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ANTIBACTERIAL POTENTIAL OF ROOT AND BARK OF *COCOS NUCIFERA* LINN. AGAINST ISOLATED URINARY TRACT INFECTION CAUSING PATHOGENS

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ABSTRACT

The study was carried out to evaluate the antibacterial potential of *Cocos nucifera* Linn. root and bark against isolated UTI (urinary tract infection) causing pathogens. In the study, the following bacteria were used: *E. coli, P. aeruginosa, S. aureus and K. pneumonia*. Amikacin taken at 1mcg/disc was selected as the standard drug. Phytochemical tests were performed for the identification of various plant constituents. Other tests which were carried out were determination of ash values and antibiotic susceptibility tests. The study revealed that the root and bark of plant *Cocos nucifera* Linn. showed antibacterial activity against all the UTI isolates. The study concluded that the aqueous extract of root of *Cocos nucifera* Linn. was found to be more effective in inhibiting the growth of UTI pathogens than the ethanolic extract and decoction.

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KEY WORDS

Cocos nucifera Linn., antibacterial, amikacin, urinary tract infection.

INTRODUCTION

Human beings have to depend on nature since his existence for survival. Man using his knowledge has discovered many remedies for ailments from nature such as plants, mineral materials and animal products. The history of drug is intimately linked with plants from the earliest times and even today plant products have extensive use in ethno medicine, traditional systems of medicines as well as in the armamentarium of the modern physician.

The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide. It is recognized that in developing countries like India, plants are the main medicinal source to treat infectious diseases[1]. The World Health Organization has estimated that 80% of the earth and 6 million inhabitants rely only upon traditional medicines for their primary health care needs and major part of the therapy involves the use of plant extracts or their active principles. Scientists in many parts of the world have carried out extensive research and have proven to humanity, the effective use of herbal medicine[2].

Cocos nucifera Linn. (Family: Palmae) is commonly referred to as Coconut or Nariyel[3]. The coconut palm is a long lived plant that may live as long as 100 years. It has a single trunk which can grow up to 20-30 meters tall. Its bark is smooth and grey, marked by ringed scars left by fallen leaf bases. Unlike some other plants, the palm tree does not have tap root hairs but has fibrous root system. The plant is native to tropical eastern regions. Today it is grown both over the Asian continent (India, Ceylon, Indonesia) and in Central and South America (Mexico, Brazil). In Africa, the largest producing countries are Mozambique, Tanzania and Ghana[4].

The coconut palm has a multitude of uses, in number and importance probably not exceeded by any other palm. It yields timber; food; fermented and unfermented drink; alcohol; vinegar; thatching materials; splints; strips and fiber for making baskets, mats, rope, hats, brushes, brooms and other articles; fuel; caulking material; utensils for household use, such as cups, bowls, spoons and the like; oil for food, cooking, illumination, for making soap, substitutes for butter and lard, ointments and oil cake for feeding domestic animals and for fertilizers. The palm is very ornamental and is frequently planted for decorative effect. The fresh leaves are extensively used for temporary decorations and large numbers of prepared young leaves are used for religious purposes on Palm Sunday.The leaflets are used for wrapping a rice confection known as suman. The most important product of the coconut palm is coconut oil. The pressed cake is valuable as a food for stock or as a fertilizer. Its value is largely due to the fact that it contains about 20 percent of protein in addition to the oil, which is not extracted. The parts of the palm used in medicine are the roots, the bark, the “bloom” of the leaf, the cabbage, the flowers and the fruit (husk, shell, water, endosperm, oil)[5].

The activities of the root include:

- The decoction of root is astringent and is used as mouth wash and gargle.
- These are also roasted, grinded and used as dentifrice.
- The decoction of root promotes flow of urine and is used in the diseases of the uterus.
- It is given also in liver complaints, bronchitis and dysentery.

- The infusion of the young roots is used as gargle for sore throat.
- The root is also used as anthelmintic.
- The root is also used as anti bacterial agent, in treatment for urinary tract infections and also in some skin infection.

The activities of the bark and stem include:

- The ash of bark and stem is antiseptic.
- It is also used for scabies and toothache.
- The shell fiber of coconut is commonly used in southern India as chewing sticks as an effective as tooth brush for plaque control.
- The crude aqueous extract of it is also found to show antibacterial effect against all gram positive cocci[6].

This study was conducted to assess the antibacterial potential of the bark and root of *Cocos nucifera Linn.* against isolated urinary tract infection causing pathogens.

MATERIALS AND METHODS

Plant collection and authentication:

The root and bark of *Cocos nucifera* Linn. was collected from the Mambakkam region of Kanchipuram district, Tamil Nadu, India. The plant parts were identified and authenticated by Prof. P. Jayaraman, Ph.D., Director, Plant Anatomy Research Centre (PARC), Tambaram, Chennai – 45 and voucher specimens (PARC/2010/511 and PARC/2011/926) was deposited at the Pharmacognosy institute for further reference.

