ORIGINAL ARTICLE

Bioactivity of *Phoenix dactylifera* seed and its phytochemical analysis

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Abstract

Aim: This study was carried out to investigate the anticancer and antibacterial activity of date seeds and its phytochemical composition. **Materials and Methods:** Qualitative phytochemical screening was performed using date seed powder using biochemical assays. Gas chromatography-mass spectrometry (GC-MS) analysis was performed to identify the phytochemical contents in the extract. MTT assay was carried out to study the cytotoxicity of the seed extracts against HCT-15 (human coloretal cancer) cells. Antibacterial activity was studied using agar well diffusion method, against *Bacillus cereus* and *Escherichia coli*. **Results:** GC-MS analysis of acetone and chloroform extract of date seeds suggests that acetone extract contains majorly of aliphatic molecules and chloroform extract contains aromatic molecules. Cytotoxicity study showed that acetone extract is highly cytotoxic with inhibitor concentration 50 (IC50) value at 20 µg/ml and chloroform extract is moderately cytotoxic with IC50 value at 100 µg/ml concentration against HCT-15 cells. Antibacterial study showed that chloroform extract had no antagonistic activity against bacteria, whereas acetone extract demonstrated significant antibacterial activity with a zone of inhibition of 17 mm and 20 mm against *B. cereus* and *E. coli*, respectively at 1 mg/ml concentration. **Conclusion:** Results of this study conclude that acetone extract of date seeds (*Phoenix dactylifera* L) contains significant potential for pharmaceutical applications, in the field of antibacterial and anticancer drug discovery.

Key words: Antibacterial activity, cyclo (-phe-pro), gancidin W, gas chromatography-mass spectrometry, marine actinomycetes

INTRODUCTION

Dates (*Phoenix dactylifera* L.) belongs to the order Arecales, family Arecaceae, monocotyledonous tree cultivated in tropical and subtropical areas.[1-3] It is popularly known for its high nutritional and medicinal values to prevent various types of diseases.[1-3] From ancient times, the date palm *P. dactylifera* has played an important role in the day-to-day life of humans and animals. Khodry, Khalas, Ruthana, Sukkary, Sefri, Segae, Ajwa, Hilali, and Munifi are the various types of dates mostly cultivated in arid and semiarid regions of the world.[3,4] For more than 7000 years, palm dates have played an important role in both food and economy of many countries.[1,4]

The dates have been used as the celebration food for many years due to its high polysaccharide content. It helps in preventing disease with its biological activities such as antioxidant, anti-inflammatory, and antibacterial property.[3,4] The production and utilization of dates increases due to its beneficial value and the nonedible contents (seed) from them can also be used in making noncaffeinated coffee and animal feeding.[5] Date seed improves the functionality of the immune system and may also lower the risk of cancer and cardiovascular conditions as it contains high amount of phenolic content and nutritional compounds such as fiber, fat, moisture, protein, ash, and vitamins.[5,6] In traditional medicine, the powdered form of date seeds is used as an ingredient for relieve ague and toothaches and also used as a folk remedies for dealing liver diseases, diabetes, and gastrointestinal disorders.[5,6]

An annual dates production was estimated as 7 million tons, among this 1 million ton represents seeds according

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to food and agricultural organization of United Nations 2010. Nearly 1,25,000 tons of dates were produced annually by Tunisia with 60% of "DegletNour" variety and seeds of these dates yield 5-12 g of oil/100 g in dry condition.[7] Report says that date seeds are free from any toxic effects and also serves as an important source for phenolic acids consisting of hydroxylated derivatives of benzoic acid (vanillic acid and protocatechuic acid) and cinnamic acid (ferulic acid, caffeic acid, and o-coumaric acid) which shows antioxidant effects.[8] Here, in this study, these date seeds were studied for its ability to target cancer cells.

MATERIALS AND METHODS

Preparation of Seed Extract of Dates

Date fruits (*P. dactylifera*) were collected from the local market in Vellore, Tamil Nadu, India. Fruit pulp was removed and the seeds were then shade dried and grounded using mortar and pestle. About 10 g of grounded seed powder was soaked in 100 ml of chloroform and acetone in individual conical flask and kept in shaker for 48 h in room temperature. Chloroform and acetone extract was then filtered using Whatman No.1 filter paper and condensed in rotary vacuum evaporator. The obtained extract is stored in 4°C for further study.[9]

Phytochemical Screening

Phytochemical analysis of phenolics, tannins, flavonoids, sterol and triterpenes, alkaloids, saponins, and anthraquinone glycoside was carried out using standard procedures.[10-12]

Water Extract

About 1 g of date seed powder was dissolved in 14 ml of distilled water and was boiled gently using mantle, then the extract was filtered using Whatman filter paper.

