Original Article

EXTRACTION OF PHENOLIC OIL FROM DATE SEED (PHOENIX DACTYLIFERA) AND EVALUATION OF ITS ANTIBACTERIAL, ANTIINFLAMMATORY, ANTIOXIDANT AND ANTICORROSION ACTIVITY

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Abstract

Aside from nutrition, one of the most intriguing aspects of date seed is the oil. Date nuts are high in fatty acids. Date seed oil contains both saturated and unsaturated fatty acids, with lauric and oleic being the most prevalent. Green extraction using a Soxhlet device as well as soaking was performed, and the results were compared to the Soxhlet procedure. For oil extraction, Deglet nour and Ajwa date seed were chosen. N-hexane, petroleum ether, and methanol were chosen as solvents. Methanol was discovered to be the most effective oil extraction solvent. Methanol extract of Deglet nour with free fatty acid 0.4 mg/mL (DN-M) yielded the highest oil yield, followed by Ajwa (AJ-PE) 0.3 mg/mL. Oil antioxidant activity was measured by DPPH, metal chelating and hydroxide scavenging activity. The DPPH test revealed that Methanol extracted DN had the highest free radical scavenging activity (82%), followed by Ajwa seed oil (78%). Deglet nour date seed oil had an 80% metal chelating activity, while AJM oil had a 69% metal chelating activity. Except for DNM, both seeds have no antioxidant ability on hydroxyl scavenging activity. DNM has a 42% anti-inflammatory effect, while DNH has a 37% anti-inflammatory effect. DN-M oil was found to be antimicrobial in nature among the two seeds. Both AJ-M and DN-M oils show microencapsulation of LAB, and on Deglet nour oil we observed a higher probiotic survival rate. In comparison to the control, the petroleum ether extract of both oils showed only 20% corrosion in hexane. GCMS reveals 25 different compounds, the majority of which are fatty acid esters. Autodock was used to investigate the anticancer potential of Benzothiazole, 2-(2-hydroxyethylthio), Acorenone, and Docosanol. Benzothiazole, 2-(2-hydroxyethylthio) was found to be a strong anticancer agent capable of inhibiting Cyclin-dependent kinases (CDK2) and revealed similar interactions as the co-crystallized ligand R-Roscovitine. Anti-proliferative compounds such as Benzothiazole, 2-(2-hydroxyethylthio), Acorenone, and Docosanol were discovered. These pave the way for future anti-cancer research with Date seed oil.

Keywords: Date seed oil, Cryopreservation, Anti-corrosion, antioxidant CDK-2

1. INTRODUCTION

Dates are a popular fruit that are known for their sweet taste and high nutritional value. The seeds of the date fruit, also known as date kernels or date stones, are a by-product of the date processing industry and are often discarded as waste. However, recent studies have shown that date seeds contain valuable compounds that can be used for various industrial and medicinal purposes. One of the most interesting aspects of date seeds is the oil that they contain. Date seed oil is rich in fatty acids, including lauric and oleic acids. These fatty acids have been shown to have beneficial effects on human health, such as reducing inflammation and improving cardiovascular health[1]. The extraction of oil from date seeds is typically done using solvents such as n-hexane, petroleum ether, or methanol. Of these solvents, methanol is the most effective, as it results in the highest oil yield. Additionally, methanol is a safer solvent compared to n-hexane and petroleum ether, which are classified as hazardous chemicals. The oil extracted from date seeds has been shown to have antioxidant activity, which can protect the body against damage caused by harmful molecules called free radicals. In addition, the oil has anti-inflammatory properties, which can help to reduce inflammation in the body. One of the most promising applications of date seed oil is in the field of medicine. Studies have shown that the oil has antimicrobial properties, which can help to protect against harmful bacteria and viruses. Additionally, the oil has been found to have anticancer potential, with certain compounds in the oil showing the ability to inhibit the growth of cancer cells[2]. Another potential use of date seed oil is in the cosmetics industry. The oil is rich in fatty acids, which can be used to moisturize and nourish the skin. Additionally, the oil has been found to have anti-aging properties, which can help to reduce the appearance of wrinkles and fine lines. In addition, date seed oil can also be used as a lubricant in industrial applications, as it has a high viscosity index and a low coefficient of friction. This makes it useful for lubricating gears and bearings in machinery[3]. Furthermore, date seed oil also can be used as a biofuel. The high calorific value of the oil makes it a suitable alternative to fossil fuels. In this study, we have tried to evaluate the antioxidant, anti-inflammatory. Anticorrosive and antimicrobial effects of date seed oil.

2. MATERIALS & METHODS

2.1. Processing Of Date Seeds

Deglet nour (DN) date seeds was collected from dates industry from Trichy and ajwa (AJ) dates seed powder (markstor ajwa dates seed powder) was bought from shop. Date seed was washed, dried and placed in hot air oven at 45°C for 16 hrs. Then ground manually to get powder.

2.2. Soxhlet extraction

The oil extracted from date palm seeds (Iraqi date palm) is extracted using the standard solvent extraction method and a Soxhlet apparatus [4](Mrabet et al., 2020). For extraction, three organic solvents are used: hexane (H), petroleum ether (PE), and methanol (M). Fresh water was used to wash the date seeds, which were then dried for one week at room temperature. This was followed by a 24-hour drying period in a 100 oC oven. The dried seeds were pulverised in a hammer mill before being powdered in a mixture grinder. Sieving was used to separate larger particles. Then, 5 g of dried date seeds were weighted and placed in a medical gauze, and 100 mL of solvent was added to the extracting flask. The Soxhlet extractor was then switched on and temperature was set at suitable temperature for 5h.

