansa* and low inhibitory effect 20mm against *Aspergillus flavus*. which may be increases with the increase of the compound concentration compared with standard antifungal agent Nizoarm. While the date oil extract showed good properties in soap in terms of viscosity, Hardness, color and moderate solubility.

Key words: Physio-chemical properties, Anti-microbial activities, Date seeds oil's.

Introduction

Investigation of soap preparation, anti-microbial activities, and properties of date seed oil comprises the research objective of this study. As [1] observes developed and in developing countries alike are increasingly acknowledging the economic and medical advantages of traditional medicinal plants.

Particularly in countries such as Saudi Arabia, the proteins, fats, minerals, and vitamins that reside within the palm tree date reflect its economic and nutritional advantages. Furthermore, the academic focus on attaining food components from a natural source represents another benefit that palm tree dates used as an antioxidant. [2] State that the increasing acknowledgement that foods drawn from non-natural sources represent a hazard to health provokes the demand of natural sources of food components.

Different parts of the selected date seeds were recognized as components of the traditional medicine in Kingdom of Saudi Arabia. They were arranged in alphabetical order together with their family, synonyms, vernacular names, habitat, description, distribution, chemical constituents and medicinal uses. The selected date seed would be subjected to detailed antibacterial screening. Recently the use of plant extracts as natural antioxidants has gained increasing interest because of the global trend of restriction in use of scientific substance [3].

As noted by [4-7], feed and food industries, medicine, cosmetics, and pharmaceuticals represent possible areas where date seeds and their components could be utilized.

Classification of Palm date

The palm date is classified as follows: *Viridiplantae*, *Phoenix dactylifera*, belongs to the family*:* *Arecaceae* which includes only one genus and one species, in Kingdom Saudi Arabia.

Kingdom:	*Plantae* (*Viridiplantae*)
Family*:*	*Arecaceae*
Genus:	*Phoenix (Arecaceae)*
Species:	*Phoenix dactylifera L. (1753)*
Binomial name*:*	*Phoenix dactylifera*.
Vern Name:	*palm date*

State that within developing countries, international trade and day-to-day health and its associated practices have prioritized aromatic and medicinal plants [8]. Contemporary academic works conducted by [9-12], are the only studies to address this area in the literature. These works note the extractable high-value constituents within date seeds.

Experimental

Material

Seeds collection

The seeds was obtained from a local date's market at Sharourah city and date Oil Manufacturing.

Extraction methods

A mass around (900 - 1000g) of seeds was utilized for oil extraction. Subsequently, an automatic machine (retsch-100) was utilized for 3 meters to crush and dry the seeds into miniscule granules after the seeds were divided from the fruits. A Soxhlet apparatus served to extract total lips from the seeds using petroleum ether. The extraction procedure lasted between six to eight hours. The oil was stored at a deep-freeze temperature of (-20 C°) within a dark container after being weighed and collected following the evaporation of the solvent on a rotary evaporator experiencing diminished pressure which generated the oil without petroleum ether.

Fatty acids composition

Preparation was performed in line with [13], regarding the oil fatty acid methyl esters. Prior to shaking, (7ml) of methanolic H_2SO_4 was added after 1 ml of the generated oil was incorporated to (7ml) of methanolic NaOH (0.5 M). Subsequently, the mixture was left untouched throughout the night. Saturated NaCl was incorporated to the reaction solution after the addition of (2ml) of petroleum ether. Following thorough shaking of the solution, division into two layers was permitted. Anhydrous Na_2SO_4 was utilized to dry (1ml) from the upper layer that was moved to a novel tube.

Identification of Free Fatty Acid

The identification of the free fatty acid (FFA) content of the oil sample must occur prior to commencing the titration procedure. The implementation of an acid-base titration technique enabled the identification of the FFA concentration within the date seeds oil. Blank titration was performed utilizing an indicator of phenolphthalein and a conical flask holding (10 ml) of isopropanol. A flask was filled with the solution from a burette consisting of (0.025 M KOH). A pre-arranged quantity of oil extracted from date seeds was used within the sample titration procedure. As per [14], the FFA concentration of raw oil was determined utilizing Eq. 2.

$$\text{Acid number} = \frac{(\text{Volume used in sample titration} - \text{Volume used in blank titration}) \times \text{Mass of KOH in g/l}}{\text{total volume of oil used}}$$

Analytical techniques

Evaluation of Viscosity

The HAAKE viscometer 6 plus (Chromatography lab–Egypt) was utilized to identify the viscosity at (30C°).

