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Preliminary phytochemical screening and anti-bacterial activity of date seed methanolic extract

Vidhya Jaganathan, Shanmugavadivu M*, Sandhya Ganesh
Department of Biotechnology, Dr. N.G.P. Arts and Science College, Coimbatore, Tamil Nadu, India
*Corresponding author: **Shanmugavadivu M.**
Assistant professor, Department of Biotechnology, Dr. N.G.P. Arts and Science College,
Kalapatti road, Coimbatore-641048, Tamil Nadu, India.
E- mail: *vadivurajesh1981@gmail.com*

Abstract

Date Palm is a high energy value crop with a good nutritional property. Seeds of this plant provide a remedy to a lot of ailments and also provide nutritional value to human. The present study involves extraction, preliminary photochemical analysis and antibacterial activity of date seeds belonging to the family Arecaceae for its medicinal value. A qualitative photochemical analysis of methanol extract of date seed was performed to identify the presence of alkaloids, carbohydrates, cardiac glycosides, saponins, phenols, flavonoids, proteins, amino acids, tannins, anthraquinones and steroids. The antibacterial activity of date seed methanolic extract against four bacterial species such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* was performed. It was identified that the methanolic extract of date seed contains alkaloids, carbohydrate, phenols, flavonoids, protein, amino acids, tannins and anthraquinones except steroids, saponins and cardiac glycoside. The methanolic extract of date seed has also showed moderate inhibition on the growth of Gram-positive and Gram-negative bacteria.

Keywords: Date seed, *Phoenix dactylifera* L, methanolic extract, phytochemicals, antibacterial activity.

Introduction

Ever since ancient times, in search for rescue for their disease, the people looked for drugs in nature. The word “herb” has been derived from the Latin word, *“herba”* and an old French word *“herbe”*[1]. Now a days, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non-woody plant. Earlier, the term “herb” was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities. Plants have been used for medicinal purposes long before prehistoric period. Recently, WHO estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants[2].Treatment with medicinal plants is considered very safe as there is no or minimal side effects. There is a need to promote them to save the human lives.

Fruits are important components of our daily diet that contain various bioactive nutraceuticals, which enhance our body’s strength to fight various illnesses.

The *Phoenix dactylifera L.* is known as date palm which is a monocotyledonous woody perennial belonging to the Arecaceae family [3]. Dates are main fruit in the Arabian Peninsula and are considered to be one of the most significant commercial crops and also have been documented in Holy Quran and modern scientific literatures. Date palm tree is known to be one of the oldest and main ancient crops in Southwest Asia and North Africa. Besides that, dates are grown in Australia, Mexico, South America, southern Africa, and the United States, especially in southern California, Arizona, and Texas [4]. It is an important plant in arid regions with more than 20 varieties reported all over the world and it has become an important fruit in some countries as a source of nutrition and economic value. Date seed is also called pits, kernels, stones or pips, form a part of the integral date fruit, which is composed of a fleshy pericarp and seed.

Date seed is a by-product of date fruit industry which is normally being discarded and used as animal feed ingredient or turned into non-caffeinated coffee by the Arabs. Date seed represent 11- 18% of date fruit weight which is composed of carbohydrates, dietary fiber, fat, ash and protein [5]. Seeds are a waste product of many industries based on the technological transformation of date fruits or on their biological transformation and they are having high value-added components [6]. Date seed is odourless and has a slightly bitter taste bland. In general, it has a light and dark brown in nature and are generally used as complementary feed materials for animals and poultry or as a conventional soil fertilizer [7]. The use of date seed in fiber-based foods and dietary supplements are suggested due to the excellent content of dietary fiber in the seed. Numerous studies have been conducted to find the benefits of the date palm, either from its fruit or seed, and it has been found that the date palm possesses several highly beneficial properties such as antibacterial, antiviral, antifungal, antioxidant, anti-hyperlidimic activity and hepato-protective activity [8]. These seeds are also shown to lower the risk of cancer and some cardiovascular conditions as well as to improve the functionality and integrity of the immune system.

