Original Article

In vitro ANTIMICROBIAL, ANTIPROLIFERATIVE ACTIVITY OF AQUEOUS EXTRACT FROM ENDOSPERM OF GERMINATED PALMYRA PALM SEED (*Borassus flabellifer* L)

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Abstract

In this study, aqueous extract of endosperm of *Borassus flabellifer* L. (Palmyra palm) seed was subjected for antimicrobial activity, antioxidant activity and antiproliferative activity. It was found to be more effective in suppressing UTI bacterial growth at the concentration of 25µl/well. Similar to how *Aspergillus niger* shown greatest zone of inhibition at concentration of 300 µl/ml, *Aspergillus brasiliensis* and *A. flavus* displayed highest zone of inhibition at 400 µl/ml. The extract was found to have good antiproliferative activity against Blood Cancer (MOLT – 4) cell lines.

Keywords: *Borassus flabellifer,* antimicrobial activity, antiproliferative activity**.**

1. Introduction

150 million / year are affected by Urinary tract infections (UTI)[1] where Recurrent UTI (rUTI) is common in most young females [2]. rUTIs are significantly increases healthcare costs [3] but it is not mostly life threatening. Uropathogenic *E. coli* (UPEC) are responsible for 70–90 % of UTIs, where is followed by *K. pneumoniae* [4] and other bacteria including *Enterococcus* sp[5]. Unnecessary and frequent usage of antibiotics lead to antibiotic resistance in most pathogenic microorganisms[6], thus treating these antibiotic resistant bacteria with a newer drug is highly essential and it made the researchers to search new drug from natural sources such as medicinal plants[7].

Since the dawn of the human period, plants have been used as an essential source of medicine. The many medical uses of plants and trees include both partial and complete use of the plant or plant. Neem, papaya and Calihariare are among a select group of plants known for their long life and great potential to prevent disease and improve people's quality of life[8]. There are 10,000 natural plant species in India and most of the plants are with medicinal value [9]. Still there are plants with potential drug source are reported every day, one such source is Palm tree. Although it is widely cultivated in many regions of India, Sri Lanka, Myanmar, and Bangladesh, the palmyra palm (*Borassus flabellifer* L.), a multifunctional tree of enormous value, is said to have originated in tropical Africa. One of the most generally cultivated genera in the Palmae family is Borassus, which is widely cultivated in tropical climates with both dry and rainy seasons[10]. As a natural sweetener, palm sap is particularly rich in minerals such as sodium, potassium, phosphorus, iron, zinc and copper as well as vitamins such as thiamin, riboflavin, pyridoxine, pantothenic acid and nicotinic acid, most of them are having antioxidant properties [11]. Palm kernel shell are being used for various applications [12]. In Asian several and West African countries, these pals are highly planted and used[13]. The current study was done to test aqueous extract from endosperm of germinated Palmyra palm seed (*Borassus flabellifer* L.) against UTI bacteria and also checked for other bioactivity studies too.

2. Materials and Method

2.1 Sample Collection

The germinated Indian palm seeds of Palmyra palm (*Borassus flabellifer*) were collected from our local area Nattathi, Tuticorin, Tamil Nadu, India. Inner embryo (endosperm) was taken out from the germinated palm seed and was cut into small pieces. These small pieces of palm seed endosperm were allowed to shade dry for more than one week. The dried endosperm samples were stored at room temperature in order to be used conveniently for this study.

2.2 Extraction

After the sample was well dried, the endosperm of palmyra palm seed were taken into a soil pot which was kept inside a small bowl and covered by a vessel containing water. This set up were heated gently for upto minimum 20 – 25 minutes. After heating process over, brown colour aqueous extract was collected into the inner small pot. Then the collected aqueous extract was further concentrated by heating them at 50 ^{0}C and then stored in a sterile container for further studies.

2.3 Collection of UTI pathogens

UTI pathogens *E. coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*- were collected from Port Trust Hospital, Tuticorin, Tamil Nadu, India. The samples were subjected for identifying their drug resistance pattern through disc diffusion method using commercial discs Streptomycin (10mcg/disc), chloramphenicol(10mcg/disc), Ampicillin(10mcg/disc), and Levofloxin(10mcg/disc). Each strain's multiple antibiotic resistance (MAR) index was determined using the formula [14]: a / b, where a is the total number of antibiotics tested and b is the number of antibiotics to which a specific isolate was resistant.

