Evaluation of Phytochemical, Antioxidant Anti-inflammatory and Antimicrobial Activity of Phoenix dactylifera

Arhama Tufail Ahd Ansari [1], Dr. Mukesh Pimpliskar [2]

[1]Environmental Science and Engineering Department, Indian Institute of Technology, Bombay, Mumbai, India
[2]KME Society's G.M. Momin Women's College Bhiwandi, India.

ABSTRACT

Natural products have been an integral part of the ancient medicine systems. Various studies have reported the nutritional benefits of P. dactylifera. However, limited studies have been done on phytochemical, antimicrobial, antioxidant, and anti-inflammatory activity of P. dactylifera. Therefore, the objective of the present study was to examine phytochemical composition, antioxidant and anti-inflammatory activity, and investigate antimicrobial activity of P. dactylifera extracts against some gram-positive, gram-negative bacteria, and fungal species. Two different solvents (aqueous and methanol) were used for extraction. The phytochemical screening showed that the P. dactylifera extract contains a mixture of phytochemicals such as flavonoids, phenolics, and alkaloids. The results of antioxidant study showed that extract of P. dactylifera and its constituents could be easily accessible source of natural antioxidants and possible food supplements in pharmaceutical industry. It was found that P. dactylifera has dose-dependent RBC membrane stabilization and can be used for anti-inflammatory action. The P. dactylifera was also found to be a potential antimicrobial agent.

Keywords: Anti-inflammatory, Antimicrobial, Antioxidant, P. dactylifera, Phytochemical.

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. According to World Health Organization (WHO), around 80% of the world's population relies mainly on traditional medicine for their primary health care. This is due to the availability, low cost, and no side effects of herbal medicines [1]. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, pharmaceutical intermediates, and chemical entities for synthetic drugs [2]. Since ancient times, in various cultures worldwide, inflammatory disorders and related diseases have been treated with plants or plant-derived formulations as plants have the ability to synthesize a wide variety of secondary metabolites. It motivates the researchers for finding new potential of such plants with variety of pharmacological activities Date palm tree belongs to Arecaceae family (Angiosperms, monocotyledon) consists of about 200 genera and more than 2,500 species. Phoenix is one of the genera with approximately 14 species native to the tropical or subtropical regions of southern Asia or Africa [3]. The name of the species dactylifera means "finger-bearing," which refers to the fruit clusters produced by this plant.Dactylifera is a grouping of the Greek word dactylus, means "finger," and the Latin wordferous means "bearing" [4]. Date fruit (Phoenix dactilyfera) is consumed as a staple food and an important component of the diet in the Middle East region. This fruit is considered highly nutritional because of its rich sugar content in the form of fructose and glucose, dietary fiber, vitamins, and minerals. [5]. Chemical constituents and biological activity of date seeds have been reported by [6]. Date palm seed is one of the rich sources of polyphenols and flavonoids [7]. Date palm seed has been extensively investigated for pharmacological activities such as immuno-stimulant [8], antidiabetic [9], and antioxidant [7], [10]. Although various studies have reported nutritional, chemical, and pharmacological activities on date seed, limited studies have been done on phytochemical, antimicrobial, antioxidant, and anti-inflammatory activity of P. dactylifera fruit. Therefore, this study aims to examine phytochemical composition, antioxidant and anti-inflammatory activity, and investigate the antimicrobial activity of P. dactylifera extracts against some gram-positive, gram-negative bacteria, and fungal species.

MATERIAL AND METHODS

Sample Collection

Dates (P. dactylifera) were procured from a market at Bhiwandi (Maharashtra), India. The selected dates were such that they were uniform in size, free from physical damage and injuries by insects and fungal infection. Dates were pitted to remove seeds. The sample was washed, cut (uniform thickness), and dried at 65˚C. Then it was ground into a fine powder and used for the analysis.

Extraction of Fruit Material

The fine powder of date fruit was extracted using both polar and non-polar solvent. Distilled water was used for aqueous extraction. The aqueous extraction was done by taking 10 grams of fruit powder and mixing it with 250 mL of distilled water in a beaker. The mixture was heated on a hot plate at 30-40 ºC and mixed with continuous stirring for 20 minutes. Then, the extract was filtered using Whatman filter paper and concentrated using water bath. Soxhlet method was used for methanol extraction as described by [11] with slight modification. 10 gram of powdered fruit was packed in a thimble which was placed into an extractor and then extracted with 250 mL of methanol. The extraction process was carried out until the solvent in the siphon tube of Soxhlet apparatus became colorless. After that, the extract was heated in hot water bath at 35 ºC until all the solvent evaporated. The dried fruit extracts were kept in refrigerator at 2-8 ºC for their future use.