The % yield can then be calculated according to weight loss of

S1/S2 X100

S1: weight of oil extraction, S2: Sample weight

2.3. Estimation of free fatty acids

The amount of potassium hydroxide (KOH) in milligrams needed to neutralise one gram of chemical material is known as the acid value, sometimes known as "acid number." The presence of carboxylic acid groups in a chemical substance, like a fatty acid, or in a combination of chemical compounds is measured by the acid number. Acidity in oil is measured as a proportion of free fatty acids. Using on a titration method, the determination of acid value and acidity was completed. The British Standards Institution describes the experimentation process (BS EN ISO 660: 2009). 25 ml of distilled water are added to 1 ml of date seed oil (DSO), which is then titrated with 0.1 N KOH. Phenolphthalein in 2 drops serves as an indication.

2.4. Qualitative Phenol test

200 µl of extract was added to the test tube contain 1 ml ethanols**.** To this 2 ml of distilled water and fewer drops of 10% ferric chloride. Formation of green colour indicated positive results.

2.5. TLC Analysis

To obtain 10 mg/mL working stock solutions, 10 L of each extract was dissolved in one millilitre methanol separately. Using an applicator, 5 L of extract solution was applied separately to a silica gel 60 precoated TLC plate (Merck, Germany). The sample poured plates were then pre-saturated with the mobile phase in a glass tank for 30 minutes. The analytes were then moved into a horizontal developing chamber (20 10) at room temperature (25°C) using the solvent system Chloroform, ethanol, and ethylacetate (5: 4:1, v/v/v). The plate was air-dried after the solvent had been run up to 80% of its total height. Some of the phenolic UV compounds studied showed absorption maxima at long-wave UV and iodine.

2.6. Total Antioxidant (DPPH) activity

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) of the extracted phytochemical at 1, 5 and 10% concentration was taken to determine antioxidant activity[5] The standards used in this test were ascorbic acid at 25-100 µg concentration. The final concentration of compound was 1 mg/ml. 2 mL of ethanol solution was taken in a test tube and 0.2 ml of DPPH reagent is added and mixed well. 0.5 ml of sample at different concentration is transferred to reagent tube. Then, the tube was wrapped in aluminium foil and incubated on the shaker at room temperature for 20 minutes. The absorbance was recorded at 517 nm. Distilled water alone used as control.

$$Inhibition\ (\%) = C - T \div C \times 100$$

2.7. Metal Chelating Activity

Antioxidant activity of extract was performed by metal chelating activity. Of the extracted Date seed oil, 100 µL was mixed with 50 µL of 2 mM ferrous chloride, and the reaction was started by adding 0.2 mL of 5 mM ferrozine. Then, absorbance was recorded at 562 nm after 10 min in a dark room. Distilled water, instead of extract, was used for the control. EDTA is used as standard. Metal chelating activity was calculated by

$$\text{Inhibition } (\%) = (1 - \text{A562 (extract)}/\text{A562 (control)}) \times 100$$

2.8. Hydrogen Peroxide Radical Scavenging Assay

The ability of plant extract to scavenge hydrogen peroxide is determined by taking 0.5ml of hydrogen peroxide (1ml of hydrogen peroxide was made upto 45ml with water), 1ml sodium phosphate buffer pH 7.4 and 0.4ml

water. 0.1 ml of the sample was added to initiate the reaction. 2 ml of dichromate acetic acid reagent was added after 60 sec to arrest the reaction to control tubes. The tubes were then heated upto 10 minutes then, allowed the containment to cool and green color developed was read at 540 nm using spectrophotometer. Oil (25 -100 µl) is added to hydrogen peroxide and absorbance at 230 nm is measured after 10 minutes against a blank solution containing phosphate buffer solution without hydrogen peroxide % of inhibition $= C - T \div C \times 100$

2.9. Encapsulation of LAB and viability

250 micro litres of LAB-containing oil were cultivated in 4.750 micro litres of 10% non-fat milk. To aid in the encapsulation of bacteria in reconstituted oil emulsions, the liquid was gently vortexed for 15 min. additionally, encapsulation was looked at with a light microscope. The resultant cultures were kept at 4°C until they were needed. The free and encapsulated bacterial cells were kept in a refrigerator at 4°C, and samples (5 ml LAB) were taken every seven days to check for vitality. An aliquot of 1000 µl was prepared by diluting for pour plating for every specimen. Following 48 hours of anaerobic incubation at 37°C, cell counts in triplicate were computed from the colonies on lactobacilli MRS agar plates, and the results were expressed as colony-forming units per milliliter (CFU/ml)[6].

2.10. Testing of iron rusting inhibition

The corrosion test was performed based on ASTM standard (ASTM Committee G-1 on Corrosion of Metals of 2017). The weight-loss technique was used in accordance with the manner outlined before by Amal Abdul Nasser and Anwar Sathiq (2011). A Pre-weighed (polished) X80 steel specimens each of dimension 3 cm × 3 × 0.5 cm were immersed into the test solutions made up of 1 M HCl without and with oil and sealed to exclude air. The set up kept under room temperature for 15 days and weight loss was taken end of 7th and 15th day. Percentage corrosion inhibition efficiency (IW, %) was calculated using $W=W_0-W_1/W_0$ W_1 and W_0 are the loss of weight values in the presence and absence of inhibitor.