Tannin Test

A volume of 2 ml of above filtrate was taken in a test tube and few drops of 1 M $FeCl_3$ were added. On addition of $FeCl_3$, appearance of green color indicates presence of condensed tannin, whereas the appearance of blue color indicates hydrolysable tannin.

Saponins

A volume of 2 ml of aqueous filtrate was taken in a test tube and shaken vigorously. Formation of froth of more than 1cm long indicates the presence of saponins.

Phenol Test

A volume of 1 ml of filtrate was taken in a test tube to which 5% of $FeCl_3$ solution was added. A dark green color indicates presence of phenolic compounds.

Acid Extract

About 1 g of date seed powder was taken in a beaker to that 6 ml of concentrated HCl was added and left to stand for 20 min. After 20 min, the extract was filter in a Whatman paper.

Flavonoids

A volume of 2 ml of acid extract was taken in a two separate test tube, to the first test tube 2 ml of distilled water and in second tube 2 ml of NaOH were added. Appearance of yellow color indicates flavonoids positive which tube gave yellow color.

Alcohol Extract

About 1 g of date seed powder was mixed with 8 ml of methanol. The mixture was left undisturbed for 30 min. After 30 min, the extract is filtered using Whatman filter paper. The filtrate was kept in mantle for evaporation, and finally, 3 ml of chloroform was added to resuspend.

Alkaloid

A few drops of alcohol extract were placed in a filter paper, and dragendorff's reagent was sprayed over the filter paper. Appearance of reddish brown color in filter paper indicates the presence of alkaloids.

Sterol and Triterpenes

A volume of 2 ml of alcohol extract was taken in a test tube to that few drops of acidic anhydride and concentrated H_2SO_4 to the wall of the test tube slowly; formation of reddish brown ring indicates positive result.

Anthraquinone Glycoside

In a test tube, take 2 ml of alcohol extract to that add 1 ml of ammonia and shake well, appearance of reddish color in aqueous layer and green color in the bottom indicates the presence of anthraquinone of ammonia and reference for this test.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The chloroform and acetone extract containing different compounds of Phoenix was subjected for (GC-MS) analysis.

The extracts was analyzed in a perkinelmer clarus 680 equipped with mass spectrometer clarus 600 (electron ionization) fitted with elite - 5MS capillary column (30 m, 0.25 mmID, 250 µm df). The GC oven was maintained with the initial temperature of 60°C for 2 min, ramp 10°C/min−300°C, temperature was maintained at 300°C for 6 min. Helium was used as a carrier gas with constant flow rate 1 mL/min, mass transfer line and source temperature were set at 240°C. Turbo mass version 5.4.2 software was used for the spectral analysis. Structure determination was done by comparison of mass spectral patterns to NIST-2008 library.[9,13]

MTT Assay

5000 HCT-15 cancer cells were seeded in a 96-well plate. About 24 h later, cells were treated with various concentration of chloroform and acetone extract (10,20,40,60,80, and 100 µg mL^{-1}) prepared from the date seeds for 24 h. After being treated with various concentrations of drugs, the cells were washed twice with drug-free medium. Once the drug treatment incubation period was done 20 µl of 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium-bromide (MTT), 5 mg/mL in phosphate-buffered saline was added to each well, the plate was incubated for 4 h at room temperature and 100 µl of dimethyl sulfoxide (DMSO) was added. The absorbance was measured in an enzyme-linked immunosorbent assay plate reader within 3 h using a test wavelength 630 nm. The percentage of surviving cells at each concentration relative to the control group was plotted.[9]