2.4 Antimicrobial activity

2.4.1 Antibacterial Activity

Antibacterial activity and Minimal inhibitory concentration were done by following the method of Samrot et al [15].

2.4.2 Antifungal Activity

By applying the disc diffusion method, *Aspergillus niger* and *Aspergillus brasiliensis* had the lowest antifungal activity and fungicidal concentration calculated by the method of Guttentag et al [16].

2.5 Antioxidant activity

Phosphomolybdenum assay was used to test the antioxidant activity of an aqueous extract from a palmyra palm seed that had germinated [17].

2.6 Antiproliferative activity

Cell lines for blood cancer (MOLT-4) were purchased from the National Centre for Cell Sciences (NCCS), Pune, India. The cell line was maintained in Dulbecco's Modified Eagle Media (DMEM), which was enhanced with 10% Fetal Bovine Serum (FBS). To keep away from bacterial contamination, the medium turned into supplemented with penicillin (100 U/ml) and streptomycin (100 µg/ml). The media containing the cell lines was kept at 37°C in a humid atmosphere with 5% CO2. Antiproliferation was determined following the method of Mosmann [18].

3. Results and discussion

3.1 Drug resistance pattern

Susceptibility of commercial antibiotics against UTI pathogens should in Table 1. All the three UTI isolated bacteria were highly resistant against selected commercial antibiotics like Streptomycin, Chloramphenicol, Ampicillin, Levofloxin. But Levofloxin was shown little susceptibility against *Klebsiella pneumonia*. In the current study, *Staphylococcus aureus* (0.75), *E. coli* (0.5), and *Klebsiella* (1.00) had increased MAR indices. The MAR index data showed that every strain of UTI tested had a very high MAR index, classifying the samples as high risk. Based on this alarming drug resistance, a new drug of less cost and less or no side effects are required to be identified and developed [3], herbal based drug might be a good solution for it[19].

Table 1. Determination of antibiotic susceptibility of commercial antibiotic against UTI pathogen

Organism name	**Streptomycin (10mcg/disc)**	**Chloramphenicol (10mcg/disc)**	**Ampicillin (10mcg/disc)**	**Levofloxin (10mcg/disc)**
E. coli	Resistance	Resistance	Resistance	Resistance
Klebsiella pneumonia	Resistance	Resistance	Resistance	Intermediate
Staphylococcus aureus	Resistance	Resistance	Resistance	Resistance

3.2 Antimicrobial activity

3.2.1 Antibacterial activity

Antibacterial activity of aqueous extract from Endosperm of Palmyra palm seed against UTI bacteria results revealed that aqueous extract was potentially effective in suppressing UTI bacteria growth with variable potency. The extract was the most effective in retarding bacterial growth of all tested pathogenic bacteria at the concentration of 10 mg. At the concentration of 25mg / well showed maximum inhibition zone of 11mm against *E. coli, Klebsiella pneumonia* respectively and 10mm against staphylococcus aureus. The Antibacterial activity of aqueous extract from Endosperm of Palmyra palm seed against UTI bacterial results were showed in the Table 2. MIC and MBC of the extract are tabulated in Table 3

Table 2. Antibacterial activity of aqueous extract against UTI pathogen

Organism name	Concentration of extract used (mg)			
	10mg	15mg	20mg	25mg
E. coli	9mm	9.5mm	10mm	11mm
K.pneumonia	9mm	9.5mm	10mm	11mm
S.aureus	8.5mm	9mm	9.5mm	10mm

Table 3 MIC and MBC value of aqueous extract

S. No	**UTI Bacteria**	**MIC (mg)**	**MBC (mg)**
1	*Escherichia coli*	50	60
2	*Staphylococcus aureus*	50	60
3	*Klebsiella pneumoniae*	50	60

3.2.2 Antifungal Activity

Antifungal activity of aqueous extract showed excellent fungal activities against isolated all fungal pathogens (Table 4). Highest zone of inhibition diameter of 16mm against *Aspergillus brasiliensis* at the concentration of 400µl/well. The minimum zone of inhibition was observed at concentration of 100µl/well in *Aspergillus flavus* and *Aspergillus brasiliensis*.