2.11. Metabolite screening by GC-MS

The extracts were analysed using gas chromatography-mass spectrometry (GC-MS) with a Shimadzu GC-MS and an RTX-5MS capillary column. The detection was carried out using an electron ionisation device (70 eV). The carrier gas, 99.99 percent helium gas, was used at a constant flow rate of 1.20 ml/min. The temperatures of the mass and heat transfer lines, as well as the injectors, were set to 250 and 200 degrees Celsius, respectively. The oven temperature was increased from 50 to 250°C at 7°C/min, held there isothermally for 2 minutes, and then increased to 250°C at 5°C/min. Samples were manually injected in split mode with a 50-600 AMU mass scan and a split ratio of 50.0. The GC-MS took 35.50 minutes to complete. To quantify the relative intensity of each volatile component, the ratio between the area of a given molecule and the total area of all recognised spots has been used[7.8,9]

2.12. IN SILICO DOCKING

2.12.1. ADME

Absorption, Distribution, Metabolism and Excretion Prediction by test was performed by using SWISS dock. ADME analysis[10] was performed on human intestinal absorption (HIA), aqueous solubility (AS), blood brain barrier (BBB), Solubility, Log P, Log S, Lipinsky rule, H donor and acceptor, molecular weight descriptors.

2.12.2. Ligand preparation

Chemical structures of the ligands were retrieved from PubChem databse and prepared as mol. file using chimera 1.6.

2.12.3. Target protein identification and preparation

The three dimensional structure of the Cyclin-dependent kinase 2 (PDB 4EK3) were obtained from the Research Collaborator for Structural Bioinformatics (RCSB) Protein data bank (www.rcsb.org) respectively. A chain of receptor was pre-processed individually by eliminating other chains (B, C, and D), ligand, and the molecules of water without hydrogen bonds. Hydrogen atoms were added and the proteins' existing ligands and molecules of water were removed using Pymol software before being saved in PDB format.

2.12.4. Prediction of active site

For the purpose of designing drugs using structures, the active site of target must be predicted. UCSF Chimera was used to identify the coordinates of the proteins' interaction sites. Docking the software AutoDock 4.2 was used to perform ligand binding simulations, and the binding energy of the protein—Schiff base adducts was determined.

3. RESULTS AND DISCUSSION

3.1. Extraction of Oil

Deglet nour and Ajwa dates were cleaned, sun-dried, roasted and mashed (Plate 1). The date powder is subjected to soxhlet extraction with Petroleum ether, Methanol and Hexane (Plate 2). The quantity of oil yield and percentage of oil yield in this study is presented in Table 1. Data reveals that methanol is found to be effective than hexnae and petroleum ether. The maximum 18 mL of oil was obtained from Deglet seed followed by Ajwa 12mL by methanolic extraction and 2.5 and 10 mL by hexane extract from Deglet and Ajwa. The petroleum ether extract of Deglet nour gave 5ml and 0.5ml from ajwa seed. The percentage of oil yield was 36, 24, 5, 20, 10 and 1% among DN-M, AJ-M, DN-H, AJ-H, AJ-PE and DN-PE. The extracted oil shows different concentration of free fatty acid determined by KOH titration (Plate 3). Table 2 represent the concentration of free fatty acid of DN estimated as 0.0785, 0.0157 and 0.482 mg/mL respectively by hexane, PE and methanol. The free fatty acid content of Ajwa seed 0.2356, 0.3927 and 0.26 mg/mL by hexane, PE and methanol. Among the three extraction Methanol extract of DN found to have maximum free fatty acid 0.482 mg followed by 0.3927 mg in ajwa seed oil obtained by petroleum ether extract.

Table 1. Percentage of oil yield from different dates seeds

S.No.	Oil Type	Oil Yield (ml)	% of oil yield
1	Deglet nour date seed + hexane (DN-H)	**2.5**	5
2	Ajwa date seed + hexane (AJ-H)	**10**	20
3	Ajwa date seed + Pet. Ether (AJ-PE)	**5**	10
4	Deglet nour date seed + Pet. Ether (DN-PE)	**0.5**	1
4	**Deglet nour date seed + Methanol (DN-M)**	**18**	**36**
5	Ajwa date seed + Methanol (AJ-M)	**12**	24

Plate 1. Processing of Date Seeds

Plate 2. Oil Extraction from Soxhlet Apparatus

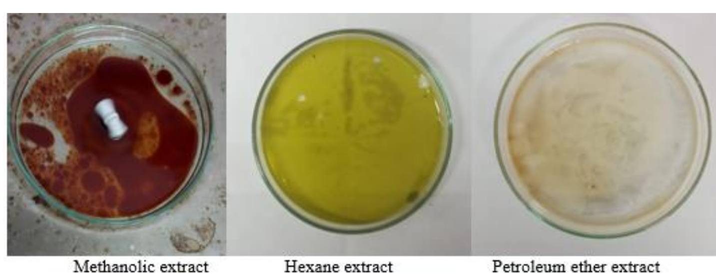


Plate 3. Fatty Acid and Phenol detection

O1 – DN-H

O2 – DN -PE

O3 – DN -M

O1 O2 O3

Table 2. Estimation of free fatty acid in different DSO

S.NO.	**OIL**	**FATTY ACID CONCENTRATION** mg/ml	**PHENOL**
1	DN-H	0.0785	-
2	AJ-H	0.2356	-
3	AJ-PE	0.3927	-
4	DN-PE	0.0157	-
5	**DNM**	**0.482**	+
6	AJM	0.26	+

3.2. Antioxidant activity

Total antioxidant activity by DPPH scavenging (Plate 4 a.) among the petroleum ether and hexane was summarized in Table 3. Extract of Deglet Noor (DN) exhibited the maximum antioxidant at 100µL 78.57, 17.02 and 82 % respectively among Hexane, PE and methanol extraction. The methanolic extract of DN seed shows 10≥30≥46≥82% inhibition. The inhibition of hexane extract were 21.53, 47.19, 62.11 and 78.57% and PE extract gave 5.26, 9.57, 12.79 and 17.02 %. The Ajwa dates demonstrated the highest DPPH inhibitory effect at 78 % hexane extract followed by 60% in methanol extract. The antioxidant activity of hexane was 11.42, 14, 71 and 78% and AJM shows 12, 22, 32 and 60% between 25-100µL. The DPPH scavenging potential of both seed found to be concentration dependent and found to effective on methanol. Both the seeds exhibited less significant antioxidant on petroleum ether extraction recorded