Antimicrobial Susceptibility Test

Antimicrobial activity of the chloroform and acetone extracts was determined using agar well diffusion method. A volume of 10 ml of sterile nutrient broth was prepared and dispensed into two different test tubes, such that each tube containing 5 ml of broth. A volume of 0.1 ml of two different strains of *Bacillus cereus* and *Escherichia coli* was inoculated to the test tube, respectively, and incubated overnight. A volume of 100 ml of Mueller-Hilton agar was prepared in a conical flask and sterilized in autoclave. Then, the sterile medium was poured into four petri plates. After solidification, respective bacterial cultures were uniformly spread over the media using cotton swab. Wells of about 6 mm in diameter were punched using cork borer. Then, the well was loaded with 100 µl of respective crude extract at various concentrations (100 µg, 75 µg, and 50 µg/ml dissolved in DMSO) DMSO was used as a negative control and streptomycin was used as positive control. The inoculated plates were then incubated at room temperature for about 24 h. The plates were observed for the presence of the inhibition zone around the wells. The size of zone obtained was measured, and the antimicrobial activity obtained was measured in terms of the average diameter of inhibition zone in millimeter.[14]

RESULTS

Phytochemical Screening

Qualitative phytochemical analysis of date seed powder demonstrated the presence of flavonoids, tannins, saponins, phenol, alkaloids, and sterols and triterpenes. Anthraquinone glycosides were not present in the powder. Among the test results, alkaloid test displayed strong color, suggesting the highest content of alkaloids; followed by tannins, saponins, phenols, and sterols and triterpenes with good color production. Flavonoid produced just enough color to infer positive, suggesting low flavonoids content in the sample. Table 1 shows the results for qualitative phytochemical analysis of date seed powder.

GC-MS Analysis

Chromatogram obtained from GC-MS analysis is shown in Figure 1a and b for acetone and chloroform. The list of compounds matched with the NIST library search in the GC-MS analysis is tabulated in Table 2. The compounds found in gas chromatogram were subjected for mass spectrometry and the results are matched with known compounds in the NIST library. The compounds found in acetone extract are aliphatic in nature, whereas the compounds found in chloroform were aromatic in nature. This suggests that these two different group of compounds found in acetone and chloroform extracts, could significantly affect its bioactivity.

MTT Assay

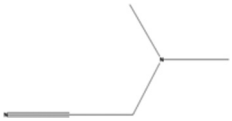
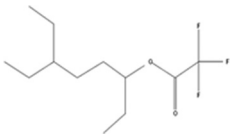
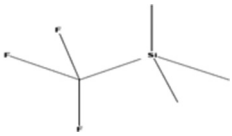
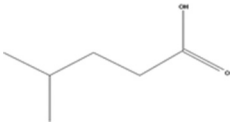
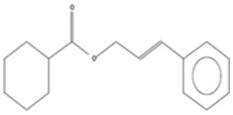
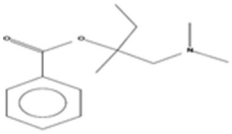
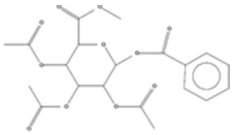
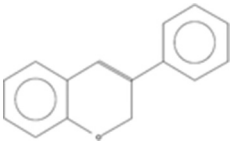
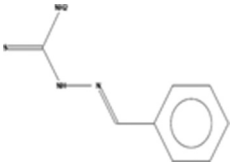
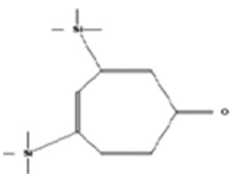
Among both the extracts, acetone extract demonstrated strong cytotoxicity and chloroform demonstrated reduced cytotoxicity against HCT-15 (colorectal cancer) cells. Acetone extract demonstrated an inhibitor concentration 50 (IC50) value of 20 µg/ml while chloroform extract showed IC50 at 100 µg/ml. Cell viability of HCT-15 cells at various concentrations of crude extracts is shown in Figure 2. The image of control HCT-15 cells and drug-treated cells are shown in Figure 3a and b, respectively.