*Evaluation of Refractive index***:**

First, distilled water was utilized to alter the refractometer. Then, the Bellingham and Stanley Ltd London (No 918095 instrument, Made in England) was utilized to ascertain the refractive index.

Evaluation of Density determination

The Puchnometer Kit (Analytic lab-Egypt) for AEP balances (AEP-250g-max250g-1;d=1mg), density determination = g/c^3) was utilized to determine the density.

*Evaluation of Color determination***:**

The Tintometer typed or Lovibond instrument (Tintomet Ltd; color laboratory–Egypt) was utilized to ascertain the color.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of date oil Gas Chromatography, Mass Spectrometer (GC.MS):

The GC-MS (Shomadzu, Japan, TQ8040) fitted with a flame ionization detector (FID) was utilized to identify the chemical composition of the date seed oil. An inner diameter of (0.25 mm), a film thickness of (0.25μm), and a length of 30 meters within the C18 column (serial number: us6551263H) was utilized for separation of the sample. A pressure of 122 KP and and injection temperature of (250C°) were applied. Hydrogen was supplied in to split mode and served as the carrier gas. A membrane filter measuring (0.22 μm) was used to filter (20ml) of every crude extract after being dissolved in (5ml) of HPLC grade methanol. A particular syringe was utilized to inject 1 microliter of the test extract into the system. A comparison of the mass spectral data was undertaken to determine the compounds.

Identification and determination of fatty acids methyl esters in the oils:

The Shimadzu instrument (GC-MS-QP-2022) fitted with electron impact mode (El 1.70 ev) was utilized to undertake GC-MS. RTX 5 (5%) phenyl-95% dimethyl polysiloxane with length of (30 m -0.25 μm) comprised the analytical column. At a flow rate of 1 mL min-1, helium served as a carrier gas. The temperature was initially set at (40C°) before augmenting at a rate of (15C°) per minute, holding for five minutes, before rising to (250C°) and holding for 10 minutes. A temperature of (250 C°) was possessed by the injector.

Identification and determination of elements in the oils

The Shimadzu instrument (AAS-6800) was utilized to carry out Atomic Absorption Spectroscopy (AAS). 10 mL of oil sample was dissolved in 1 mL of carbon tetra chloride for the potassium, calcium, magnesium, sodium, and iron. Centrifugation at (2500 to 3000) revolutions per minute for two minutes occurred after (10 mL-1 10%) v/v nitric acid was incorporated to the solution and thoroughly mixed for roughly 10 minutes. In line with the corresponding standards, the nominated elements were identified via AAS following the separation of the supernatant. As outlined by [15], selenium underwent the fore mentioned process yet utilized (0.25 gm) of nick sulphate dissolved in 10 mL of (10%) v/v nitric acid.

Anti-microbial activity of date oil

Bacterial microorganisms

Escherichia coli	ATCC 25922(Gram -ve bacteria**)**
Staphylococcus aureus	ATCC 25923(Gram +ve bacteria)
Aspergillus niger	ATCC 9763(Fungi)
Candida Albicans	ATCC 7596 (Fungi)

National Collection of Type Culture (NCTC), Colindale, England.
American Type Culture Collection (ATCC) Rockville, Maryland, USA.

Anti-Microbial activity

The anti-microbial efficiency of the tested compound was investigated against the standard Pathogen strains, *E.coli* (Gram negative), *Staphylococcus aureus* (Gram Positive), *Candida albicans* (Unicellular Fungi), and *Aspergillus flavus* (Multicellular fungi), kindly supplied by Microbiology and Immunology Dep., Faculty of medicine (Boy), Al-Azhar University. Pre-activation of pathogens were carried out by inoculating in the nutrient broth medium for 24 h. at (37C°) for bacterial strains, while fungal strains was inoculating in Potato Dextrose Broth (PDB) medium for 48 h at (28C°) under shaking condition. Screening of the tested compound was preliminary take place at a concentration of 100 µl/ well using agar well by diffusion method [16]. The evaluation of tested compound to inhibit the microbial proliferation was assayed based on the inhibition zone diameter (mm) in compared to the standard antibacterial and antifungal agents such as Cephradine and Nizoarm respectively [17].

