Research Article

Evaluation of Antimicrobial Activity of Immature Palmyra Palm (*Borassus flabellifer linn.*) Fruits

Krishnamoorthy Renuka, Chandiran Sharmila, Sorimuthu Pillai Subramanian*
Department of Biochemistry, University of Madras, Guindy Campus, Chennai- 600 025, India.
***Corresponding author's E-mail:** subbus2020@yahoo.co.in

Received: 20-01-2019; **Revised:** 25-02-2019; **Accepted:** 05-03-2019.

ABSTRACT

The frequency in the prevalence of diseases due to pathogenic microorganisms has increased alarmingly due to the development of resistance to existing drugs and the present scenario necessitate the continuous search for new classes of antimicrobial agents preferably from natural resources. Palmyra palm (Palm tree) stands for an icon among various cultures owing to the utility that it bestows from various parts of the tree which renders immense benefit to mankind. Recently, we have reported the phytochemicals present in the immature palm fruits and its total phenolic and flavonoids contents. The present study was carried out to evaluate the possible antibacterial and antifungal efficacy of an ethanol extract of immature Palmyra Palm (*Borassus Flabellifer linn*.) fruits by well diffusion (bacteria), disc diffusion (fungi) methods. The MIC, MBC and MFC were also determined by established procedures. The bacterial and fungal strains were chosen based on their clinical significance. Totally five Gram-positive, five Gram-negative and eight fungi were screened using graded concentrations of immature fruits extract. The results obtained on the zone of inhibition and visible growth lead to the conclusion that the immature fruits extract possess a broad spectrum of antibacterial and antifungal activity. The present study also provides evidence for the use of immature palm fruits in the traditional medicine for the treatment of microbial infections and forms the basis for the isolation bioactive compounds with significant antimicrobial activity from the immature fruits for food preservation and therapeutic applications.

Keywords: Palmyra Palm fruits, antimicrobial, McFarland standard, minimum inhibitory concentration, minimum fungicidal concentration.

INTRODUCTION

The majority of microorganisms such as soil born, intestinal, industrial and commercial application oriented elicit crucial contributions to the welfare of the world's inhabitants by aiding to maintain the balance of living things and chemicals in the environment. Though only a minority of microorganisms is pathogenic, they pose a major threat to human health care in terms of morbidity and mortality in both developed and developing countries [1]. Subsequent to the serendipitous discovery of penicillin in the year 1928, antibiotics have been recognized as the only means of effective to control the pathogenesis of microorganisms. Along with the usage of new antibiotics as therapeutics, there is an emerging menace of drug-resistance among the pathogenic microorganisms worldwide [2]. Despite the progress made in the understanding of etiology, epidemiology, pathology and control, the incidence of epidemics due to drug resistant microorganisms, the emergence of hitherto unknown disease causing microbes to pose critical problems in the treatment of public concerns [3]. Hence there is an increased demand for the search of new lead molecules as antimicrobial agents. Rational drug design does not always result in potential antimicrobials. Most of the enzyme inhibitors that have been designed and synthesized in the past elicit only moderate antimicrobial activity probably owing to the complex issues associated with their uptake by the cells and bioavailability [4].

Phytochemicals are ecologically derived secondary metabolites synthesized by the plants from the primary metabolites such as carbohydrates, lipids and amino acids to protect them against environmental challenges such as UV- irradiation, extreme cold, drought, microbial attack, wound, sugar and nutrient deficiency. They often contribute to the unique odor, taste and color in plants [5, 6]. Based on the chemical nature, the secondary metabolites are mainly classified into alkaloids, steroids, saponins, tannins, lectins, pectins, terpenoids, anthraquinones, flavonoids, glycosides and phenolic compounds. Interestingly, these secondary metabolites are known to bring out significant pharmacological and beneficial effects to alleviate chronic diseases such as cancer, diabetes and cardiovascular diseases due to their antioxidant, anti-inflammatory and regulatory actions [7, 8]. Recent advances in the field of medicinal chemistry lead to the discovery of isolating the active phytochemicals from various parts of the medicinal plants for treating human infectious diseases. Numerous structural analogs of plant secondary metabolites have been successfully generated and widely used for their pharmacological actions [9]. However, the therapeutic efficacy of medicinal plant extracts is usually synergetic in nature [10]. Since earliest times, several plant extracts have been known to exert significant antimicrobial properties against human pathogens. Approximately 30% of the plants exist in the world have been subjected into pharmacological screening and a substantial number of new antibiotics derived from them have been introduced in the clinical practice [11]. Palmyra palm, commonly known as a palm

tree and botanically termed as *Borassus flabellifer* Linn. belongs to the family "*Arecaceae*" is one such medicinal plant that has not been subjected to systemic scientific scrutiny for its therapeutic applications [12]. The immature Palmyra palm fruits have been widely used in traditional medicine for the treatment of various ailments [13, 14]. Recently, we have reported the phytochemical screening and *in vitro* antioxidant properties of immature Palmyra palm fruits extract. The immature Palmyra palm fruits have been widely used as an antimicrobial agent in traditional medicine. In the absence of systematic reports in the scientific literature regarding the antimicrobial activities of immature Palmyra palm fruits, the present study was aimed to screen the antibacterial and antifungal properties of immature Palmyra palm fruits against clinically important bacteria and fungi.