Extraction:

The types of solvent used for extraction were water (aqueous solvent) and ethanol (non-aqueous solvent). The coarsely powdered root and bark of *Cocos nucifera* Linn. was used for the extraction procedure for the preparation of extracts. To obtain the ethanolic extract, shade dried and coarsely powdered root and bark of *Cocos nucifera* Linn. was extracted with 99.9% ethanol by cold maceration in a narrow mouthed bottle for seven days. After completion of extraction, it was filtered and the solvent was removed by distillation under reduced pressure. The residue was then weighed and yield was recorded. Similarly, to obtain the aqueous extract, shade dried and coarsely powdered root and bark of the plant was extracted with water. To make a decoction, the shade dried root and bark of *Cocos nucifera* Linn. was treated with water and boiled for 10 minutes. After boiling, the liquid was cooled and filtered and the solvent was removed by distillation under reduced pressure.

Phytochemical tests were performed for the identification of plant constituents such as flavanoids, steroids, glycosides, carbohydrates, proteins, alkaloids, tannins, quinones, saponins and phenols. Other tests which were carried out were determination of total ash, acid insoluble ash and water insoluble ash.

Collection of urine sample:

A total of three urine samples from two female patients & one male patient were collected from Bose Clinical Laboratory, Madurai, India in separate sterile wide mouthed bottles. Before collecting the sample, the women were instructed to swab the vulva and men to retract the foreskin and cleanse the glans penis. Mid stream urine was then collected in sterile wide mouthed containers.

Isolation and identification of bacteria from urine sample:

For the isolation of UTI (urinary tract infection) bacterial strains, loop full of urine samples were streaked in to the nutrient agar, macon key agar, blood agar and chocolate agar plates & incubated at 37 ± 2 degree Celsius for 24 hours. Next day individual colonies were selected and identified on the basis of morphological characteristics, gram staining & biochemical characters.

Antibiotic susceptibility testing:

Anti biogram of the UTI isolates was ascertained on Muller Hinton agar using disc diffusion method and CLSI (Clinical and Laboratory Standards Institute) standards. Antibiotics most commonly used for the treatment of UTI were employed. i.e. Ofloxacin, Amikacin, Ampisulbactam, Moxifloxacin, Cephotaxime, Ciprofloxacin, Methicilin, Linezolid, Gentamycin, Cefuroxime, Norfloxacin, Ceftriaxone, Nalidixic acid and Cefepime. The diameter of the zone of inhibition produced by each antibiotic was measured and recorded and the isolates were classified as resistant, intermediate or sensitive based on the standard interpretation chart.

Antibacterial activity of plant extract:

The extract was concentrated under vacuum. Whatmann filter paper No.1 disc (6mm diameter) impregnated with crude extract (100mg dissolved in 1ml of DMSO (dimethyl sulfoxide) i.e. 1mcg/disc) was prepared. Preliminary disc diffusion assay method was performed to determine the antibacterial activity. Bacterial suspension was spread over the surface of Muller Hinton agar using sterile cotton swabs. Disc impregnated with the extracts were applied on the solid agar medium by pressing slightly & incubated at 37 degree Celsius ± 2 degree Celsius for 18 to 24 hours. After that the zone of inhibition was measured & expressed as mm in diameter.

In the present study the following isolated bacteria were used: *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Staphylococcus aureus*. The standard drug used was Amikacin 1mcg/disc.

RESULT

Aqueous and ethanolic extraction of the root of *Cocos nucifera* Linn. gave the yield values of 3.3% w/w and 4.1% w/w respectively whereas the decoction gave an yield value of 3.1% w/w (Table 1).

Table 1
Colour, consistency and percentage yield values of root of Cocos nucifera Linn.

Extract	Colour	Consistency	Yield (% w/w)
Aqueous	Brown	Crystal	3.3
Ethanolic	Brown	Crystal	4.1
Decoction	Dark brown	Crystal	3.1

Aqueous extraction of the bark of Cocos nucifera Linn. gave the yield value of 3% w/w whereas the decoction gave an yield value of 2.6% w/w (Table 2).

Table 2:
Colour, consistency and percentage yield values of bark of Cocos nucifera Linn.

Extract	Colour	Consistency	Yield (% w/w)
Aqueous	Brown	Powder	3.0
Decoction	Brown	Powder	2.6

Table 3 indicates the ash values of the root of *Cocos nucifera* Linn. Determination of total ash, acid insoluble ash and water insoluble ash revealed mean values of 0.07% w/w, 0.03% w/w and 0.19% w/w respectively.

Table 3
Ash values of the root of Cocos nucifera Linn.

Ash values	1 (%w/w)	2 (%w/w)	3 (%w/w)	4 (%w/w)	5 (%w/w)	Mean (%w/w)
Total ash	0.07	0.06	0.06	0.07	0.07	0.07
Acid insoluble ash	0.03	0.03	0.03	0.02	0.03	0.03
Water soluble ash	0.19	0.19	0.18	0.19	0.19	0.19

Table 4 indicates the ash values of the bark of *Cocos nucifera* Linn. Determination of total ash, acid insoluble ash and water insoluble ash revealed mean values of 0.07% w/w, 0.02% w/w and 0.17% w/w respectively.