Total antioxidant activity by metal chelating activity (Plate 4 b.) among the petroleum ether, methanol and hexane was summarized in Table 4. The metal chelation potential of both seed found to be concentration dependent and effective on methanol extract (80% DN and 69% AJ) moderately on hexane extract. The Deglet Noor dates demonstrated the maximum of metal chelation effect recorded at 100µL oil were 46, 8 and 80 percentage among hexane, PE and methanol. The percentage of inhibition of ferrous ion were recorded as 14, 36, 56 and 80% in methanol and 12, 27,31 and 46% by hexane extraction. Petroleum ether extract of Deglet Noor seed oil exhibited less significant metal chelation recorded as 1.03, 3.41, 5.07 and 8.34%. Among the three extract of Ajwa seed, no metal chelation was noted on oil extracted by petroleum ether but significant activity is observed in methanol were recorded as 18, 28,40 and 69% and 9, 8,12 and 14% by hexane extracted oil.

Hydrogen peroxide scavenging assay (Plate 4 c.) represent all the tested oil failed to inhibit the free radicals developed by hydrogen peroxide (table 5). The antioxidant contents vary in different date seed types and found activity only on DN seed oil extracted by methanol. The percentage of inhibition were 12, 36, 58 and 68%. The antioxidant content of Deglet nour dates is higher than the contents of other dates, which indicates better oxidative stability. Another study found a significant amount of gallic acid in date seeds[11]

Plate 4. Antioxidant activity

4 a. DPPH 4 b. Metal chelating 4 c. Hydrogen peroxide assay

Table 3. Percentage of DPPH Free Radical Inhibition assay of DSO

S. No.	Sample Code	Concentration µg/ml			
		25µl	50 µl	75 µl	100 µl
1	DN-H	21.53	47.19	62.11	78.57
2	DN-PE	5.26	9.57	12.79	17.02
3	**DNM**	**10**	**30**	**46**	**82**
4	AJ-H	11.42	14	71	78
5	AJ-PE	0.95	1.28	2.98	4.45

6	AJM	12	22	32	60

Table 4. Percentage of Metal chelating Free Radical Inhibition assay of DSO

S. No.	Sample Code	Concentration µg/ml			
		25µl	50 µl	75 µl	100 µl
1	DN-H	12	27	31	46
2	DN-PE	1	3	5	8
3	**DNM**	**14**	**36**	**56**	**80**
4	AJ-H	9	8	12	14
5	AJ-PE	-			
6	AJM	18	28	40	69

Table 5. Percentage of hydrogen peroxide Free Radical Inhibition assay of DSO

S. No.	Sample Code	Concentration µg/ml			
		25µl	50 µl	75 µl	100 µl
1	DN-H	-	-	-	-
2	AJ-H	-	-	-	-
3	AJ-PE	-	-	-	-
4	DN-PE	-	-	-	-
5	DNM	12	36	58	68
6	AJM	-	-	-	-

3.3. Anti-inflammatory Activity of DSO

Anti-inflammatory activity of Deglet Nour seed oil was evaluated against denaturation of egg albumin (Plate 5) method and represented on figure 1. The highest inhibition rate was showed in methanolic extracts calculated as 42% and 37% by hexane at the concentration of 500 µg/ml. whereas less significant on PE extract. Date seed has a stronger anti-denaturation action (125 g) than diclofenac sodium (IC50 = 225.04 g/mL). The interaction of proteins with polyphenolic chemicals enhanced their thermostability, as per the studies of Czubinski and Dwiecki, (2017). According to earlier research, phenolic substances showed anti-inflammatory effects in a variety of experimental paradigms[12,13]

3.4. Antibacterial activity of DSO

The date seed oil extract of DN possesses antibacterial activity against the tested organisms; however, Ajwa seed oil extract do not showed any effective antibacterial (Table 6). Across all the extracts from two different varieties, methanolic extract of Deglet Noor seed oil exhibited (Plate 6). The significant zone of inhibition was recorded on DN M and founded that 15,16 and 14 mm on Escherichia coli, Klebsiella pneumonia and Proteus vulgaris. The

activity is moderate compare to standard tetracyclin. The flavonoid of methanolic extract of date seed with antioxidant and antibacterial had been reported similarly by According to Holetz et al. (2002) Acorenone is an antibacterial compound of some plant oils[14,15].

3.5. Probiotic viability of DSO

Encapsulation of bacterial cells within extracted AJM, DNM oil emulsions was performed. The bacteria were cultivated in non-fat milk and enclosed in synthetic oil emulsions made of broken down oil bodies. The formulation compositions and processing variables affected the morphology and size of oil emulsions containing LAB. In our optimum temperature, spherical oil emulsions of approximately 100 to 250 µm in diameter and control shows 50µm in diameter (Plate 7a).Viability of bacteria in storage at 4°C for 7 days was substantially elevated from after encapsulation and Compared with free cells (Plate 7b). The surviving ability of encapsulated and free LAB stored in nonfat milk at 4°C is shown in Table 7. The viable cell count of encapsulated bacteria dropped from18X10^{7} (control) 12X10^{7}whereas 120X10^{7} DNM and 16X10^{7}CFU in AJM date seed. The viability of free LAB was significantly reduced in the culture of control. Methods of oil encapsulation are frequently used to increase the survivability of probiotic microorganisms in industrial applications. Researchers have systematically used oil to coat and microencapsulate probiotic bacteria is much needed[16]