Phytochemical Screening

The phytochemical analysis of both aqueous and methanolic extract of P. dactylifera was done by standard methods as described by [12].

Test for Carbohydrates

A quantity of 0.1 g each of the extracts was shaken vigorously with water and then filtered. To the aqueous filtrate, few drops of Molisch reagent were added, followed by vigorous shaking. 1 mL of concentrated sulphuric acid was carefully added to form a layer below the aqueous solution. A violet ring appeared at the interphase of the test tube, indicating the presence of carbohydrates.

Test for Steroids

The amount of 0.5 g of extract was dissolved in 10 mL anhydrous chloroform and filtered. The filtrate was mixed with 1 mL of acetic anhydride followed by the addition of 1 mL of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green colouration as indicative of steroids.

Test for Flavonoids

0.2 g of extracts was heated with 10 mL of ethyl acetate in boiling water for 3 min. The mixture was filtered consecutively, and the filtrates were used for the flavonoid test. 4 mL of the filtrates was shaken with 1 mL of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration was observed at the ammonia layer, which turned colorless when 2 drops of diluted hydrochloric acid were added to the solution. This result indicated the presence of flavonoids.

Test for Alkaloids

0.5 g of extracts was boiled with 5 mL of 2% HCl on a steam bath for 10 min and filtered. To 5 mL of the filtrate, 2 mL of dilute ammonia solution was added. After that, 5 mL of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 mL of acetic acid. Draggendorff's reagent was added to the extract. The formation of reddish-brown precipitate was regarded as positive for the presence of alkaloids.

Test for Saponins

0.5 g of extract was added 5 mL of distilled water in a test tube. The solution was shaken vigorously and observed for a stable, persistent froth. Formation of foam indicated the presence of saponins.

Test for Tannins

About 0.5 g of the extract was boiled in 10 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green colouration.

Test for Cardiac Glycosides

0.5 g of extract diluted to 5 mL in water and added 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with 1 mL of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxy sugar characteristic of cardenolides.

Test for anthraquinones

0.5 g of extract was placed in a dry test tube, and 20 mL of chloroform was added. This was heated in steam bath for 5 min. The extract was filtered while hot and allowed to cool. To the filtrate, 10% ammonia solution was added with an equal volume of filtrate. This was shaken, and the upper aqueous layer was observed for bright pink colouration as indicative of the presence of anthraquinones.

Antioxidant Assay

The antioxidant activities of P. dactylifera powder were measured using three different methods, namely (i) DPPH(2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging assay, (ii) Hydrogen peroxide scavenging (H_2O_2) assay, and (iii) Reducing power method (RP).

DPPH Scavenging Assay

The free radical scavenging potential of both methanolic and aqueous extracts were determined according to the procedure of [13] with some modifications. An aliquot of 200 µL of sample solution of various concentrations (100–500µg/mL) were mixed with 2mL of methanolic solution of DPPH (0.5mM). The reaction mixture was incubated at 37°C for 1 h in the dark. The free radical scavenging potential of the extracts were expressed as the disappearance of the initial purple color. The absorbance of the reaction mixture was recorded at 517 nm using UV–Visible spectrophotometer. Ascorbic acid was used as the positive control. DPPH scavenging capacity was calculated by using the following formula:

Hydrogen peroxide Scavenging Assay

The ability of the extract to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of [14]. Aliquot of 0.1 mL of extracts (100–500µg/mL) was transferred into the eppendorf tubes, and their volume was made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4) followed by the addition of 0.6 mL of H_2O_2 solution (2 mM). The reaction mixture was vortexed, and after 10 min of reaction time, its absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. The ability of the extracts to scavenge the H_2O_2 was calculated using the following equation:

Reducing Power Assay

The reducing power was determined according to the [15] method with some modifications. Aliquot of 0.2 mL of various concentrations of the extracts (100–500µg/mL) were mixed separately with 0.5 mL of phosphate buffer (0.2 M, pH 6.6) and 0.5 mL of 1% potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 20 min. After cooling at room temperature, 0.5 mL of 10% trichloroacetic acid was added, followed by centrifugation at 3,000 rpm for 10 min. Supernatant (0.5 mL) was collected and mixed with 0.5 mL of distilled water. Ferric chloride (0.1 mL of 0.1%) was added, and the mixture was left at room temperature for 10 min. The absorbance was measured at 700 nm. Ascorbic acid was used as positive control.