MATERIALS AND METHODS

Plant material

The immature (prior to the development of endosperms) Palmyra palm fruits that have weighed between 30 to 50 gm were collected from the trees near Chengalpet, Tamil Nadu during the month of December 2017. The plant material was authenticated by a taxonomist at the Centre for Advanced Studies in Botany, University of Madras. A voucher specimen was deposited in the Herbarium (CAS-2017-07).

Preparation of the fruits extract

The immature fruits were washed thoroughly under running tap water, rinsed in distilled water, cut into slices, dried in an electric oven and powdered by using an electric grinder to a coarse powder which was stored in an airtight container at 5^0C until further use. The powdered fruits were delipidated with petroleum ether (60-80^0C) for overnight to selectively remove the lipids. A known amount of the delipidated extract was placed in the extraction thimble which was suspended above the flask containing the ethanol and below the condenser. The fruits extract was subjected to Soxhlation at a temperature of 50-55^0C. At the end of the extraction process, the flask containing the ethanol extract was removed and the solvent was evaporated using a rotary evaporator and the semisolid obtained was dried in a vacuum desiccator. The weight of the extract was measured and the percentage yield of the fruit material was calculated [15].

Bacterial, fungal strains and growth medium

The bacterial and fungal strains used in the present study are standard laboratory strains procured from the stock cultures of the Division of Microbiology, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulattur and maintained at 20^0C on Muller Hinton Agar (MHA) (Himedia) and Potato Dextrose Agar (PDA) (Himedia) media for bacteria and fungus respectively. The essential composition of the MHA media to maintain bacterial cultures include beef extract, acid hydrolysate of casein, starch and agar. The medium was prepared by adding 2 gm of beef extract, 17.50 gm of acid hydrolysate of casein, 1.50 gm of starch and 17 gm of agar in one liter of distilled water. The final pH of the medium was adjusted to 7.3 ± 0.1 at 25^0C [16]. The composition of the PDA media used for fungal cultures comprises of potato infusion, dextrose and agar. Briefly, the medium was prepared by adding 200 gm of potato infusion, 20 gm of dextrose and 20 gm of agar in one liter of distilled water and the final pH was adjusted to 5.6 ± 0.2 [17].

The Gram-positive bacteria used in the present study include, *Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis, Streptococcus pyogenes and Streptococcus pneumonia* and the Gram - negative bacteria include, *Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Shigella dysenteriae and Pseudomonas aeruginosa*. The fungal cultures chosen for the present study include *Candida albicans, Saccharomyces cerevisiae, Aspergillus fumigates, Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Penicillium chrysogenum and Penicillium notatum.*

The bacterial cultures were maintained on slopes of MHA medium and sub-cultured every 15th day to prevent pleomorphic transformation. The bacterial cultures were appropriately diluted in sterile normal saline solution to obtain the cell suspension at 10^6 to 10^8 CFU/ml. Likewise, the fungal strains were subcultured on slants of PDA at 28^0C for 7days and the colonies were suspended in 1ml of sterile normal saline. The resulting mixture of conidia and hyphal fragments was vortexed and the turbidity of each homogenous suspension was adjusted to match that of a 0.5 McFarland standard, as read at 530nm. At this turbidity, the fungi density was maintained at $3x10^6$ to $5x10^6$ CFU ml^{-1}.

Determination of antibacterial and antifungal activity

Preparation of inoculum

The suspension for inoculation was prepared from the broth culture. Few colonies of similar morphology of the selected bacteria from twenty-four hours old culture were transferred with the aid of a sterile inoculating loop to a Muller- Hinton broth and were incubated until adequate growth of turbidity equivalent to McFarland 0.5 turbidity standard (10^8 CFU/ml) was achieved. The turbidity was corrected by adding physiological saline. The isolates were subcultured on MH Agar and incubated at 35^0C for 7–14 days. The growth was scraped aseptically, crushed and macerated thoroughly in sterile distilled water. Similarly, the fungal inoculums were prepared from 5 to 10 days old cultured grown on PDA medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using the sterile spatula. The spore density of each fungus was adjusted with spectrophotometer absorbance at 595 nm to obtain a final concentration of approximately10^5 spores/ml. The fungal suspension was standardized

spectrophotometrically to an absorbance of 0.600 at 450 nm.