Plants produce many important compounds such as phenolics and flavonoids which possesses antioxidant and antimicrobial properties. Phenolic and antioxidant contents are rich in date seed, but they are generally neglected and treated as a waste product. Ethnobotanical records indicate the potential of Ajwa date seeds and flesh having phenolics, antioxidants, and some other nutrients [9]. The drug resistance against microbial pathogens is increasing significantly world-wide. Bacterial resistance against antimicrobial agents is one of the major difficulties in treatment. The present mode of treatment of bacterial infection/disease is based on antibiotics, which is expensive and also causes adverse side effects. Natural products and their constituents is good approach in the control of infection as they are inexpensive, effective without side effects. Date seed and its constituents play a significant effect in the prevention or treatment of bacterial diseases.

The aim of this research was to screen the phytochemicals present in the methanolic extract of date seed and to determine its antibacterial activity against both gram-positive and gram-negative bacteria.

Materials and Methods

Collection of plant material: The dates (*Phoenix dactylifera L.*) were procured from local fruit market. Seeds were separated from date fruits and dried in shadow. The dried seeds were ground to powder.

Preparation of methanolic extract: 5g of date seed powder was dissolved in 100ml of methanol and kept in orbital shaker overnight at 120 rpm. Then the mixture was filtered using Whatmann No.1 filter paper and the filtrate was evaporated at room temperature to get dried powder. The yield of dried extract was calculated using the formula,

$$\text{Yield (\%)} = (W1 \times 100)/W2$$

Where, W1 was the weight of extract after evaporation and W2 was the dry weight of the sample.

Preliminary phytochemical Screening:

Phytochemical examinations were carried out for the methanolic extract as per the standard methods [10,11].

Detection of Carbohydrates:

Fehling's Test:

1ml of crude extract was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A

and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Benedict's test:

1ml of crude extract was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Detection of Proteins (Xanthoprotein Test):

1ml of crude extract was treated with few drops of conc. nitric acid. Formation of yellow colour indicates the presence of proteins.

Detection of Amino acids (Ninhydrin Test):

1ml of crude extract when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple colour suggesting the presence of free amino acids.

Detection of Alkaloids (Wagner's Test):

1ml of crude extract was dissolved in dilute HCl. and filtered. Filtrates were treated with Wagner's reagent (iodine in potassium iodide). Formation of brownish/ reddish precipitate indicates the presence of alkaloids.

Detection of Cardiac glycosides (Keller-Killani test):

1ml of crude extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Detection of Saponins (Froth Test):

1ml of crude extract was diluted with distilled water to 2ml and this was shaken vigorously for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Detection of Flavonoids (Lead Acetate test):

1ml of crude extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Test for Tannins (Ferric chloride test):

1ml of crude extract was mixed with ferric chloride solution would give blackish red colour indicating the presence of tannins.

Test for Terpenoids (Salkowshi test):

1ml of crude extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 minutes. Development of a greyish colour indicates the presence of terpenoids.

Detection of steroids (Liebermann Burchard test)

1ml of crude extract was treated with few drops of acetic anhydride, boiled and cooled, conc. H_2SO_4 was added from the sides of the test tube showed a brown ring at the junction of two layers and the upper layer turns green which showed the presence of Steroids.

Detection of Anthraquinones (Ammonium hydroxide test):

One drop of concentrated ammonium hydroxide was added to 1ml of crude extract. After two minutes, formation of red colour indicated the presence of anthraquinone

Detection of phenols:

1ml of crude extract was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

Anti-bacterial activity

The agar disc diffusion method was employed for the determination of antibacterial activity of the methanolic extract of date seed powder [12]. The test organisms, *Staphylococcus aureus; Streptococcus pyogenes; Pseudomonas aeruginosa; Klebsiella pneumonia* was spread on Mueller Hinton Agar. Filter paper discs (6 mm in diameter) were soaked with 20 µl of the 5mg/ml methanolic extract and placed on the inoculated plates. The plates were incubated at 37°C for 24h. The diameter of the inhibition zones were measured in millimeters. Streptomycin antibiotic discs were used as positive control and were evaluated for their antibacterial activities and their results compared with date seed methanolic extract. Methanol was served as negative control.

Results and Discussion

Preparation of crude extract:

The yield of date seed methanolic extract was calculated and found to be 23.64%.

Biologically active compounds usually occur in low concentration in plants. An extraction technique that gives higher yield and minimal changes to the functional properties of the extract has to be employed [13]. The ethanolic extract of date seed gave better yield and it was reported to be 32.8% [14].

