Indian J.L.Sci. 4 (1) : 95-100, 2014

ISSN : 2277-1743 (Print)
ISSN : 2278-7879 (Online)

ANTIMICROBIAL AND SYNERGISTIC ACTIVITY OF PHOENIX DACTYLIFERA SEEDS

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ABSTRACT

Due to the various side effects and resistance of microorganisms towards available antibiotics, new effective drugs are formulated. In this experiment, seeds of Phoenix Dactylifera (Safawy dates) were washed, dried, powdered and extracted using a soxhlet extractor in three solvents and diluted in DMSO. Microorganisms (6 gram negative and 4 gram positive) were cultured and tested for their antimicrobial activity using agar well diffusion method on Mueller Hinton Agar. The zone of inhibition was measured in mm after incubation for 24hrs at 370C. B.subtilis and C.diphtheriae were most sensitive while S.pyogenes and P.aeruginosa were most resistant. MIC-MBC values were 2.5 and 1.25mg/ml respectively. Salmonella paratyphi A and S.dysentriae showed synergism with honey while S.aureus and Salmonella paratyphi B showed antagonism. Synergism was seen with C.diphtheriae for Gentamicin, S.pyogenes for Chloramphenicol, Salmonella paratyphi A and S.dysentriae for Rifampicin while antagonism with S.dysentriae for Chloramphenicol. Phytochemical screening revealed the presence of carbohydrates, proteins, amino acids, flavonoids, phenols, glycosides and phytosterols in the sample.

KEYWORDS : P.Dactylifera Seeds, Antimicrobial, Antibiotic Synergy, Honey Antimicrobial, Phytochemical

Throughout the history of mankind, infectious diseases have affected millions of people worldwide. Antibiotics discovery proved to be an effective weapon against these infections. However due to improper use of drugs antibiotic resistance has emerged (1), (2). There are three approaches to overcome the resistance of microorganisms: Use of antibiotics, Use of natural plant extract and combination therapy i.e. association of antibiotics with plant extracts (3). The secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, saponins, sterols, glycosides etc. present in various plants are found to have antimicrobial properties in vitro (4), (5), (6). Crude plants extracts have been found to be more effective pharmacologically than isolated active compounds; due to the synergistic effects of various components present in the extracts (7).

A middle stoned (8), Dioecious i.e. the male and female parts are on separate plants, perennial and monocotyledon fruit trees (9) of Phoenix dactylifera belong to the Arecaceae family (10), comprising of 200 genera and 3000 species worldwide (11), (12). Dates represent an important economical and ecological culture for many countries (13). Dates have been used as a detersive and astringent in intestinal troubles, treatment for sore throats, colds, bronchial catarrh, fevers, gonorrhea, edema liver, can be applied to wounds, lesions, inflammation, prescribed in case of asthma and abdominal troubles, and to counteract alcohol intoxication (14), (15), (16). The grounded paste of seeds from the date palm is effective in treating ague (17). Date contains six essential vitamins A, C, B1, B2, nicotinic acid and folic acid (18), (19).

In most ancient cultures honey has long been used for both nutritional and medical purposes. It is believed to be a nutrient, a drug and an ointment. Apitherapy, an alternative branch developed in recent years offers treatments based on honey and other bee products against many diseases including bacterial infections (20).

Hence this study was initiated to estimate the phytochemical composition and the antimicrobial properties inherited by seeds of Phoenix Dactylifera, its synergistic activity of with raw unprocessed honey and standard antibiotic disks.

MATERIALS AND METHODS

Collection of Plant material and extraction: The Phoenix dactylifera (Safawy dates) seeds were washed thoroughly with DW, dried in open air for a week, crushed and powdered using a mortar pestle and extracted using a soxhlet extractor (21) in acetone, methanol and ethanol solvents and diluted in DMSO(3). For roasted extract dried seeds were roasted until slightly burned (22) before powdering. Raw unprocessed honey was diluted in DW and stored in airtight container at 40C before analysis.