Preparation of the McFarland standard

The preparation of McFarland Standard was carried out by mixing appropriate proportions of 1ml of 36N sulfuric acid in 99 ml of water and 1% anhydrous Barium chloride solution in 100 ml of water. The reaction between the two chemicals results in turbidity which in turn is due to the formation of a fine precipitate of barium sulfate. The most commonly used 0.5 McFarland solutions as a standard for Antibiotic Susceptibility test (AST) was freshly prepared by mixing 0.5 ml $BaCl_2$ in 99.5 ml of 1% H_2SO_4 solution. The solution was shaken well so that the precipitate distributed homogenously in the solution to obtain specific optical densities. A 0.5 McFarland turbidity standard provides an optical density comparable to the density of a bacterial suspension 1.5x 10^8 colony forming units (CFU/ml). The 0.5% McFarland turbid solution is used as a standard solution to which the cultures bacterial suspensions are compared and standardized. The approximate number of bacteria in a liquid suspension or broth culture was determined by comparing the turbidity of the cultured test suspension with that of the McFarland Standard [18].

The antibacterial activity of the ethanol extract of immature palm fruits was evaluated by agar well diffusion method. The stock solution of the immature palm fruits extract (2.5 mg/ml) was prepared in sterile distilled water [19]. Dilutions of the stock solution containing 50, 100, 150, 200 and 250 mg were also prepared in sterile distilled water. The pure 24 hrs old bacterial cultures were aseptically transferred to a sterile saline solution into different 10ml test tubes. They were matched with 0.5 McFarland standards.

The inoculums with respective tested bacteria were homogenously seeded onto the 90mm Petri dishes containing 20 ml of cooled molten Muller Hinton agar medium using a sterile cotton swab in such a way as to ensure thorough coverage of the plates and a uniform thick lawn of growth following incubation. The inoculums were then spread evenly by using a spreader (sterile cotton swab). Thereafter, with the help of a 9mm sterile cork borer, the bores were made on the agar medium plates. Each concentration was marked at the back of the agar bores prior to filling. Using a sterile pipette, 100µl of sterile distilled water was added to the control wells. Similarly, 100µl of each dilution of the fruit extracts was added into the wells. The plates were kept for 1 h at room temperature to allow free diffusion of the extract into the agar medium. Subsequently, all the plates were incubated at 37^{0}C for 18-24 h. Following incubation, the plates were examined for signs of microbial growth. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the wells. The diameters and the bore sizes were measured to the nearest mm. Chloramphenicol (30 µg/ml) was used as a positive control. Each experiment was carried out in triplicate.

Antifungal activity of the ethanol extract of immature Palmyra palm fruits extract was evaluated by the disc diffusion method. The inoculums with respective fungi were homogenously seeded onto the 90mm Petri dishes containing 20ml of cooled molten SDA medium using sterile pipette in such a way as to ensure thorough coverage of the plates and a uniform lawn of growth following incubation [20]. These inoculated plates were left to dry for at least 15min. The fruit extract was dissolved in sterile distilled water to obtain the different concentrations of 0.175, 0.375, 0.75, 1.5 and 3 mg/disc. Amphotericin B at concentration 10 µg/disc was used as a positive control and was dissolved in dimethyl sulphoxide (DMSO). Sterile filter paper discs (6mm in diameter) were impregnated with 10µl of each different concentration of fruit extract. The discs were allowed to dry and then placed on the agar surface of each Petri dish. DMSO was used as negative control. The Zone of inhibitions (in mm) was measured after 48-72h at 28^{0}C. The complete antifungal analysis was carried out under strict aseptic conditions. Each assay was repeated for a minimum of three times.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) assays

A serial of 2-fold macro-broth dilution method was performed to determine the MICs and MBCs of immature palm fruits extract for the respective tested bacterial suspensions (concentration) as recommended by the Clinical and Laboratory Standards Institute (CLSI) [21]. The minimum inhibitory concentration (MIC) of immature palm fruits extract against the fungal strains was determined using broth micro dilution method as described by the National committee for clinical laboratory standards for fungi (M27-A2). The stock solutions of immature palm fruits extract was diluted suitably as required from the stock solution. The ranges should be prepared one step higher than the final dilution range required (if a final dilution range of 0.5, 1, 2, 4, 8, and 16 mg/ml is required then a range of 1, 2, 4, 8, 16 and 32 mg/ml should be prepared) to compensate for the addition of an equal volume of inoculums. Two rows of 12 capped test tubes were arranged in the test tube rack. In a sterile 30 ml, universal screw capped bottle, 8 ml of MH broth (bacteria), 8ml PDA broth (fungi) containing the required concentration of immature palm fruits extract for the first tube in each row was prepared from the appropriate stock solution already made. The contents of the universal bottle were mixed using a sterile pipette and transferred 2 ml to the first tube in each row. Using a fresh sterile pipette, 4 ml of broth was added to the remaining 4 ml in the universal bottle, mixed well and transferred 2 ml to the second tube in each row. Dilutions were continued in this way to as many as 10 tubes. Subsequently, 2 ml of broth free from extract was added to the last tube in each row. The density of the bacterial suspension was adjusted (10^8 CFU/ml) to equal that of the 0.5 McFarland standard by adding sterile distilled water as detailed above. The bacterial suspension was suitably

diluted (10^6 CFU/ml) and added to the tubes containing MH broth. Chloramphenicol (30mg) was used as positive control. After incubation at 37^0C for 24 h, the turbidity of the tubes was assessed visually by comparison to uninoculated control.