Table 4. Antifungal activity of aqueous extract

Organism name	Concentration of extracts			
	100µl	200µl	300µl	400µl
Aspergillus flavus	1mm	10mm	12mm	14mm
Aspergillus brasiliensis	10mm	12mm	14mm	16mm
Aspergillus niger	0mm	1mm	1mm	11mm

The MFC of *Aspergillus niger* was confirmed at the concentration of 300µl/ml. At the concentration of 400µl/ml showed MFC of both *Aspergillus flavus* and *Aspergillus brasiliensis.* The results were showed in the Table 5.

Table 5. Minimum fungal concentration of aqueous extract

Name of Fungi	**MFC CONCENTRATION**
Aspergillus niger	300µl/ml
Aspergillus flavus	400µl/ml
Aspergillus brasiliensis	400µl/ml

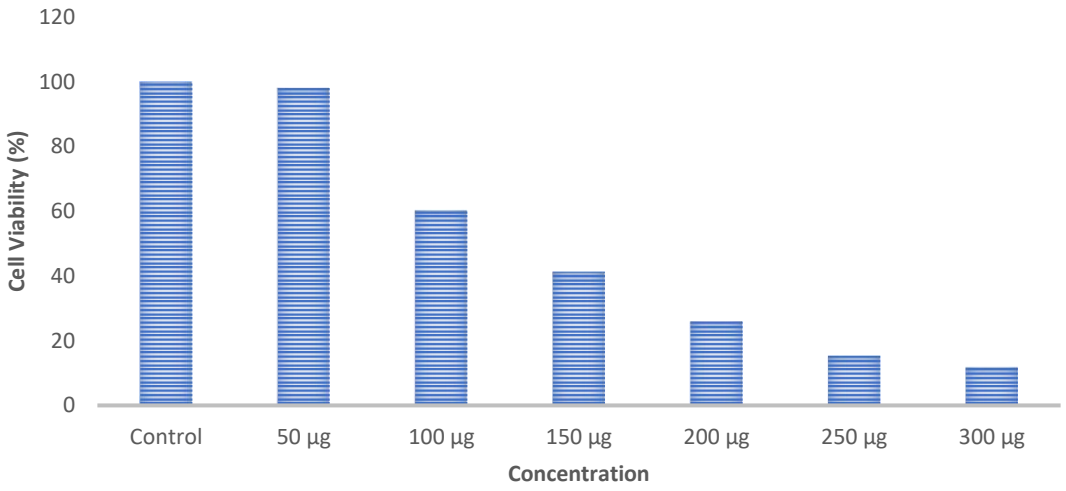
3.3 Antioxidant Activity

The extract was found to show antioxidant activity of 62.35 % at 300 µl concentration (result not shown here), which was determined by phosphomolybdenum assay. It is not a good activity when compared to the available commercial antioxidants. There are more reports on antioxidant activity exhibited by medicinal plants Various activity have been studied[20 - 29].

3.4 Antiproliferative activity

The cancer cell line studied in this experiment was shown to respond well to the anti-proliferative properties of the extract. (Fig 1). IC50 value was found to be around 150 µg/ml. AlQathama *et al* [30] reported that *Grewa tenax* to have inhibitory effects against A375 human melanoma cells. These characteristics support the use of medicinal in holistic medicine or as a new source of active chemicals.

Fig 1 Antiproliferative activity of aqueous extract



Conclusion

Plant-based alternatives to antibiotics are intriguing options for the prevention and long-term treatment of recurrent UTI. The water extract of germinated palmyra palm kernels is inexpensive, readily available, safe, and has few side effects. It also helps to reduce the risks associated with antibiotic resistance as well as the negative effects of antibiotics and other diseases. The aqueous extract from germinated palmyra palm seed's endosperm

had excellent antimicrobial and antiproliferative activities. However, the separation of natural bioactive substances with attractive biological and pharmacological effects from germinated palm kernel juice extract needs further investigation.

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