Testing for anti-bacterial Activity

The anti-bacterial activity of prepared extracts is evaluated via the implementation of the cup-plate agar diffusion technique[18].A temperature of (45C°) was sustained as (100 ml) of molten sterile nutrient agar was mixed well with (1ml) of the standardized bacterial stock suspension (108 – 109 C.F.U/ml).Sterile Petridishes served as the receptacles for (20 ml) aliquots of the inoculated nutrient agar. Agar discs were excited and a sterile cork borer (No.4) was utilized to cut 4 cups with a diameter of (10 mm) within each of the mentioned plates once the agar was left to set an automatic microliter pipette was utilized to fill alternate cups containing (0.1ml) samples of every extract and for

two hours, such cups diffused at room temperature for a period of 18 hours, the plates were placed in an upright position and incubated at a temperature of (37C°). Against every test organism, two replicates were undertaken for every extract. At the same time elsewhere, methanol was incorporated into the positive control serving as a substitute for the extracts. The mean values and average were recorded and evaluated with regard to the diameters of the growth inhibition zones following incubation.

Determination of Minimum Inhibitory Concentration (MIC) by agar diffusion method:

In line with [19], an altered agar diffusion technique was utilized to identify the Minimum Inhibitory Concentration (MIC). A diminishing range of extract concentrations from (100 mg/ml - 3.125 mg/ml) was attained by preparing a two-fold serial dilution of every extract. As above mentioned, the discs placed on the surface of the MHA plate were incorporated with (10 µl) previously seeded with bacterial cells. For a period of 24 hours, incubation occurred at a temperature of (37C°). The MIC was ascertained by taking the value for the lowest concentration of extract displaying a zone of inhibition.

Soap preparation from date seed oil

1- Weight of (6 ml) of oil in a cup, then add (50 ml) of ethanol to it to dissolve the oil.
2- Add to the previous solution (30 ml) of (30%) sodium hydroxide solution.
3- Heat the previous mixture on a water bath with a temperature between (80-85C°) without boiling, and stir the mixture for 20 minutes, then test the complete saponification.
4- Prepare a solution consisting of (40g) of sodium chloride dissolved in (200ml) of water. Put it in an ice bath and then divide it in half.
5- After complete saponification, add a solution to half the amount of the saline solution then
6- Cool in an ice bath, filter, and rinse with the other half of the saline solution.
7- Leave the soap to dry and see the resulting weight.

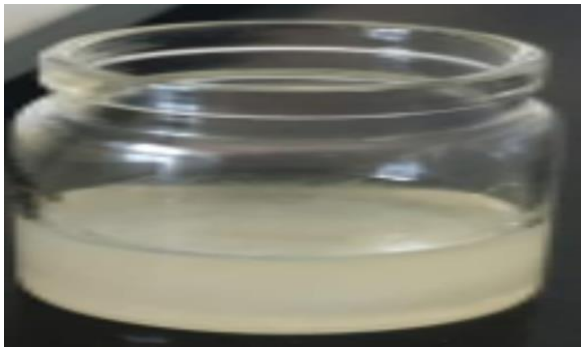
Statistical analysis

As per [20-21], the utilization of ANOVA for one-way classified data followed by Duncan's multiple range test enabled the comparison of the significance of variances between means at each point in time.

Results and Discussion

Date seeds are portrayed in Fig (1). Nonetheless, a moderate viscosity ratio, a white color, and a liquid state at room temperature are among the properties of oil extracted from date seeds as shown in Fig (2). In addition, the presence of fatty acids in tube number 1 was verified via a copper acetate test which revealed a blue oil color and thus, a positive outcome. On the other hand, negative findings were revealed in tubes (A and B).

Fig. 1: Plantae (Viridiplantae) Date seed.



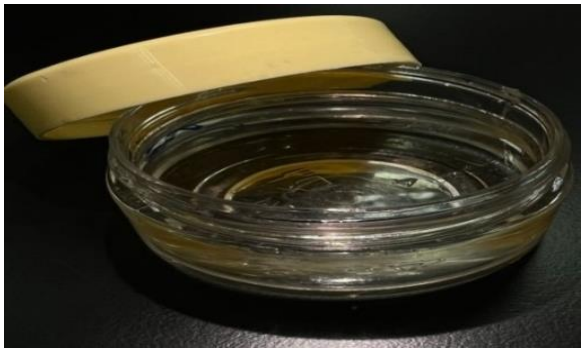


Fig. 2: Plantae (Viridiplantae) Date seed oil's.