Minimum fungicidal concentration (MFC)

To determine the MFC, Amphotericin B was included in the assays as a positive control (10 µg/ disc) for fungi. After incubation at 28^0C for 42-78h, the turbidity of the contents in the tubes was assessed visually by comparison to uninoculated control. The MIC is expressed as the lowest concentration of the extract where bacterial and fungal growth and/or fungal growth with no visible growth after incubation. All the assays were tested in triplicate.

RESULTS AND DISCUSSION

The continuous evolution of drug resistance to most of the currently available antimicrobial drugs has necessitated the search for the novel and effective therapeutic agents especially from natural resources. The development of antibiotic resistance is multifactorial, such as the unique nature of the microbes to antibiotics, indiscriminate use of various antimicrobial drugs, host characteristics and environmental factors. Substances and extracts isolated from different natural resources, especially medicinal plants have always been a rich arsenal for controlling the microbial infections and spoilage [22]. The synergistic effects of the plant secondary metabolites are actively considered to develop the antimicrobial activity and reduction of adverse side effects of currently available antimicrobial agents.

The yield of the ethanol extract of immature Palmyra palm fruits was around 42%. Earlier we have reported the major phytochemicals present in the immature fruits. Additionally, the immature fruits are found to contain significant levels of phenolic (104.00±0.02µg gallic acid equivalents/100 mg of fruits extract) and flavonoids (98.45±0.03µg quercetin equivalents/100 mg of fruits extract) [23]. In the present study, the antimicrobial activity of the ethanol extract of immature Palmyra palm fruits was screened against clinically important five Gram - positive, five Gram - negative and eight fungi. The efficacy was qualitatively and quantitatively evaluated by the diameter of the inhibition zones, minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC) [24]. The data obtained were presented as table 1, 2, 3 and 4 respectively. The zone of inhibition for both the bacteria and fungi were presented in mm and the minimum bactericidal and fungicidal concentrations were assessed visually by comparison to uninoculated control. The findings were compared with the growth inhibition results obtained for the standards (Chloramphenicol for the bacteria and Amphotericin B for fungi)

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. When compared to the disc diffusion method, the agar well diffusion method is considered to be superior because of the fact that the antimicrobial agent diffuses freely in the solid nutrient medium and inhibits the growth of the microbial strains [25]. The disc diffusion test or agar diffusion test or Kirby–Bauer test is a test of the antibiotic sensitivity of fungi. Briefly, it uses the antibiotic discs to evaluate the extent to which fungi are affected by selected antibiotics. In this test, wafers containing the antifungal agents are placed on an agar plate where the fungus has been streaked. The area around the wafer where the fungus has not grown enough to be visible is called a zone of inhibition [26].

Chloramphenicol (CAM), a well known antibacterial drug, was originally isolated in 1947 from *Streptomyces venezuela* and was introduced in clinical practice since 1949 [27]. CAM is effective parenterally as well as orally and has excellent cell penetration potential. It consists of a p-nitrobenzene moiety, 2-amino-1, 3-propanediol moiety and a dichloroacetyl tail. CAM is active against a broad spectrum of bacteria, usually behaving as a bacteriostatic drug, although it exhibits bactericidal activity against the most common causes of meningitis, Haemophilus influenza, Streptococcus pneumonia, and Neisseria meningitides [28]. CAM selectively inhibits protein synthesis by binding to the peptidyl transferase (PTase) center of the bacterial ribosome and abrogating essential ribosomal functions like peptide-bond formation [29], termination of translation [30] and translational accuracy [31].

Amphotericin B (AMB) was used as a reliable standard drug to compare the antifungal activity of the immature Palmyra palm fruits extract. AMB is considered as a reference drug in evaluating the antifungal activity of unknown drugs developed for the treatment of serious invasive mycoses. It is a polyene antifungal agent and its mode of action is based on the formation of a complex with the ergosterol in the fungal cell membrane, causing destabilization and subsequent release of a vital cell component [32, 33]. Liposome encapsulated AMB's lower affinity for mammalian cells and its enhanced distribution volume readily accounts for its decreased toxicity and the broad range of antifungal efficacy [34, 35, 32].