Anti-inflammatory Activity

The anti-inflammatory activity of aqueous and methanolic extract of P. dactylifera was estimated by membrane stabilization method. Acetylsalicylic acid available in the commercial name of Ecosprin ʀ-75 was used as a source of acetylsalicylic acid. The blood was collected from a healthy human volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment and collected in heparinized vacutainer. The blood was washed three times with 0.9% saline and centrifuged simultaneously for 10 minutes at 3000 rpm. The packed cells were washed with 0.9% saline, and a 40% v/v suspension made using isotonic

phosphate buffer composed of 154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4 used as Stock erythrocyte or RBC suspension. Membrane stabilization test was done according to the method described [16] with slight modifications. The test sample consisted of stock erythrocyte (RBC) suspension 0.030 mL mixed with 5mL of hypotonic solution (154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4) containing P. dactylifera extract ranging from concentration 100-500 µg/mL. The control sample consisted of 0.030 mL RBC suspension mixed with hypotonic buffered solution alone. The standard drug acetylsalicylic was treated similarly to test at 100-500 µg/mL concentrations. The mixtures were incubated at 10 minutes at room temperature, centrifuged for 10 minutes at 3000 rpm, and absorbance of the supernatant was measured spectrophotometrically at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated by the following equation.

Where;
A_1=Absorbance of hypotonic buffered solution alone
A_2= Absorbance of test /standard sample in hypotonic solution.

Antimicrobial Activity

The agar well diffusion method was used for the antimicrobial test. The strains used for the present study were Escherichia coli, Staphyloccocus aureus, and Candida albicans. Mueller Hilton agar and dextrose medium were prepared according to manufacturer's specification. The media were autoclaved and dispensed into sterile petri-dishes and allowed to solidify. Inoculum of each isolate was streaked on the agar plate. Four wells of 6mm each were made in each plate using a sterile cork borer. The wells were filled with 0.1 mL of different concentrations (500 µg/mL, 1000 µg/mL) of the extract with the aid of sterile pipettes per well. Diameters of zones of inhibition were measured after 24 hours of incubation at 37°C.

RESULTS AND DISCUSSION

Phytochemical Screening

Phoenix dactylifea (date) fruit powder was extracted with methanol and water and analyzed for their phytochemical content. Preliminary phytochemical analysis of both extracts (aqueous and methanol) are shown in Table. 1. Screening of phytochemicals of P. dactylifea showed the availability of natural compounds such as alkaloid, flavonoid, anthraquinone, carbohydrates, saponin, steroid, and tannin by both the aqueous and methanol extracts.

Table.1. Preliminary phytochemical screening of P. dactylifea

Phytochemical Test	Ethanol Extract	Aqueous Extract
Carbohydrate + +		
Flavonoids + +		
Saponin + +		
Alkaloid + +		
Cardiac Glycosides + +		
Steroid + +		
Anthraquinone + +		
Tannins + +		