Figure 1. Percentage of inhibition of albumin denaturation

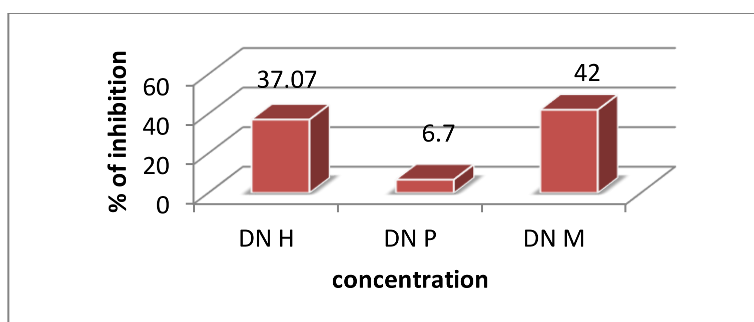
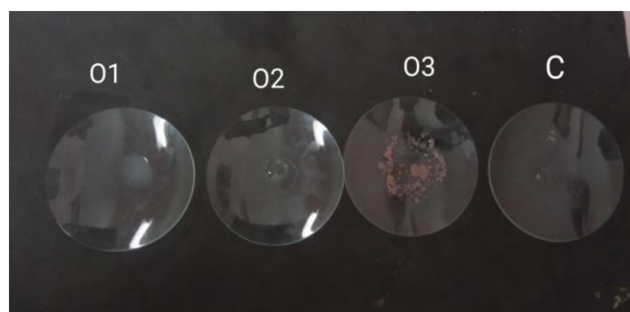


Table 6. Zone of inhibition of oil against selected pathogens (mm in diameter)

S.NO.	SAMPLE CODE	Escherichia coli	K. pneumonia	Proteus vulgaris
1	DN H	12±0.02	10±0.012	8±0.01
2	AJ	0	0	0
3	**DNM**	**15±0.01**	**16±0.012**	**14±0.02**
4	TC	26±0.001	26±0.001	26±0.001
5	NC	0	0	0

Plate 5. Anti-inflammatory test



O1-DNH

O2- DNPE

03- DNM

C – CONT.

Plate 6. Anti-bacterial test (well diffusion method)

Escherichia coli   Klebsiella pneumoniae   Proteus vulgaris

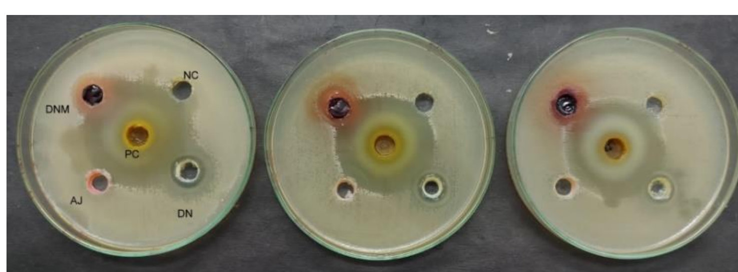


Plate 7. Viability of LAB by encapsulation

a) Encapsulation   b) Viability of LAB

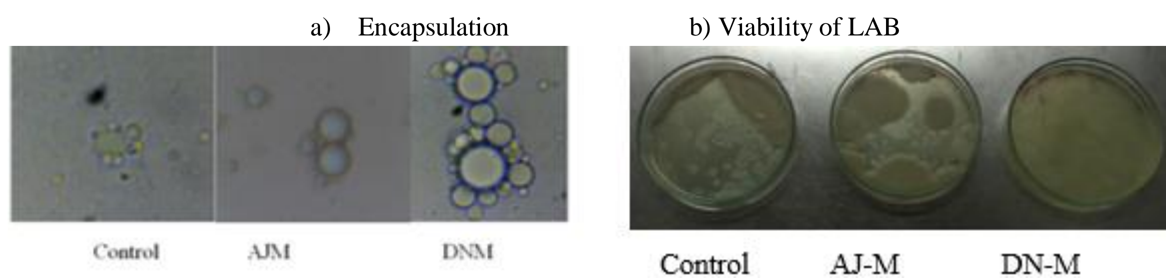


Plate 8. Anti-Corrosion Test

C - Control

O1- DN-H

O2 – DN-PE

O3- AJ-H

O4 – AJ-PE

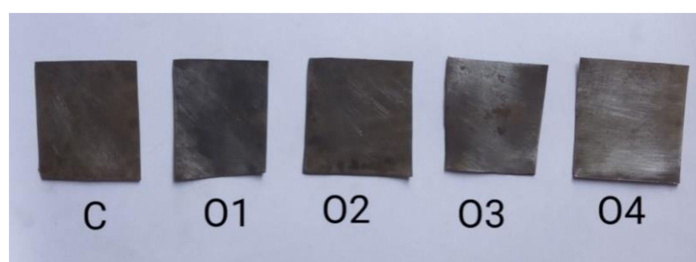


Table 7. Cryopreservation Test

S.NO.	SAMPLE CODE	Viability Of Cells CFU
1	AJM	$16X10^7$

2	DNM	$120X10^7$
3	Control	$12X10^7$

3.6. Corrosion inhibition on metal

Data on weight loss and corrosion rate of MS, and inhibition of oil compound at 500 ppm concentration from weight loss analysis in 1 M H_2SO_4 solution after 240 h are shown in Table 8. Weight loss analysis was performed on MS steel coupons individually submerged in 200 mL of the H_2SO_4 and the data is given in Table 8. The percentage of weight loss were higher in control and lesser in DNPE seed oil. The percentage of anticorrosion were 5, 3.2, 1, 2.8 and 1.6 respectively by the samples control, DN-H, DN-PE, AJ-H and AJ-PE. According to Deepak *et al.,* (2017) oil make it the attractive choice for most carbon steel corrosion applications.