Table 1 shows the antibacterial activity of immature Palmyra palm fruits extract against the Gram- positive and Gram- negative bacteria. From the results, it is evident that the ethanol extract of palm fruits showed an inhibitory zone in a dose dependent manner. However, there was no significant difference between the levels of the zone of inhibition at the concentration of 200 µg and 250 µg. The sensitivity of the fruit extract was found to be in the order of *Bacillus subtilis* > *Streptococcus pyogenes* > *Staphylococcus epidermidis* > *Streptococcus pneumoniae* and *Staphylococcus aureus* against the Gram - positive bacteria and *Klebsiella pneumoniae* > *Shigella dysenteriae* > *Salmonella typhi* > *Escherichia coli* and *Pseudomonas*

aeruginosa for the Gram - negative bacteria. The observed difference in the sensitivity of the fruit extract among the different bacteria may be due to morphological differences between them. Further, the Gram - positive bacteria were known to be more vulnerable since they possess only an outer peptidoglycan layer, which is not an effective permeability barrier and may facilitate the infiltration of hydrophobic compounds [36].

The antifungal activities of the immature palm fruits extract against the selected pathogenic fungi are presented as Table 2. The data obtained evidenced that the fruits extract showed the antifungal activity similar to that of antibacterial activity. The highest antifungal activity (diameter of the zone of inhibition 24 mm) was demonstrated against *Candida albicans* while the lowest activity was observed against *Saccharomyces cerevisiae*. The results of the *in vitro* antifungal assay also revealed that the growth of fungal strains was severely affected by the fruits extract by forming clear inhibition zones. The antibacterial and the antifungal activity of the immature fruits extract were comparable with Chloramphenicol and Amphotericin B respectively.

The minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of immature fruits extract as well as the standard antibiotics are shown in Table 3 and 4. The MIC, MBC and MFC may be defined as the minimum concentration of the antimicrobial agent which exhibits the maximum zone of inhibition and allows no visible growth respectively. The MIC values of fruits extract against both the Gram - positive and the Gram - negative bacterial strains vary from the 1 to 5 mg and the efficacy was comparable with the standard drug. However the lowest MIC values were shown by *Bacillus subtilis* in Gram - positive bacteria and by *Klebsiella pneumoniae* in Gram - negative bacteria. The highest MIC values were shown by *Staphylococcus aureus* in Gram - positive bacteria and by *Salmonella typhi* in Gram - negative bacteria. Likewise, the MBC values also represent the significant antibacterial activity of the fruit extract (Table 3).

Table 1: Antibacterial activity of *immature palmyra palm fruits (Borassus Flabellifer Linn)* - Zone of inhibition in diameter (mm)

S. No.	Bacterial species	Control	50 µg	100 µg	150 µg	200 µg	250 µg	Chloramphenicol (30 µg)
Gram Positive								
1.	*Staphylococcus aureus*	-	2.0	5.0	10.5	16.5	18.0	23.0
2	*Bacillus subtilis*	-	4.0	10.0	14.5	25.0	27.0	26.0
3	*Staphylococcus epidermidis*	-	3.0	9.0	15.5	19.0	21.0	25.0
4	*Streptococcus pyogenes*	-	4.0	10.3	16.0	23.0	23.5	20.0
5	*Streptococcus pneumoniae*	-	1.5	4.0	10.0	15.0	20.0	20.0
Gram Negative								
6	*Escherichia coli*	-	2.25	7.2	15.0	20.5	21.5	21.0
7	*Klebsiella pneumoniae*	-	4.0	9.2	17.0	22.5	26.5	25.0
8	*Salmonella typhi*	-	1.0	10.	18.0	20.5	22.5	24.0
9	*Shigella dysenteriae*	-	3.0	9.2	15.0	22.5	26.0	25.0
10	*Pseudomonas aeruginosa*	-	2.0	6.0	14.0	19.0	20.5	20.0

Table 2: Antifungal activity of *immature palmyra palm fruits* extract against selected fungal species determined by disc diffusion assay

S. No.	Strains	Control	0.175 mg/disc	0.375 mg/disc	0.75 mg/disc	1.5 mg/disc	3 mg/disc	Amphotericin B
1	*Candida albicans*	-	9.2	11.6	12.5	22.0	24.0	25.0
2	*Saccharomyces cerevisiae*	-	-	7.0	10.5	14.0	15.0	18.0
3	*Aspergillus fumigatus*	-	12	14.5	12.5	19.0	21.5	23
4	*Aspergillus flavus*	-	9.0	12.0	14.5	17	20.0	22.5
5	*Aspergillus niger*	-	7.0	8.5	12.0	18.5	21.0	23.0
6	*Aspergillus ochraceus*		6.0	9.0	10.5	17.0	19.0	20.0
7	*Penicillium chrysogenum*	-	7.0	10.0	15.0	18.5	19.5	22.0
8	*Penicillium notatum*	-	10	13.0	16.0	20.0	23.5	24.0