Table 1: Qualitative phytochemical composition of date seed powder

Phytochemicals	Inference
Flavonoids	+
Tannins	+++
Saponins	+++
Phenol	+++
Alkaloids	++++
Sterols, triterpenes	+++
Anthraquinone glycosides	-

+: Low positivity, +++: Moderate positivity, ++++: High positivity, -: Negative

Table 2: List of NIST library matches for compounds present in crude extracts

R.t	Reverse	Forward	Compound name	Structure
Chloroform				
12.8	727	310	184 Acetonitrile, (dimethylamino)-	
18.9	696	478	3-ethyl-6-trifluoroacetoxyoctane	
20.1	687	321	Trifluoromethyltrimethylsilane	
21.2	674	413	Pentanoic acid, 4-methyl-	
22.3	547	339	Cyclohexanecarboxylic acid, 3-phenylprop-2-enyl ester	
25.5	574	326	9-oxononanoic acid	
Acetone				
10.33	702	390	2-butanol, 1-(dimethylamino)-2-methyl-, benzoate	
12.9	657	344	Methyl benzoyl-beta-D-glucuronide triacetate	
22.9	704	497	3-phenyl-2H-chromene	
23.9	594	365	Hydrazinecarbothioamide, 2-(phenylmethylene)-	
28.13	603	303	2,4,6-cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	

Antimicrobial Susceptibility Test

Among the two extracts, only acetone extract demonstrated antibacterial activity, whereas chloroform extract did not show any antibacterial activity. Acetone extract demonstrated strong antagonism against *E. coli* and *B. cereus* at various concentrations as tabulated in Table 3. Acetone extract showed highest zone of inhibition of 20 mm and 17 mm against *E. coli* and *B. cereus* at 1 mg/ml concentration. Zone of inhibition of acetone extract at various concentrations against *B. cereus* is shown in Figure 4. The test pathogen (*B. cereus)* was not susceptible to the positive control (streptomycin), probably due to the development of resistance toward streptomycin, but was still susceptible to the studied plant extract.

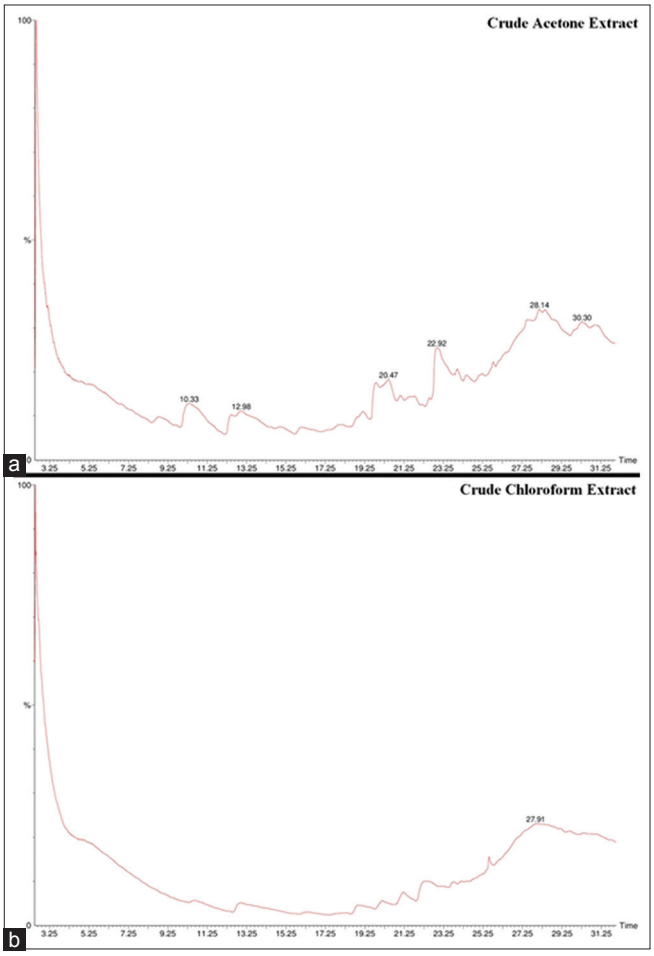


Figure 1: Gas chromatography-mass spectrometry chromatogram of acetone and chloroform extract of *Phoenix dactylifera* (a) crude acetone extract, (b) crude chloroform extract

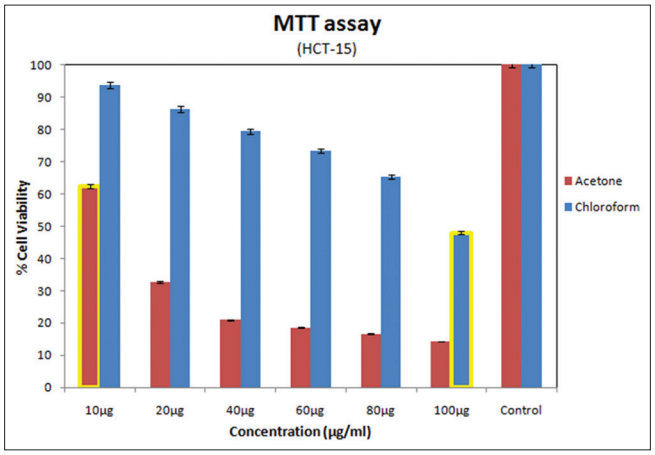


Figure 2: Cell viability of HCT-15 cells at various concentrations of crude extract