Fig. 3: Copper acetate test.

Physio-chemical properties of date seed oil

The principal of physio-chemical properties of the extracted oil and date seeds are presented in Table (1) and were evaluated via adopting standard techniques. The properties revealed viscous liquid state at ambient temperature and semi-solid state at temperatures under (10C°).

A standard GC chromatogram of the separated fatty acids is displayed in Table (2) and Fig (4) where the internal normalization technique is utilized in descending order to ascertain the percentage. The GC-MS was used to identify the chemical structure of compounds within the date oil.

Table-1: Reflects the summarized physio-chemical properties of the oils.

Property	Unit	Measured value
Kinetic viscosity	° Cst	20
Acid value	Mg KOH/g oil	1.42
Flash point	C °	245
Density	g cm^{-3}	0.9188
Refractive index		1.468
Color	(red-yellow).	2.8 - 13.5
Acid value	(MgKOH/goil	2.67
Specification value	mg/g	0.279
Iodine value	gm I2/100 gm fat	74.31
Peroxide value	mg Peroxide /Kg oil	7.4
Iron	Ppm	1.27
Magnesium	Ppm	0.359
Calcium	Ppm	29
Sodium	Ppm	70
Polyphenols	mg/100g	589

Fig. 4 and Table-2, shows a typical gas chromatography GC of the separated fatty acids, in which percentage (%) content calculated by using internal normalization method in descending order. Chemical structure of compounds presents in date oil was detected by Gas Chromatography- Mass Spectrometer (GC-MS).

The chemical constituents detected in date oil were: Oleic acid, Octanoic acid, methyl ester , Decanoic acid, methyl ester ,Dodecanoic acid, methyl ester Methyl Tetradecanoate ,Hexadecanoic acid, methyl ester,9-Octadecenoic acid (Z)-, methyl ester,9,12-Octadecadienoic acid (Z,Z), Lauric acid, methyl ester ,Linolenic acid, methyl ester,cis-13-Eicosenoic acid, methyl ester ,5,8,11,14-Eicosatetraenoic acid, methyl ester,

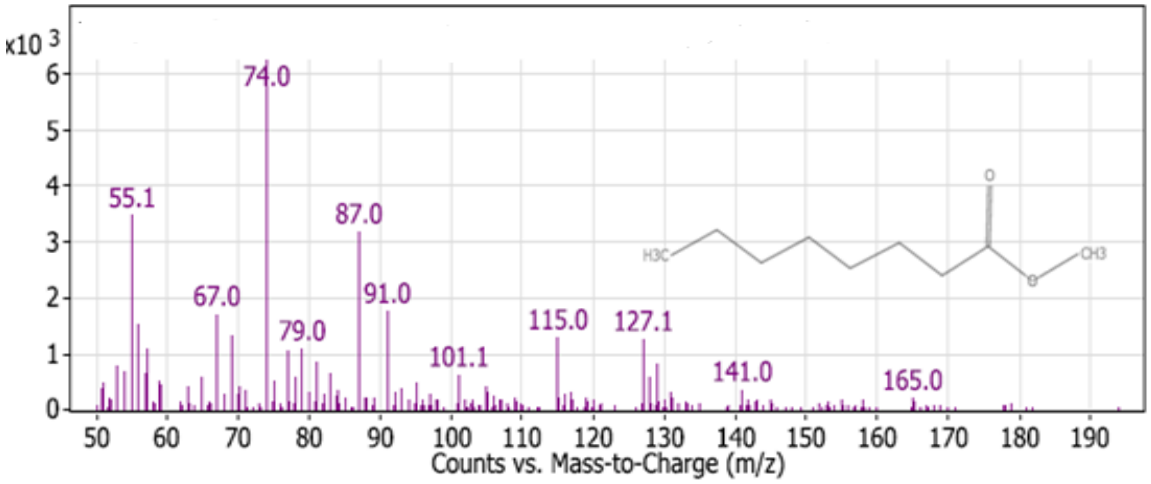
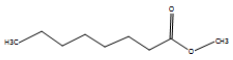
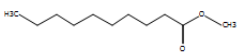
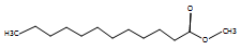
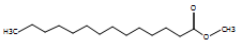
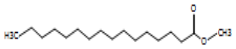
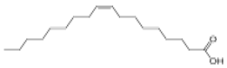
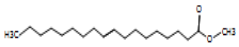
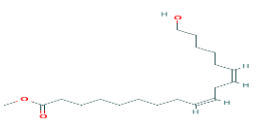
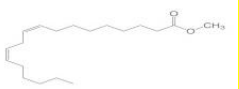
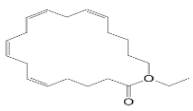


Fig. 4: Chemical structure of date seed oil detected by (GC-MS).