Table 3: MICs and MBCs of *immature palmyra palm fruits* extract on Gram positive and Gram negative bacteria

Bacterial species	Minimum Inhibitory Concentration (MIC)		Minimum Bactericidal Concentration (MBC)	
	Fruit extract (mg/ml)	Chloramphenicol (µg/ml)	Fruit extract (mg/ml)	Chloramphenicol (µg/ml)
Gram positive				
Staphylococcus aureus	5	3	3	2
Bacillus subtilis	1	2	3	4
Staphylococcus epidermidis	3	1	4	4
Streptococcus pyogenes	4	2	2	3
Streptococcus pneumoniae	2	2	1	2
Gram negative				
Escherichia coli	3	2	4	8
Klebsiella pneumoniae	1	2	2	3
Salmonella typhi	4	2	8	4
Shigella dysenteriae	2	3	6	4
Pseudomonas aeruginosa	3	2	3	2

Table 4: Antifungal activity of *immature palmyra palm fruits* extract against fungal species determined by MIC and MFC.

Fungal species	MIC		MFC	
	Fruit extract (mg ml^{-1})	Amphotericin B (µg ml^{-1})	Fruit extract (mg ml^{-1})	Amphotericin B (µg ml^{-1})
Candida albicans	1.5	1.0	2	2
Saccharomyces cerevisiae	8	3	8	4
Aspergillus fumigatus	3.0	1.8	5	4
Aspergillus flavus	2.8	2	4	3
Aspergillus niger	4.5	2.5	5	5
Aspergillus ochraceus	5.0	2	3	2
Penicillium chrysogenum	2	3.5	3	2
Penicillium notatum	2.5	6	4	4

The fungal strains used in the present study to evaluate the antifungal activity of the immature palm fruits were selected on the basis of their clinical importance. Among the fungi, the lowest MIC values (1.5 mg/ml) were shown by *Candida albicans* and the highest MIC value (8mg/ml) was elicited by *Saccharomyces cerevisiae* (Table 4). It is pertinent to note that the *Candida* infections have been associated with the highest rates of morbidity as well as associated mortality of more than 38% [37,38].

In the traditional medicine different parts of the Palmyra palm trees are being used for their medicinal and beneficial properties. Jamkhande et al., (2016) [39] have reported the antibacterial, antifungal and antioxidant properties of Palmyra palm leaves. The different solvent extracts of palm fruit seed coat and roots have been reported for the antimicrobial properties [40, 41]. The aqueous extract of the palm fruit pulp was reported to exhibit significant antibacterial activity as evidenced from the MIC and MBC values against various clinically important bacteria [42]. Oboh et al., (2016) [43] have reported the antimicrobial activity of Palmyra palm syrup. Recently, Reshma et al., (2017) [44] have reported the presence of 2, 3, 4- tri hydroxy -5- methylacetophenone, nicotinamide and uracil for the first time from the Palmyra palm syrup.

Though, *In vitro* antimicrobial assays represent an alternative method, as they have been used successfully to identify promising treatment regimens for both bacterial and fungal infections [45-47], it has several limitations such as the efficacy of the simulated antimicrobial regimens were tested in an artificial medium that does not necessarily reflect *in vivo* conditions or accounts for the potential contribution of the host immune response. Likewise, the applicability of test variables selected for the testing antimicrobial agents to actual *in vivo* infection is not fully understood. Further, the concentrations of individual phytochemicals may vary in different plants which results in unique medicinal properties for a specific plant [42]. The mode of preparation of plant extract has also been linked to their antimicrobial properties [48]. Thus it is necessary to isolate the active ingredients present in the immature fruits and evaluate their antimicrobial properties by various methods to develop successful antibiotics. Further studies are in progress to isolate the active principles present in the immature palm fruits and evaluate the pharmacological and beneficial properties.

CONCLUSION

To the best of the author's knowledge, this study is the first scientific report on the antibacterial and antifungal efficacy of immature Palmyra palm fruits. The results of the present study provide a scientific basis that the fruit extract might open new promising opportunities for the development of more efficient, non-toxic and cost-effective natural antimicrobial agents for the control of various pathogenic microorganisms in the food, pharmaceutical industry and new clinically effective antimicrobial agents.