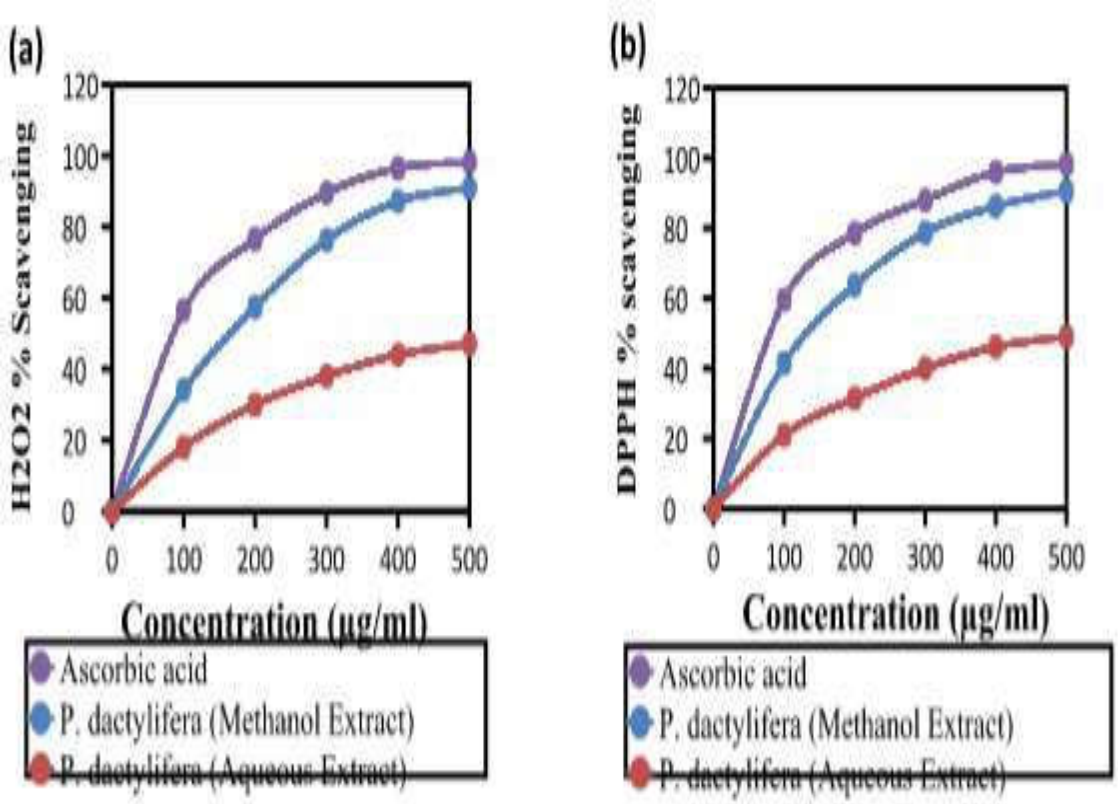
Antioxidant Assay

Three different methods and modifications have been used to measure antioxidant activity. A single method is not enough to assay the whole antioxidant activity; therefore, application and combination of several tests recommended providing a better description. The scavenging activity of both extracts were expressed as % inhibition and was compared with standard antioxidant as ascorbic acid as shown in Figure. 1. As shown in Figure. 1, concentration of both extract samples increases the % inhibition as the concentration of sample increases. It indicates the hydrogen donating ability of the sample antioxidants such as phenolic compounds. Among the three-antioxidant activity assay, the methanol extract of P. dactylifea showed more

% inhibition than that of aqueous extract. Antioxidant activity is recognized due to the wide range of phenolic compounds present in dates, including p-coumaric, ferulic, sinapic acids, flavonoids, and procyanidins [17].

Anti-inflammatory Activity

Anti-inflammatory activity of P. dactylifea was analyzed by HRBC (Human Red Blood Cells) membrane stabilization assay. Study was conducted for both the methanolic and aqueous extract at concentration range of 100-500 µg/mL. In this assay, acetylsalicylic acid was considered standard. As shown in Figure. 2, with increase in the concentration inhibition percentage of hemolysis also increases. The results were comparable with the reference drug, which was also subjected to the same treatment. These results showed the effective bioactivity of P. dactylifea for the pharmaceutical usage as anti-inflammatory drugs. For the methanolic extract highest inhibition percentage of hemolysis of 49% was observed at 500 µg/mL concentration, while for the same concentration of aqueous extract highest inhibition percentage of hemolysis was 23%. Acetylsalicylic acid showed maximum inhibition tendency of hemolysis 87.2% at a 500 µg/mL concentration. The lysosomal enzymes released during inflammation generate different disorders. The extracellular activity of these enzymes is responsible for acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or stabilizing the membrane. Since RBC membranes are similar to lysosomal membrane components, the prevention of hypotonicity-induced RBC membrane lysis is taken as a measure of anti-inflammatory activity of drug. Compounds with membrane-stabilizing properties are well known for their ability to interfere with the early phase of inflammatory reactions, namely the prevention of the release of phospholipases that trigger the formation of inflammatory mediators. The extract of this plant demonstrates significant membrane-stabilizing properties, which suggests that extract of P. dactylifea may offer some beneficial effects in the management of inflammatory conditions.