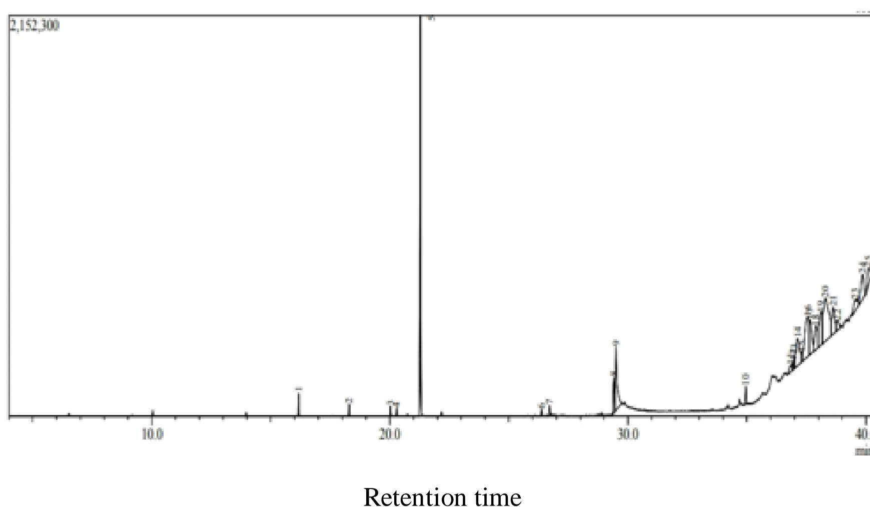
Table 8. Percentage of corrosion inhibition

S. No.	Sample code	Initial weight	Final weight (after 1 week)	Weight loss	% of loss
1	CONTROL	2.07 g	1.97 g	0.10 g	5
2	DN-H	1.92 g	1.86 g	0.06 g	3.2
3	DN-PE	2.28 g	2.26 g	0.02 g	1
4	AJ-H	2.21 g	2.15 g	0.06 g	2.8
5	AJ-PE	1.90 g	1.87 g	0.03 g	1.6

3.7. Metabolite profiling by GCMS

The metabolite profile of the methanolic oil extract of samples identified by GCMS (Figure 2.) and the detected compounds are listed in Table 9. About 43.78% was1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester and 4.3% of 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester , 3.94% of 9,12-Octadecadienoic acid (Z,Z) were major compound. 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, 1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester, Benzothiazole, 2-(2-hydroxyethylthio), Oleic Acid, Bis(2-ethylhexyl) phthalate, Acorenone and 9-octadecenoic acid (z)-, tetradecyl ester, Docosanol were selected for docking based on their Lipinski rule 5. Table 10 reveals all the compounds are pharamcologically active and safe to use. Though the compounds are poorly soluble all are highly absorbed by Gastro intestinal and do not cross Blood brain barrier. The composition results were close to those found for samples from other geographical origin and results agree well with other findings previously reported by Besbes *et al*. (2005)[17]

Figure 2. GC/MS analysis of DNM oil extracted



Retention time

Table 9. NIST library matched compounds

S.NO.	PEAK	RETENTION TIME	AREA %	COMPOUND
1	1	16.174	2.52	Tetradecane
2	2	18.284	1.37	OCTADECANE
3	3	20.03	1.12	Diethyl Phthalate
4	4	20.284	0.92	OCTADECANE
5	5	21.283	43.78	1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester
6	6	26.375	0.73	Benzothiazole, 2-(2-hydroxyethylthio)-
7	7	26.708	1.11	n-Hexadecanoic acid
8	8	29.404	3.94	9,12-Octadecadienoic acid (Z,Z)-
9	9	29.5	7.02	Oleic Acid
10	10	34.953	1.99	Bis(2-ethylhexyl) phthalate
11	11	36.83	0.72	dodecanoic acid, 1,2,3-PROPANETRIYL ESTER
12	12	36.935	0.91	Acorenone
13	13	36.99	1.45	9-OCTADECENOIC ACID (Z)-, 2-HYDROXY-1-HYDROXYMETHYL)ETHYL ESTER
14	14	37.131	2.99	Dodecanoic acid, 2,2,3,3,4,4,4-heptafluorobutyl ester
15	15	37.29	1.09	Octadecanoic acid, 2,3-dihydroxypropyl ester
16	16	37.569	4.3	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
17	17	37.665	3.76	dodecanoic acid, 1,2,3-PROPANETRIYL ESTER

18	18	37.898	2.53	3-(Octanoyloxy)propane-1,2-diyl bis(decanoate)
19	19	38.105	3.61	DODECANOIC ACID, 1,2,3-PROPANETRIYL ESTER
20	20	38.297	4.7	Dodecanoic acid, 1,2,3-propanetriyl ester
21	21	38.626	2.97	9-octadecenoic acid (z)-, tetradecyl ester
22	22	38.805	1.04	Docosanol, TBDMS derivative
23	23	39.545	1.17	DODECANOIC ACID, 1,2,3-PROPANETRIYL ESTER
24	24	39.856	2.73	Dodecanoic acid, 1,2,3-propanetriyl ester
25	25	40.155	1.55	Dodecanoic acid, 1,2,3-propanetriyl ester