Table-2: Chemical Structure Detected by Gas Chromatography Mass Spectrometer (GC.MS) of date seed oil.

Name	Structure	Formula	Area	Area Sum %
Date oil				
Octanoic acid, methyl ester		**C9H18O2**	**59121.4**	**0.1**
Decanoic acid, methyl ester		**C11H22O2**	**110873.86**	**0.19**
lauric acid		**C12H24O2**	**2272947.8**	**35.76**
Dodecanoic acid, methyl ester		**C13H26O2**	**22080735**	**37.52**
Methyl tetradecanoate		**C15H30O2**	**4010799.8**	**6.82**
Palmatic acid		**C16H32O2**		**0.42**
Hexadecanoic acid, methyl ester		**C17H34O2**	**2738990.5**	**4.65**
Oleic acid		**C18H34O2**	**25413566**	**38.25**
9-Octadecenoic acid (Z)-, methyl ester		**C19H36O2**	**27413472**	**64.59**
9,12-Octadecadienoic acid (Z,Z)-, methyl ester		**C19H34O2**	**2272947.8**	**3.86**
Linolenic acid, methyl ester		**C19H32O2**	**18978.08**	**2.33**
cis-13-Eicosenoic acid, methyl ester		**C21H40O2**	**77043.84**	**0.13**
5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-		**C21H34O2**	**60419.65**	**0.1**

Anti-Microbial activity

As shown in Table (3), the date seed oil shown demonstrated antibacterial activity high effect against a *Escherichia coli* (Gram negative) and moderated effect against *Staphylococcus aureus* (Gram positive), respectively. Compared with standard drug, Cephradine. In this way, the date seed oil had moderate inhibitory effect against *Candida albicansa* and low inhibitory effect against *Aspergillus flavus*. which may be increases with the increase of the compound concentration compared with standard antifungal agent Nizoarm Fig (5, 6, 7& 8).

Table-3: Anti-microbial activity Petroleum ether of date seed oil extract against the standard organism.

Sample No.	Sample Code	Inhibition Zone (mm)			
		E.coli	***Staphylococcus aureus***	***Candida albicans***	***Aspergillus flavus***
		ND	**ND**	**2**	**2**
Nizoarm		**-**	**-**	**40**	**20**
Cephradine		**30**	**20**	**-**	**-**

Key: Nizoarm (Antifungal agent) and Cephradine (Antibacterial agent) were used as positive control, ND: not determined

Interpretation of results

**M.D.I.Z: Mean diameter of growth inhibition zone in (mm). Average of 2 replicates; M.I.Z.D (mm): >18 mm: Sensitive (14–18)mm: Intermediate,< 14 mm: Resistant; (-)No inhibition zone.

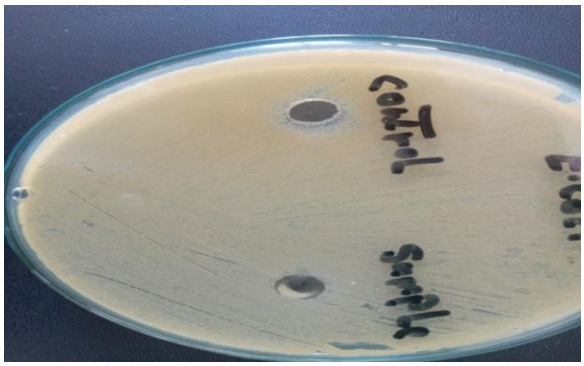


Fig. 5: In vitro antimicrobial activity of methanolic extract of date seed oil's against Escherichia coli.