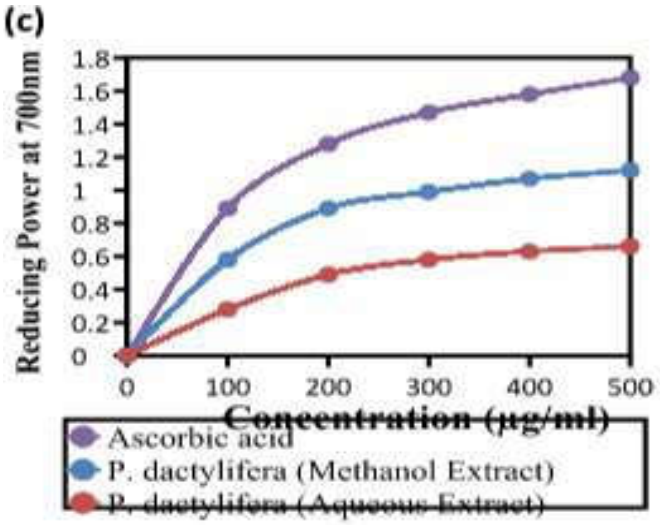


Figure. 1. Antioxidant activities of P. dactylifea extracts: (a) DPPH free radical scavenging activity; (b) hydrogen peroxide scavenging activity; (c) reducing power

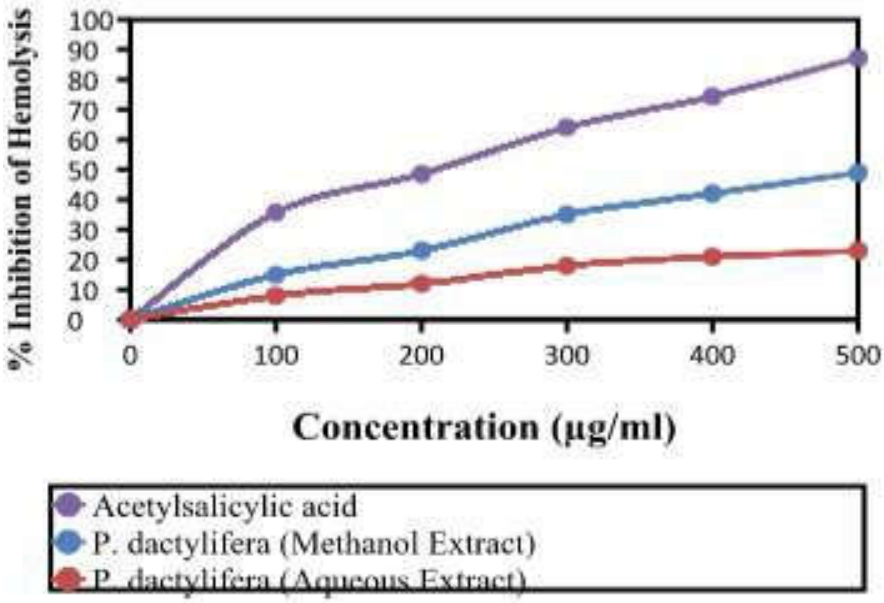


Figure. 2. Anti-inflammatory activities of P. dactylifea extracts

Antimicrobial Activity

The results of the antimicrobial activity of P. dactylifea are shown in Table. 2. From the result, highest zone of inhibition was observed in methanolic extract for E. coli. As shown in Table. 2, P. dactylifea extracts contain significant antibacterial activity against gram-negative bacteria like Escherichia coli. It was also effective against gram-positive bacteria like Staphylococcus aureus and fungal species like Candida albicans. In the present study, P. dactylifera extract has antibacterial effect against E. coli, indicating that this extract can be used for treating enteric diseases.

Table. 2. Antimicrobial activities of P. dactylifea extracts

Culture	Concentration (µg/mL)	Zone of inhibition (in mm)	
		Ethanol Extract	Aqueous Extract
E. coli	500	18	12
	1000	20	16
S.aureus	500	16	10
	1000	19	17
C.albicans	500	13	8
	1000	19	15

CONCLUSION

Our present investigation concluded that P. dactylifera is a medicinal plant with enormous biological activities. This is due to the potential constituents like flavonoids, terpenoids, alkaloids, saponins, and steroids. P. dactylifera demonstrated high phytochemical content and are potent antioxidants and could be used as a supplement to prevent oxidative stress. Phytochemical analysis proved the availability of natural chemical constituents. The anti-inflammatory assays carried out on the fruit extracts indicate that the plant is an excellent source of anti-inflammatory drugs. The P. dactylifera was also found to be a potential antimicrobial agent. Dates fruits in the control of disease create optimism towards the novel therapeutic strategy. Keeping all information in hand as antioxidant, anti-inflammatory, and antimicrobial, further research based on clinical trial is required to authenticate the exact mechanism of P. dactylifera and their constituents in disease prevention.