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Test Organisms

Ten bacterial isolates (Six gram negative and four gram positive) Salmonella paratyphi A, Salmonella paratyphi B, Shigella, Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Bacillus subtilis, Corynebacterium diphtheriae, Streptococcus pyogenes, Pseudomonas aeruginosa were obtained from the Department of Microbiology, Kischinchand Chellaram College, Mumbai.

Antibiotic disks: 30 mg Chloramphenicol, 10 mg Gentamicin, 10 mg Ampicillin and 5 mg Rifampicin disks of Hi Media Limited stored at 40C were used.

Agar well diffusion and Disk Diffusion method: Agar well diffusion method (23) (24)(25)(26) was employed for the antimicrobial testing.300ul of 0.10 absorbance culture were added to 25 ml of M-H Agar and poured into sterile plates(27)(28)(29).30ul of extracts were added in 8mm wells. Two controls of DMSO and the respective solvent were maintained. For synergistic activity 15ul each of the two extracts were added to the well (30).Plates were incubated for 24h at 370C after which synergism and antagonism was determined by measuring the inhibition zone (mm) using a transparent scale (28).For disk diffusion method 100ul cultures were swabbed on the solidified plate and Antibiotic disks soaked in seed extract for 10 minutes were placed.

Minimum inhibitory concentration (MIC) and MBC Determination

Minimum inhibitory concentration(MIC) is defined as the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test(31).2ml of dsMH Broth(32) and 2ml of extract were serially diluted to obtain 5, 2.5, 1.25, 0.625 and 0.03125 mg/ml respectively.100ul of 0.01 O.D culture were added to all tubes except negative control.The first tube showing no turbidity after incubation for 24h at 370C was taken as the MIC. A loopful of inoculum was streaked on M-H Agar plates and incubated for 24h at 370C, and then bacterial growth was evaluated. The lowest concentration of plant extract showing no growth was taken as the MBC (33)

Phytochemical Analysis

Phytochemical analysis of the crude powder as well as acetone, methanolic and ethanolic extracts of date seeds were determined as follows:

1. Detection of carbohydrates: The sample were dissolved in 5 ml DW and filtered.

a) Molisch's Test: 2 drops of alcoholic α-naphthol solution were added to the filtrate. Violet ring at the junction is positive.

b) Benedict's Test: Benedict's reagent and filtrate were heated gently. Orange red precipitate is positive for reducing sugars.

c) Fehling's Test: Hydrolysation and neutralisation of filtrate were carried out by dil. HCl and NaOH simultaneously followed by heating with Fehling's A & B solutions. Red precipitate is positive.

2. Detection of saponins

a) Froth Test: The samples were diluted in 10 ml DW and shaken in a graduated cylinder for 15 minutes. 1 cm layer of foam is positive.

b) Foam Test: The samples were diluted with 2 ml DW and shaken. If the foam produced persists for ten minutes than positive.

3. Detection of flavonoids

a) Alkaline Reagent Test: The samples were treated with few drops of NaOH solution. Flavonoids are present if an intense yellow colour is formed, which becomes colourless on addition of dilute acid.

b) Lead acetate Test: Yellow coloured ppt on treatment with few drops of lead acetate solution is positive.

4. Detection of proteins and amino acids

a) Xanthoproteic Test: Yellow colour on reacting with few drops of conc. Nitric acid is positive.

b) Ninhydrin Test: Blue colour when boiled with Ninhydrin reagent is positive.

5. Detection of phytosterols

a) Liebermann Burchard test: The samples were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Brown ring with conc.H_2SO_4 is positive.