REFERENCES

1. Richard JF, Yitzhak T, Antibiotics and Bacterial Resistance in the 21st Century, Perspectives in Medicinal Chemistry, 6, 2014, 25–64.
2. Saswati S, Madhab KC, The multifaceted roles of antibiotics and antibiotic resistance in nature, Hans-Peter Grossart Front Microbiol., 4, 2013, 47.
3. Nicholas Israel NT, Emerging and Neglected Infectious Diseases: Insights, Advances, and Challenges, Biomed. Res. Int., 2017, 1-15.
4. Kalyani G, Sharma D, Yogesh V, Vishal S, Deshmukh, A review on drug designing, methods, its applications and prospects, International Journal of Pharmaceutical Research & Development., 5(05), 2013, 15 -30.
5. Bennett RN, Wallsgrove RM, Secondary metabolites in plant defence mechanisms, New Phytol., 127, 1994, 617-33.
6. Rao SR, Ravishankar GA, Plant cell cultures: chemical factories of secondary metabolites.,Biotechnol Adv., 20, 2002,101-53.
7. Ravishankar GA, Venkataraman LV, Food applications of plant cell cultures, Curr Sci, 57, 1990, 381-3.
8. Ravishankar GA, Rao SR, Biotechnological production of phytopharmaceuticals, J Biochem. Mol. Biol Biophys., 4, 2000, 73-102.
9. Mouhssen Lahlou, The Success of Natural Products in Drug Discovery, Pharmacology & Pharmacy., 4, 2013, 17-31.
10. Amenu D, Antimicrobial Activity of Medicinal Plant Extracts and Their Synergistic Effect on Some Selected Pathogens, American Journal of Ethnomedicine., 2014, 018-029.
11. Dias DA, Urban S, Roessner U, A historical overview of natural products in drug discovery, Metabolites, 16,2(2),2012,303-36.
12. Artnarong S, Masniyom P, Maneesri J, Isolation of yeast and acetic acid bacteria from palmyra palm fruit pulp Borassus flabellifer linn, Int Food Res J., 23, 2016, 1308-14.
13. Paschapur MS, Patil S, Patil SR, Kumar R, Patil MB, Evaluation of the analgesic and antipyretic activities of ethanolic extract of male flower inflorescences of Borassus flabellifer L, Arecaceae. Int J Pharm Pharm Sci., 2009a, 1, 98-106.
14. Paschapur MS, Patil MB, Kumar R, Patil SR, Evaluation of anti-inflammatory activity of ethanolic extract of Borassus flabellifer L. male flowers (inflorescences) in experimental animals, J. Med. Plants. Res., 2009b, 3: 49.
15. Shagufta N, Safia J, Saiqa I, Farkhanda M,Farah A, and Aamir A, Antibacterial activity of curcuma longa varieties against different strains of bacteria, Pak. J. Bot., 42(1), 2010, 455-462.
16. Mueller JH, Hinton J, A protein-free medium for primary isolation of the Gonococcus and Meningococcus, Proc. Soc. Exp. Biol. Med., 48,1941, 330–333.
17. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken HR, Manual of Clinical Microbiology, 6th Ed. ASM Press,Washington DC, 1995; 15-18.
18. McFarland J, Nephelometer: an instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines, J Am Med Assoc., 14, 1907,1176-8.
19. Suzita R, Son R, Khozirah S, Yaya R, Antibacterial Activity of Ethanolic Extract of Syzygium polyanthum L. (Salam) Leaves against Foodborne Pathogens and Application as Food Sanitizer, Biomed Res Int., 2017,1-13.
20. Apisariyakul A, Vanittanakom N, Buddhasukh D, Antifungal activity of turmeric oil extracted from Curcuma longa (Zingiberaceae). J Ethnopharmacol., 15,49(3),1995,163-9.
21. Wikler MA, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard. 8th edition. Document M07-A8, Pacific Beach Biosciences Inc., 2008.
22. Ody P, The Complete Medicinal Herbal. Dorling Kindersley Ltd., New York, USA, 1993,132-171.
23. Renuka K, Roshana Devi V, Subramanian S, Phytochemical screening and evaluation of in vitro antioxidant potential of immature palmyra palm (Borassus flabellifer linn.) Fruits, Int J Pharm Pharm Sci., 10(8),2018, 77-83.
24. Onoh RC, Umeora OUJ, Egwuatu VE, Ezeonu PO, Onoh TJP, Antibiotic sensitivity pattern of uropathogens from pregnant women with urinary tract infection in Abakaliki, Nigeria, Infect Drug Resist., 6,2013, 225–233.
25. Mounyr B, Moulay S, Saad Koraichi I, Methods for in vitro evaluating antimicrobial activity: A review, Journal of Pharmaceutical Analysis, 6, 2016, 71–79.
26. Brown DF, Kothari D, Comparison of antibiotic discs from different sources, J. Clin. Pathol., 28, 1975, 779–83.
27. Pongs O, Chloramphenicol, in: F.E. Hahn, (ed.) Antibiotics V, Springer Verlag, New York, 1979, 26-42.
28. Balbi HJ, Chloramphenicol: A review, Pediatr. Rev., 25,2004, 284-288.
29. Wimberly BT, The use of ribosomal crystal structures in antibiotic drug design, Curr Opin Investig Drugs., 10,2009, 750–765.
30. Polacek N, Gomez MJ, Ito K, Xiong I, Nakamura Y, Mankin A, The critical role of the universally conserved A2602 of 23S ribosomal RNA in the release of the nascent peptide during translation termination, Mol. Cell, 11, 2003,103-112.
31. Thompson J,Connor MO, Mills J, Dahlberg A, The protein synthesis inhibitors, oxazolidones and chloramphenicol,

cause extensive translational inaccuracy in vivo, J. Mol. Biol., 322,2002, 273-279.