Phytochemical screening:

Preliminary phytochemical screening of methanolic extract of date seed showed the presence of alkaloids, tannins, flavonoids, proteins, amino acids, terpenoids and phenols. Whereas, cardiac glycoside, saponins, anthraquinones and steroids were absent (Table-1).

Table-1 Phytochemical screening of date seed methanol extract

Phytochemical tests	Date seed (Methanol extract)
Alkaloids	+
Cardiac glycoside	-
Tannins	+
Flavonoids	+
Proteins	+
Amino acids	+
Terpenoids	+
Saponins	-
Steroids	-
Anthraquinones	-
Phenols	+

The alkaloids, saponins and tannins present in plant extracts were found to be effective in treating common pathogenic strain infections [15,16]. The phytochemical components in ethanolic extract of date seed showed the presence of saponins, terpenoids, phenols, glycosides [17]. The petroleum ether extract of date seed showed only the presence of diterapenes, whereas the ethyl acetate extract showed the presence of all except carbohydrates and proteins and chloroform was proved to be the poor extractant for bio-active molecules[18]. Flavonoids are known to be synthesized by plants in response to microbial attack. Hence, it should not be surprising that they have been found to be effective antimicrobial substances against a wide array of microorganisms, when tested *in-vitro* [19]. The biological activities of the date seeds such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic activities etc., were due to significant contribution of these secondary metabolities [20]. Tannins were also reported to have various physiological effects like anti-irritant, anti-secretolytic, antiphlogistic, antimicrobial and anti-parasitic effects. Phytotherapeutically, tannin containing plants were used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins [21]. Plant phenols are vital compounds used in eliminating the causes and effects of skin aging, skin diseases, and skin damage, including wounds and burns [22]. Phenols and flavonoids becomes very important plant constitutes because of their antimicrobial activity [23].

Antibacterial activity:

In the present investigation, the antibacterial activity of methanolic extracts of date seed was performed against four bacterial species, *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella pneumoniae.* Maximum zone of inhibition was observed against *Streptococcus pyogenes* (17mm). *Staphylococcus aureus* and *Klebsiella pneumoniae* were inhibited with 15mm zone of inhibition, followed by *Pseudomonas aeruginosa* with 14mm (Table-2) & (Fig-1).

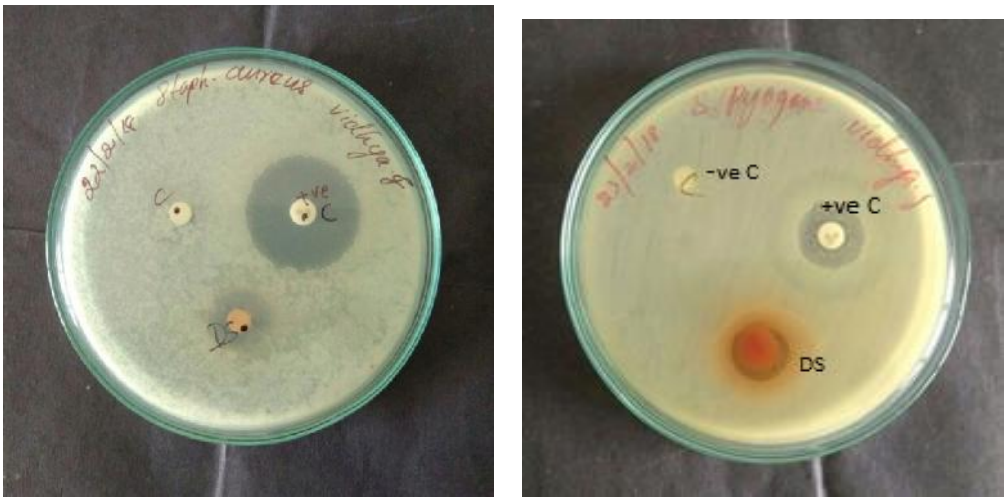
Table-2: Antibacterial activity of methanolic extract of date seed

Bacterial species	Zone of inhibition	
	Streptomycin	Date seed extract
Staphylococcus aureus	23mm	15mm
Streptococcus pyogenes	16mm	17mm
Pseudomonas aeruginosa	21mm	14mm
Klebsiella pneumoniae	20mm	15mm