6. Detection of glycosides: The samples were hydrolysed with dil. HCl.

Table 1: Phytochemical Analysis

Tests	P	P*	E	E*	M	M*	A	A*	H	C
Molisch's tests	-	-	+	+	+	+	-	+	+	-
Benedicts test	-	-	+	+	-	-	-	-	+	-
Fehlings test	-	-	+	+	-	-	-	-	+	-
Froth test	-	-	-	-	-	-	-	-	-	-
Foam test	-	-	-	-	-	-	-	-	-	-
Xanthoproteic test	-	-	+	+	+	+	+	+	-	-
Ninhydrin test	-	-	+	+	+	+	+	+	-	-
Alkaline reagent test	+	+	+	+	-	-	+	+	+	-
Lead acetate test	+	+	+	+	-	-	+	+	+	-
Ferric chloride test	+	+	+	+	+	+	+	+	-	-
Modified borntrager's test	+	+	+	+	+	+	+	+	+	-
Liebermann burchard test	+	+	+	+	+	+	+	+	+	-
Tests for alkaloids	+	+	+	+	+	+	+	+	+	-
Tests for tannins	+	+	+	+	+	+	+	+	+	-

Key: P, E, M and A represent powder ethanol methanol and acetone extracts of seeds respectively.
(*) represent roasted seed

Table 2: Synergistic Activity of Antibiotics With Ethanol Extract of Roasted Seed

Test organisms	Gen	Gen*	C	C*	R	R*
SPA	-	-			22	26
S. dysentriae	-	-	22	17	-	-
C.diphtheriae	20	28	-	-	-	-
S.pyogenes	-	-	25	32	-	-

Figure1:Antimicrobial Activity of *P. dactylifera* Seeds With Crude and Roasted Seed Extract of Ethanol, Methanol and Acetone

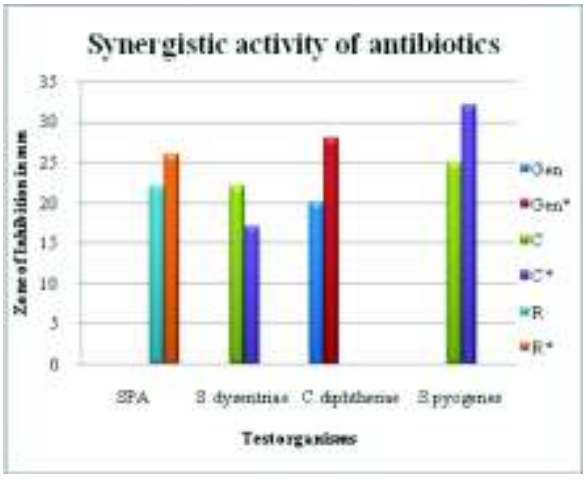

Figure 2: Synergistic Activity of Antibiotics With Ethanol Extract of Roasted Seed.

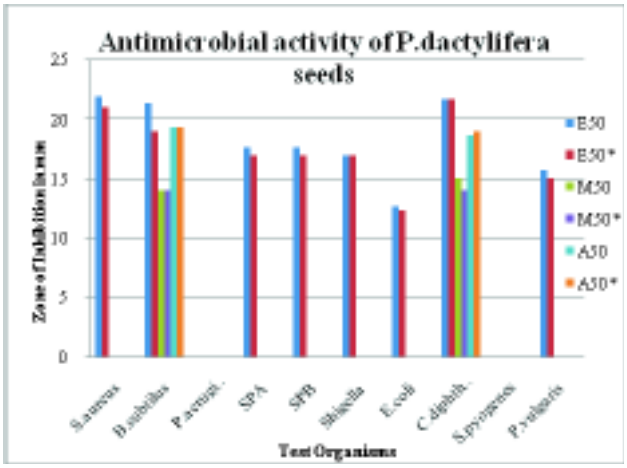

Table 3: Antimicrobial and Synergistic Activity of Ethanol, Methanol and Acetone Extract of Roasted and Crude Seed