DISCUSSION

Objective of this study was to analyze the anticancer and antibacterial activity of *P. dactylifera* seed extract. Results of qualitative phytochemical analyses of date seed powder match with the study by Abiola *et al.* (2015). Our study showed that the date seed powder contains alkaloids, flavonoids, tannins, saponins, phenol, and sterols and triterpenes. These phytochemicals were quantitatively analyzed in the study by Abiola *et al.* (2015). Anticancer property of date's fruit was studied by Omar *et al.* (2004). According to this Omar *et al.* (2004), glucan molecule present in the date's fruit was purified and it demonstrated significant antitumor activity against ascites sarcoma-180. Similarly, our study is the first report on anticancer activity of *P. dactylifera* seed extract against HCT-15 (colon cancer)

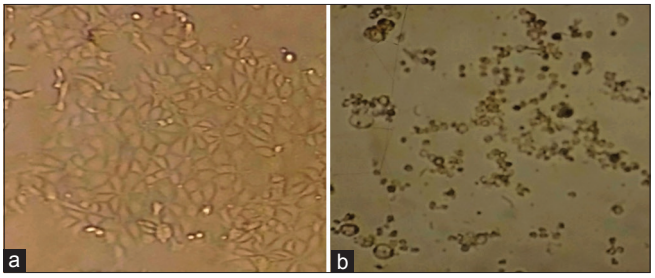


Figure 3: Morphology of (a) control HCT-15 cells; (b) acetone extract treated HCT-15

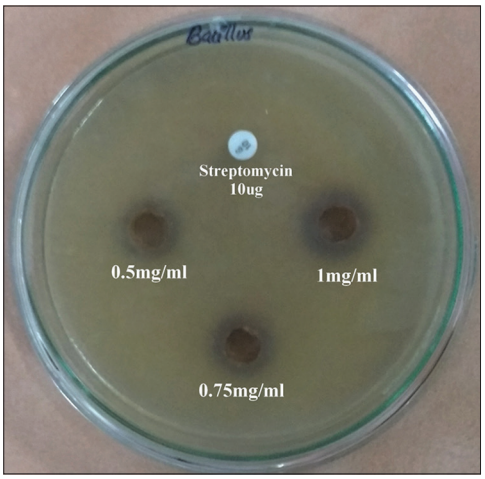


Figure 4: Zone of inhibition of acetone extract against *Bacillus cereus*

Table 3: Zone of Inhibition of acetone extract at various concentrations

Concentration Pathogen	500 µg	750 µg	1000 µg	Positive control
Escherichia coli (MTCC: 1687)	15 mm	18 mm	20 mm	15 mm
Bacillus cereus (MTCC: 0430)	14 mm	15 mm	17 mm	-

MTCC: Metro Toronto Convention Centre

cells. This study suggests that not only does the fruit but also its seed have medicinal value. Furthermore, study by Mohammad *et al.* (2013) demonstrated that administration of date seed extract significantly increased the paraoxonase and arylesterase activity in hypercholesterolemic rats. Eimad *et al.* (2015) also suggested that date seeds have potential to be used as ingredient in food additives, cosmetics, and pharmaceutical industries. All these reports prove that date's seed has potential to be used in pharmaceutical, cosmetic, and industrial applications. The current study on the anticancer and antibacterial activity adds significance to the medicinal property of the date seeds.

CONCLUSION

Based on the observed results of this study, it can be concluded that date seeds have potential for antibacterial and anticancer activity. In particular, the aliphatic compounds present in the acetone extract contain potential for both antibacterial and anticancer activity, at a significant concentration. This study is the first report on anticancer activity of *P. dactylifera* seed extract. Comparing the acetone and chloroform extract, acetone extract has significant bioactivity and further purification and characterization of the compounds present in acetone extract would lead to identification potential anticancer and antibacterial drug molecules.

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