ACKNOWLEDGMENT

The authors gratefully acknowledge the KME Society's G.M. Momin Women's College for providing financial support for this work.

REFERENCES

[1]. D. Murugesan and R. Deviponnuswamy, "Innovare Academic Sciences potential anti-inflammatory medicinal plants - A review,"Int. J. Pharm. Pharm. Sci., vol. 6, no. 4, pp. 43–49, 2014.
[2]. N. S. Ncube, A. J. Afolayan, and A. I. Okoh, "Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends,"African J. Biotechnol., vol. 7, no. 12, pp. 1797–1806, 2008.
[3].Luiseach Nic Eoin, "Immunity: RALF to the rescue,"Nat. Plants, vol. 2, no. 5, p. 1, 2016.
[4]. Z. Ashraf and Z. Hamidi-Esfahani, "Date anddata processing: A review,"Food Rev. Int., vol. 27, no. 2, pp. 101–133, 2011.
[5]. W. Al-Shahib and R. J. Marshall, "The fruit of the date palm: Its possible use as the best food for the future?,"Int. J. Food Sci. Nutr., vol. 54, no. 4, pp. 247–259, 2003.
[6]. F. Al Juhaimi, K. Ghafoor, and M. M. Özcan, "Physical and chemical properties, antioxidant activity, total phenol and mineral profile of seeds of seven different date fruit (Phoenix dactylifera L.) varieties,"Int. J. Food Sci. Nutr., vol. 63, no. 1, pp. 84–89, 2012.
[7]. O. Djaoudene et al., "Phoenix dactylifera L. seeds: A by-product as a source of bioactive compounds with antioxidant and enzyme inhibitory properties,"Food Funct., vol. 10, no. 8, pp. 4953–4965, 2019.
[8]. Saryono et al., "Date seeds (Phoenix dactylifera L.) consumption as anti-inflammatory and immunostimulant: A systematic review,"IOP Conf. Ser. Earth Environ. Sci., vol. 250, no. 1, 2019.
[9]. A. F. El-Fouhil, A. M. Ahmed, and H. H. Darwish, "Hypoglycemic effect of an extract from date seeds on diabetic rats,"Saudi Med. J., vol. 31, no. 7, pp. 747–751, 2010.

[10]. E. dine T. Bouhlali, C. Alem, J. Ennassir, M. Benlyas, A. N. Mbark, and Y. F. Zegzouti, "Phytochemical compositions and antioxidant capacity of three date (Phoenix dactylifera L.) seeds varieties grown in the South East Morocco,"J. Saudi Soc. Agric. Sci., vol. 16, no. 4, pp. 350–357, 2017.
[11]. S. B. Nikhal, "IJPSR (2010), Vol. 1, Issue 7 (Research Article)," vol. 1, no. 7, pp. 78–81, 2010.
[12]. A. . Harborne, "Harborne, J.B. (1998) Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis.," 1998.
[13]. T. Kulisic, A. Radonic, V. Katalinic, and M. Milos, "Use of different methods for testing antioxidative activity of oregano essential oil,"Food Chem., vol. 85, no. 4, pp. 633–640, 2004.
[14]. R. J. Ruch, S. jun Cheng, and J. E. Klaunig, "Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from chinese green tea,"Carcinogenesis, vol. 10, no. 6, pp. 1003–1008, 1989.
[15]. M. Oyaizu, "Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine.,"Japanese J. Nutr. Diet., vol. 44, no. 6, pp. 307–315, 1986.
[16]. U. A. Shinde, A. S. Phadke, A. M. Nair, A. A. Mungantiwar, V. J. Dikshit, and M. N. Saraf, "Membrane stabilizing activity - A possible mechanism of action for the anti-inflammatory activity of Cedrus deodara wood oil," Fitoterapia, vol. 70, no. 3, pp. 251–257, 1999.
[17]. A. Mansouri, G. Embarek, E. Kokkalou, and P.Kefalas, "Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (Phoenix dactylifera),"Food Chem., vol. 89, no. 3, pp. 411–420, 2005.