Cultures	E50	E50*	M50	M50*	A50	A50*	C1	C2	C3	C4	H	HE	HE*	HM	HA
S.aureus	22	21	-	-	-	-	-	-	-	-	-	-	-	-	-
B.subtilus	21.33	19	14	14	19.33	19.33	-	-	-	-	-	-	-	-	-
P.aeruginosa	-	-	-	-	-	-	-	-	-	-	40	24	25	25	35
SPA	17.67	17	-	-	-	-	-	-	-	-	35	40	40	38	40
SPB	17.67	17	-	-	-	-	-	-	-	-	37	33	35	30	33
Shigella	17	17	-	-	-	-	-	-	-	-	30	34	33	40	38
E.coli	12.67	12.33	-	-	-	-	-	-	-	-	-	-	-	-	-
C.diphtheriae	21.67	21.67	15	14	18.67	19	-	-	-	-	-	-	-	-	-
S.pyogenes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P.vulgaris	15.67	15	-	-	-	-	-	-	-	-	-	-	-	-	-

Inhibition zone diameter in millimeters on the numbers show. The reading is a mean of three different readings. (*) represents roasted seed extracts. E, M and A indicates Ethanol, Methanol and Acetone extract and C1 C2, C3 their respective controls. C4 and H represents DMSO and honey respectively

Table 4: Determination of MIC and MBC of Ethanol Extract of Crude and Roasted Seed

Cultures	E	E*
S.aureus	MIC- 1:4; MBC- 2.5%	MIC- 1:4; MBC- 2.5%
B.subtilus	MIC- 1:4; MBC- 2.5%	MIC- 1:4; MBC- 2.5%
SPA	MIC- 1:4; MBC- 2.5%	MIC- 1:4; MBC- 2.5%
SPB	MIC- 1:4; MBC- 2.5%	MIC- 1:4; MBC- 2.5%
Shigella	MIC- 1:4; MBC- 2.5%	MIC- 1:4; MBC- 2.5%
E.coli	MIC- 1:4; MBC- 1.25%	MIC- 1:4; MBC- 1.25%
C.diphtheriae	MIC- 1:4; MBC- 1.25%	MIC- 1:4; MBC- 1.25%
P.vulgaris	MIC- 1:4; MBC- 2.5%	MIC- 1:4; MBC- 2.5%

a) Modified Borntrager's Test: Filtrate with $FeCl_3$ solution was immersed in BWB for 5 minutes, cooled, extracted in benzene and reacted with ammonia to give rose pink colour (34).

RESULTS AND DISCUSION

The strain used by Najla'a Nabhan Yassein inhibited all the test organisms (Escherichia coli, Proteus vulgaris and Pseudomonas aeruginosa) except Kleibsiella pneumonia (due to the presence of capsule)above 100mg/ml; however a zone of inhibition for as low as 10mg/ml was observed with ethanol extracts indicating it to be a better solvent than saline. Javed Aamir et.al observed inhibition zones at 50mg/ml of methanol extracts with Escherichia coli, Staphylococcus aureus, Salmonella paratyphi A and Pseudomonas aeruginosa due to difference in the date species chosen. The MIC and MBC values are very less compared to previous studies on other varieties giving MIC values as high as 100mg/ml except for the date seed species tested by Mehrdad Shakiba et.al which showed similar values ranging between 2.5mg/ml and 1.25mg/ml respectively indicating that the species of Phoenix dactylifera collected by Mehrdad Shakiba et.al could possibly be Safawy dates.

Antimicrobial activity was observed with B.subtilis and C.diphtheriae being the most sensitive while S.pyogenes and P.aeruginosa being the most resistant of all the test organisms. Salmonella paratyphi A and S.dysentriae showed synergism with honey. Synergism was seen with C.diphtheriae for Gentamicin, S.pyogenes for Chloramphenicol, Salmonella paratyphi A and S.dysentriae for Rifampicin while antagonism with S.dysentriae for Chloramphenicol. Phytochemical screening revealed the presence of carbohydrates, proteins, amino acids,

flavonoids, phenols, glycosides and phytosterols in the sample.

CONCLUSION

The seeds of Phoenix dactylifera were found out to be a potential antimicrobial agent. The synergistic activity with antibiotics shows that the dose of antibiotics can be reduced if given in combination with the crude extract of Phoenix dactylifera.

ACKNOWLEDGMENT

I would like to extend my gratitude and sincere thanks to my college, our encouraging principal, my mentor, all the teaching and non-teaching staff, my family and friends.

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