Table 10. ADME properties of compounds in Date Seed Oil

Compound	Mol. wt.	H donor	H acceptor	Log P	Log S	Solubility	GI absorption	BBB	CYP1A2 inhibitor	Lipinski
1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	390.56 g/mol	0	4	0.93	-6.06	Poorly soluble	High	NO	NO	YES
1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester	276.28 g/mol	1	7	0.93	0.87	Very soluble	High	NO	NO	YES
Benzothiazole, 2-(2-hydroxyethylthio)-	211.30 g/mol	1	2	2.25	-2.61	Soluble	High	NO	YES	YES
Oleic Acid	282.46 g/mol	1	2	5.71	-5.41	Moderately soluble	High	NO	YES	YES
Bis(2-ethylhexyl) phthalate	390.56 g/mol	0	4	6.17	-6.06	Poorly soluble	High	NO	NO	YES
Acorenone	220.35 g/mol	0	1	3.71	-3.83	Soluble	High	Yes	No	Yes
9-octadecenoic acid (z)-, tetradecyl ester	478.83 g/mol	0	2	10.62	-9.87	Poorly soluble	Low	No	No	Yes
Docosanol, TBDMS derivative	440.86 g/mol	0	1	9.75	-9.79	Poorly soluble	Low	No	No	Yes

3.8. MOLECULAR DOCKING

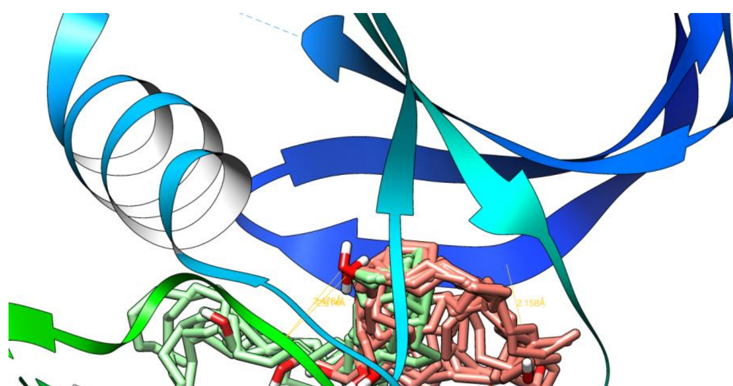
3.8.1. Molecular docking of Docosanol with Cyclin-dependent kinase 2 (PDB 4EK3)

A crucial protein kinase involved in the control of the cell cycle is called cyclin-dependent kinase 2 (CDK2). The development and metastasis of cancer are linked to CDK2's aberrant activity. A saturated 22-carbon aliphatic alcohol with antiviral properties is docosanol. Docosanol, a saturated 22-carbon aliphatic alcohol, has antiviral effects on a variety of lipid-enveloped viruses, including the herpes simplex virus (HSV). Based on their physicochemical and ADME characteristics, compounds discovered by GCMS were further sorted out. Interaction of Docosanol derivative with target protein (Fig 3.) analysis revealed that selected compounds interact with the functionally important B residues such as ILE, LEU and ASP of the active site pocket of CDK2. The Ligand shows formation of Intra-molecular hydrogen bonds in a protein required for stability and overall conformation. However, the compound formed 1-3 hydrogen bonds to the active pocket residues with potential score of -5.5, -4.5 and -4.4 Kcal (Table 11)

Table 11. Molecular Docking of Docosanol derivative

S. NO.	SCORE	H BOND DISTANCE A	RESIDUES
1	-5.5	2.158	ILE
2	-4.5	2.332	LEU
3	-4.4	2.614	ASP

Figure 3. Interaction of Docosanol and CDK2



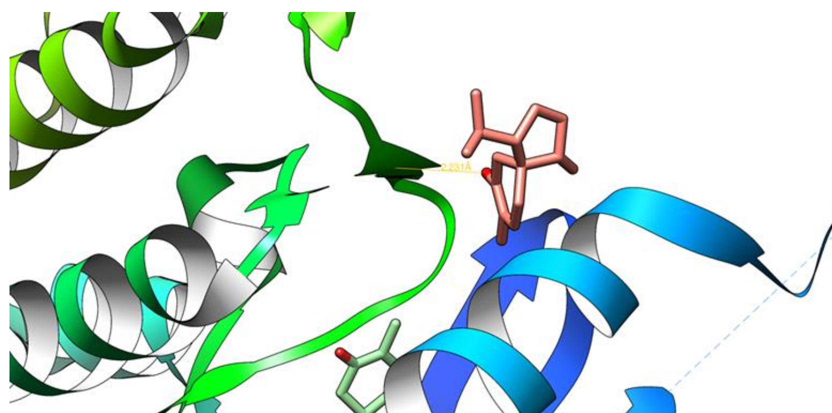
3.8.2. Molecular docking of Acorenone with Cyclin-dependent kinase 2 (PDB 4EK3)

The molecular docking shows interaction between ligands, Acorenone derivative with target protein given in Fig 4. Acorenone is an enone and an enol ether. It has a role as a metabolite. Investigation showed that specific substances interact with CDK2's active site pocket residues, including ALA. A protein's ability to establish intra-molecular hydrogen bonds, which are necessary for stability and overall structure, is demonstrated by the ligand. However, the compound formed 1-3 hydrogen bonds to the active pocket residues with potential score of -4.6 Kcal (Table 12). The hypothesised configuration of acorenone B is thought to be produced from trans-cis-farnesol via the -bisabolyl cation and reported as an AchE inibitor, according to Zalkow[18] et al.