32. Navarro JM, Flores P, Garrido C, Martinez V, Changes in the contents of antioxidant compounds in pepper fruits at ripening stages, as affected by salinity. Food Chem., 96,2006, 66-73.

33. Massacci ASM, Nabiev L, Pietrosanti SK, Nematov TN, Chernikova K,Thor, Leipner J, Response of the photosynthetic apparatus of cotton (Gossypium hirsutum) to the onset of drought stress under field conditions studied by gas-exchange analysis and chlorophyll fluorescence imaging. Plant Physiol Biochem., 46,2008, 189-95.

34. Seigler DS. Plant Secondary Metabolism. Boston MA: Chapman and Hall, Kluwer Academic Publishers, 1998,711.

35. Edreva AM, Velikova V, Tsonev T, Phenylamides in plants, Russ J Plant Physiol., 54,2000, 287-301.

36. Burt S, Essential oils: their antibacterial properties and potential applications in foods-a review, Int. J. Food Microbiol., 94,2004,223-253.

37. Edmond MB, Wallace SE, McClish DK, Pfaller MA, Jones RN, Wenzel RP, Nosocomialbloodstream infections in United States hospitals: a three-year analysis. Clin Infect Dis., 29(2), 1999, 239-44.

38. Wenzel RP, Nosocomial candidemia: risk factors and attributable mortality, Clin Infect Dis., 20(6), 1995, 531-4.

39. Jamkhande PG, Suryawanshi VA, Tukaram M, Kaylankar, Shailesh L, Patwekar, Biological activities of leaves of ethnomedicinal plant, Borassus flabellifer Linn. (Palmyra palm): An antibacterial, antifungal and antioxidant evaluation, Bulletin of Faculty of Pharmacy, 54, 2016, 59–66.

40. Govinda RD, Nagendra SY, Kaladhar DSVGK, Preliminary Studies on In Vitro Antimicrobial Activity and Phytochemical Analysis of Tender Seed Coat Aqueous Crude Extract of Borassus Flabellifer Linn, Asian Journal of Biochemical and Pharmaceutical Research Issue, 3 (1), 2011,517-523.

41. Chayanika S, Najam A, Shakil, Vidyanath Jha, Rajinder Kumar Gupta, Screening of Nutritional, Phytochemical, Antioxidant and Antibacterial activity of the roots of Borassus flabellifer (Asian Palmyra Palm), Journal of Pharmacognosy and Phytochemistry., 3(4),2014, 58-68.

42. Ayertey E, Tandoh MA, Mills-Robertson FC, Kumah DB, Larbie1 C, Aninkorah NP, Borassus aethiopum Fruit Pulp Extract has Antimicrobial Activity on elected Clinical Microbial Strains, Journal of Complementary and Alternative Medical Research 6(3), 2018, 1-8.

43. Oboh FO, Lorenta I, Monday I, Stephen E, Physico-chemical and Nutritional Characteristics, and Antimicrobial Activity of Oil Palm Syrup, Raffia Palm Syrup and Honey, IOSR Journal of Pharmacy and Biological Sciences., 11, (1),2016,73-78.

44. Reshmaa MV, Jubi Jacob, Syamnath VL, Habeeba VP, Dileep Kumar BS, Ravi S, Lankalapalli, First report on isolation of 2,3,4-trihydroxy-5-methylacetophenone frompalmyra palm (Borassus flabelliferLinn.) syrup, its antioxidant andantimicrobial properties, Food Chemistry., 228, 2017,491–496.

45. Bayston R, A model of catheter colonisation in vitro and its relationship to clinical catheter infections. J Infect., 9(3), 1984, 271-6.

46. Bayston R, Zdroyewski V, Barsham S, Use of an in vitro model for studying the eradication of catheter colonisation by Staphylococcus epidermidis. J Infect., 16(2), 1988, 141-6.

47. Guggenbichler JP, Berchtold D, Allerberger F, Bonatti H, Hager J, Pfaller W,Dierich MP, In vitro and in vivo effect of antibiotics on catheters colonized by staphylococci. Eur J Clin Microbiol Infect Dis., 11(5),1992, 408-15.

48. Nazif NM, Phytoconstituents of Zizyphusspina-christi L. fruits and their antimicrobial activity, Food Chem., 76, 2002, 77-81.

Source of Support: Nil, **Conflict of Interest:** None.