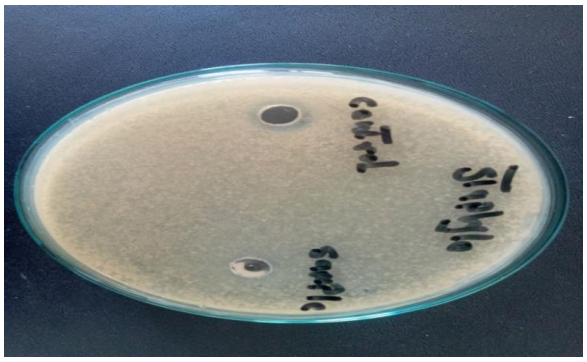


Fig. 6: *In vitro* antimicrobial activity of methanolic extract of date seed oil's against *Staphylococcus aureus.*

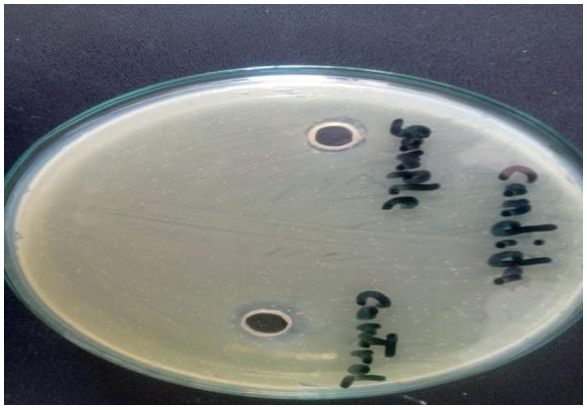


Fig. 7: In *vitro* antimicrobial activity of methanolic extract of date seed oil's against *Candida albicans*.

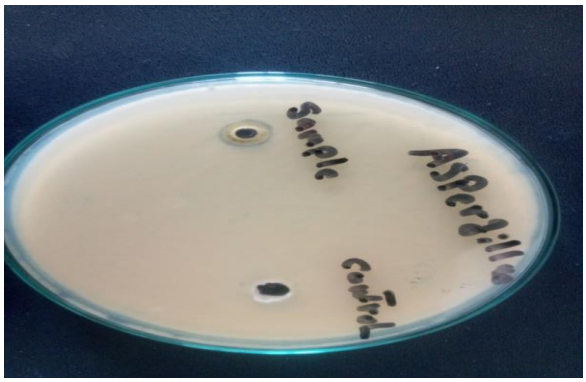


Fig 8: In *vitro* antimicrobial activity of methanolic extract of date seed oil's against *Aspergillus niger.*

Soap preparation from date seed oil:

Fig (9) shows soap prepared from date seed oil extraction.

Fig. 9: Soap prepared from date seed oil.

The evidence presented in the literature by prior studies is reflected by the findings of this research that ascertained the anti-microbial activity and physico-chemical properties of date seed oil as well as the chemical components of fatty acids. Oleic acid (Omega-9) and lauric acid are provided reliably by date seed oil. Notably, Saudi Arabian date seed oils contained 35.76% and 38.25% of lauric acid and oleic acid at a ratio of 1.169. The academic work conducted by [10], coroborates these results. Another study measured a ratio of oleic acid to lauric acid of 2:1 with percentages of 42.5% & 21%, respectively.

Anti-fungal and anti-bacterial activity were revealed within date seed oil, per findings of this research resulting in producing H_2O_2, *E. coli*, anti-

fungi, and *S. aureus* are inhibited via the polyphenol compounds residing within the oil. In addition, in accordance with the environmental conditions and chemical structure, the polyphenols are active intermediaries directly affecting the induction of oxidative stress in bacteria. Anti-microbial activity and microorganisms can be inhibited by polyphenols, per the literature [22].Bioactive behavior against fungi and bacteria is linked to the secondary metabolites polyphenol residing within date seeds, as compared and identified in certain academic works[23-26].

Conclusion

Marginal variances in the principal components of date seed oil were discovered by comparing the prior literature undertaken in distinct geographical locations shows that date seed oil have physio-chemical properties beside high level of free fatty acid (FFA) and minerals. Also producing H_2O_2, anti-fungi, *S. aureus* (Gram positive), and *E. coli* (Gram negative) which can be inhibited by the high percentage of polyphenols residing within date seed oil. The induction of oxidative stress in bacteria is directly contributed via active intermediary role of polyphenols performance. Further, cosmetics and soap preparation could be influenced by date seed oils.

Acknowledgment

The author is thankful to the Deanship of Scientific Research at Najran University and staff members Faculty of Science & Art at Sharourah.

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