Figure-1: Antibacterial activity of methanolic extract of date seed

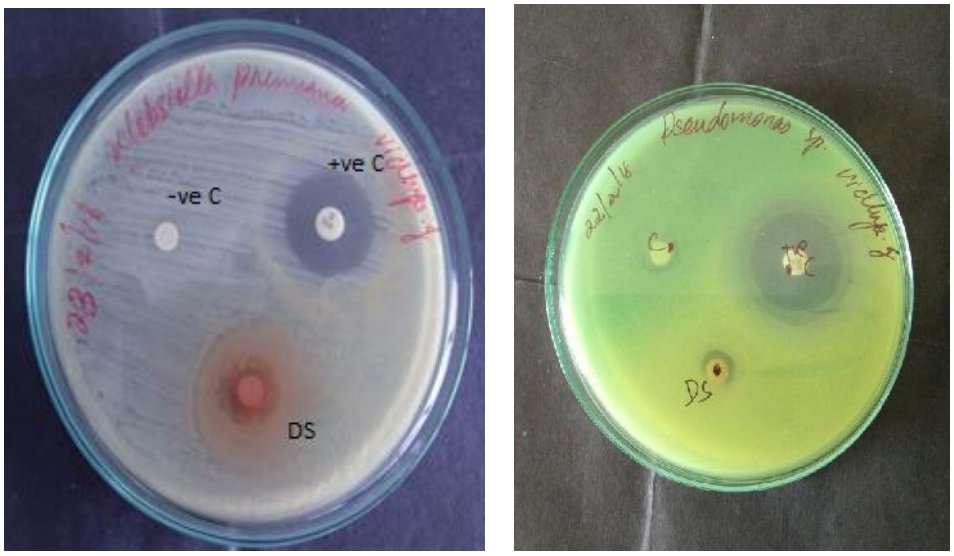
Gram positive bacteria

a) *Staphylococcus aureus* b) *Streptococcus pyogenes*



Gram negative bacteria

c) *Klebsiella pneumonia* d) *Pseudomonas aeruginosa*



DS - Methanolic extract of date seed
+ve C - Streptomycin (control)
-ve C - Methanol (control)

A similar study on methanol extract of date seed showed maximum zone of inhibition against against *S. pyogenes* (29.3 mm) followed by *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* and *S. Flexeneri* [24]. Ethanolic extract of date seed showed maximum zone of inhibition against *Staphylococcus aureus* (18mm) [25]. The high concentration of the date seed ethanolic extract was found to be effective against *Escherichia coli* (20.4mm) and thus it was very useful in the management of gastro-intestinal infection due to *E. coli* [14]. The acetone and methanol extracts of date seed inhibited the growth of Gram positive and Gram negative bacteria moderately [26, 27].

The inhibition of bacterial growth by these extracts could be due to the presence of some active compounds which may act alone or in combination [28]. The methanol extract of date seed had been evaluated for their antimicrobial efficacy against both Gram negative and Gram positive bacterial clinical isolates [29]. Tannin in date seeds also known as antimicrobial agent, which have been reported to prevent the development of microorganisms by precipitating microbial protein. The growth of many fungi, yeasts, bacteria, and viruses were inhibited by this compound [30].

Conclusion

The current mode of treatment of various diseases is based on synthetic drugs that are expensive which alters genetic and metabolic pathways and also shows adverse side effects. Thus, safe and effective approach is needed to prevent the diseases development and progression. To sort out those problems, natural products are good remedy in the treatment of diseases and they are affordable and effective without any adverse effects. Date seed, an agro-waste contains a number of bioactive components that are helpful in mitigating various physiological threats. The inedible parts of fruits and vegetables are often discarded as waste. However, these are rich source of dietary fiber, phenolic and antioxidants. The phytochemicals present in date seeds extract have well known curative activity against several human pathogens and therefore, could suggest the use of it for treatment of various diseases. According to the results obtained from this study, it is suggested that date seed must be used in treatment of the infections as an antibiotic for of gram positive and gram negative bacteria. Therefore, further research is needed to characterize isolated components and search for bioactive constituents with antimicrobial, antioxidant and other health-promoting activities.

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