Table 12. Molecular Docking of Acorenone

S. NO.	SCORE	H BOND DISTANCE A	RESIDUES
1	-4.6	2.231	ALA

Figure 4. Interaction of Acorenone and CDK2



3.8.3. Molecular docking of Benzothiazole, 2-(2-hydroxyethylthio) with Cyclin-dependent kinase 2 (PDB 4EK3)

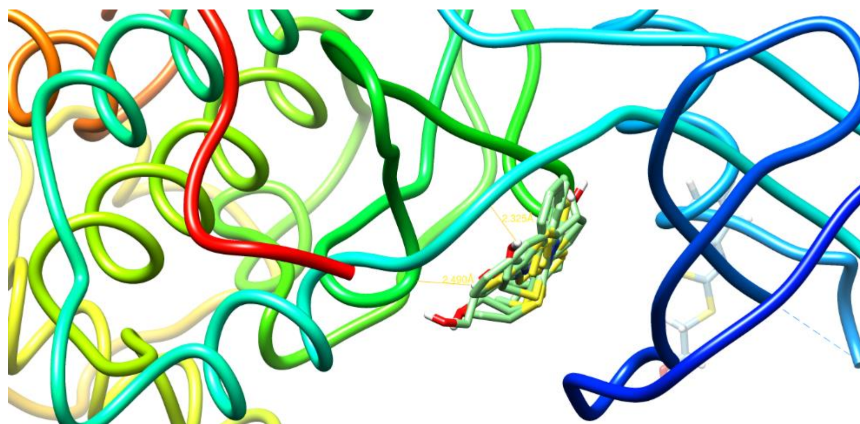
The molecular docking shows interaction between ligands, Benzothiazole, 2-(2-hydroxyethylthiol with target protein given in Fig 5. analysis revealed that selected compounds interact residues such as LEU and GLN of the active site pocket of CDK2. The Ligand shows formation of Intra-molecular hydrogen bonds in a protein required for stability and overall conformation. However, the compound formed 1-3 hydrogen bonds to the active pocket

residues with potential score of -6.2 and -5.9 Kcal (table 13). Benzothiazole, 2-(2-hydroxyethylthio) Neem cake Oil is previously reported as Anthelmintic in nature [19]

Table 13. Molecular Docking of Benzothiazole, 2-(2-hydroxyethylthio)

S. NO.	SCORE	H BOND DISTANCE A	RESIDUES
1	-6.2	2.326	LEU
2	-5.9	2.490	GLN

Figure 5. Interaction of Benzothiazole, 2-(2-hydroxyethylthio) and CDK2



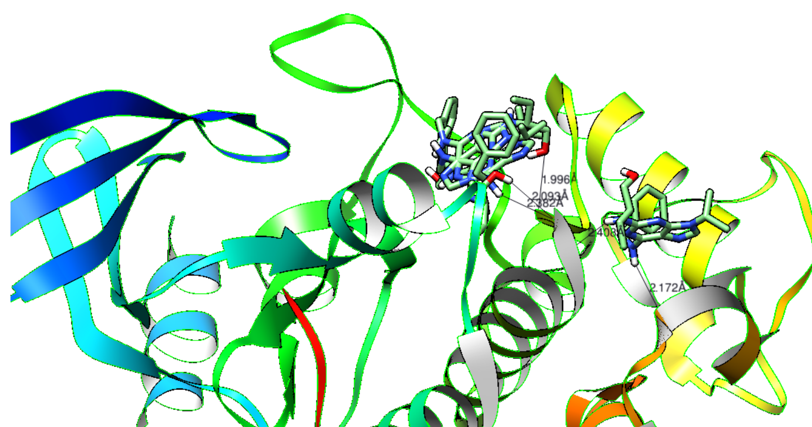
3.8.4. Molecular docking of Roscovitine with Cyclin-dependent kinase 2 (PDB 4EK3)

The molecular docking shows interaction between ligands, **Roscovitine** with target protein given in Fig 6. Analysis revealed that selected compounds interact residues such as ALA, ARG, THR, VAL of the active site pocket of CDK2. The Ligand shows formation of Intra-molecular hydrogen bonds in a protein required for stability and overall conformation. However, the compound formed 4 viable hydrogen bonds to the active pocket residues with potential score of -6.5, -6.7 and -6.8 Kcal (table 14). R-roscovitine, also known as Seliciclib or CYC202, is a cyclin-dependent kinase (CDK) inhibitor that preferentially inhibits a number of enzyme targets, notably CDK2, CDK7, and CDK9 by blocking ATP binding sites and prevents the replication process.

Table 14. Molecular Docking of Roscovitine

S. NO.	SCORE	H BOND DISTANCE A	RESIDUES
1	-6.7	1.996	ALA
2	-6.8	2.03	ARG
3	-6.5	2.408	THR
4	-6.5	2.172	VAL

Figure 6. Interaction of Roscovitine and CDK2



SUMMARY AND CONCLUSION

In summary, this research examines the potential uses and benefits of date seed oil. The study used a Soxhlet device and soaking method to extract oil from Deglet nour and Ajwa date seeds using solvents such as n-hexane, petroleum ether, and methanol. The study found that methanol was the most effective solvent for extracting oil and that the oil had high antioxidant activity, metal chelating activity, and anti-inflammatory properties. Additionally, the study found that Deglet nour date seed oil had the highest oil yield, and had strong antimicrobial properties, with higher survival rate of probiotic bacteria when encapsulated in the oil. GCMS analysis revealed the presence of 25 different compounds in the oil, mostly fatty acid esters. The study also found that some compounds in the oil had the potential to inhibit Cyclin-dependent kinases (CDK2) and have anti-proliferative effects, suggesting potential use as anti-cancer agents in future research**.**

Contributorship

RM- design, concept, MRS and SPS – analyses and writeup BS- communication**;**

External financial aid – NIL

CONFLICT OF INTEREST - NIL for all authors

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