Table 4
Ash values of the bark of Cocos nucifera Linn.

Ash values	1 (%w/w)	2 (%w/w)	3 (%w/w)	4 (%w/w)	5 (%w/w)	Mean (%w/w)
Total ash	0.08	0.06	0.07	0.08	0.08	0.07
Acid insoluble ash	0.03	0.02	0.02	0.03	0.02	0.02
Water soluble ash	0.16	0.18	0.18	0.18	0.16	0.17

Qualitative chemical analysis for the presence of phytoconstituents in different extracts of root of *Cocos nucifera* Linn. revealed that flavanoids, glycosides, carbohydrates, tannins and saponins were found to be present whereas

steroids, proteins, alkaloids, phenols and quinines were found to be absent (Table 5).

Table 5
Phytochemical screening of plant constituents of root of Cocos nucifera Linn.

Constituents	Aqueous extract	Ethanolic extract	Decoction
Flavanoids	+	+	+
Steroids	-	-	-
Glycosides	+	+	+
Carbohydrates	+	+	+
Proteins	-	-	-
Alkaloids	-	-	-
Phenols	-	-	-
Tannin	+	+	+
Saponin	+	+	+
Quinones	-	-	-

+ indicates presence, - indicates absence

Qualitative chemical analysis for the presence of phytoconstituents in different extracts of bark of *Cocos nucifera* Linn. revealed that steroids, glycosides, carbohydrates, phenols, tannins and saponins were found to be present whereas flavanoids, proteins, alkaloids and quinones were found to be absent (Table 6).

Table 6
Phytochemical screening of plant constituents of root of Cocos nucifera Linn.

Constituents	Aqueous extract	Decoction
Flavanoids	-	-
Steroids	+	+
Glycosides	+	+
Carbohydrates	+	+
Proteins	-	-
Alkaloids	-	-
Phenols	+	+
Tannin	+	+
Saponin	+	+
Quinones	-	-

+ indicates presence, - indicates absence

Table 7 reveals the biochemical characterization of isolated bacteria from patients suffering from urinary tract infection

Table 7
Biochemical characterization of isolated bacteria from UTI patients

Characteristics	*P. aeruginosa*	*E. coli*	*P. vulgaris*	*S. aureus*
Gram staining	-	-	+	+
Mannitol	Acid	Acid	-	-
Motility	Motile	Motile	Motile	Non-motile
Indole test	-	+	-	-
Methyl red test	+	+	-	-
Citrate test	-	-	-	-
Urease test	+	-	-	-
Oxidase test	+	-	-	-
Catalase test	+	-	-	+
H_2S test			-	

(+): positive, (-): negative

Table 8 indicates the susceptibility of UTI isolates to various antibiotics.

Table 8
Susceptibility of UTI isolates to various antibiotics

Micro organism	Antibacterial resistant profile														
	OF	A	AMP	ML	CE	CIP	ME	LI	GEN	CEF	CEFA	NOR	CEFR	NAL	CEFE
S.aureus	S	S	S	S	I	I	I	R	R	R	R				
	16	18	16	17	17	16	13								
E.coli	S	S	R		I	R			R	R	R	R	R	R	
	16	18			16										
P.aeruginosa	S	S	R		I	I			R	R	I	S	R	R	
	16	18			17	18					16	17			
P.vulgaris	I	S	S		I	R			R	S	I	I	R	R	R
	13	18	16		17					18	16	15			

S – Sensitive I – Intermediate R – Resistance

OF	-	Ofloxacin	A	-	Amikacin
AMP	-	Ampisulbactam	ML	-	Moxifloxacin
CE	-	Cephotaxime	CIP	-	Ciprofloxacin
ME	-	Methicilin	LI	-	Linezolid
GEN	-	Gentamycin	CEF	-	Cefuroxime
CEFA	-	Ceftazidime	NOR	-	Norfloxacin
CEFR	-	Ceftriaxone	NAL	-	Nalidixic acid
CEFE	-	Cefepime			

Tables 9 and 10 reveal the invitro screening for antibacterial activity of root and bark extracts of *Cocos nucifera* Linn. against UTI pathogens.

Table 9

Invitro screening for antibacterial activity of root extracts of C. nucifera Linn. against UTI pathogens

Organisms	Standard	R-alcohol	R-aqueous	R-decoction
E.coli	17.58 ± 1.021 ***	0	13.40 ± 0.8099 ***	13.17 ± 0.7607 ***
P.vulgaris	17.58 ± 1.021 ***	14.90 ± 0.8173 ***	13.18 ± 0.6940 ***	11.25 ± 0.8550 ***
P.aeruginosa	17.58 ± 1.021 ***	15.07 ± 0.7548 ***	15.20 ± 0.6841 ***	14.40 ± 0.8462 ***
S.aureus	17.58 ± 1.021 ***	13.47 ± 0.8847 ***	15.05 ± 0.7583 ***	13.95 ± 0.6442 ***

Figure 1
Bar graph showing comparison of antibacterial sensitivity of root of Cocos nucifera Linn. against UTI bacterial pathogens

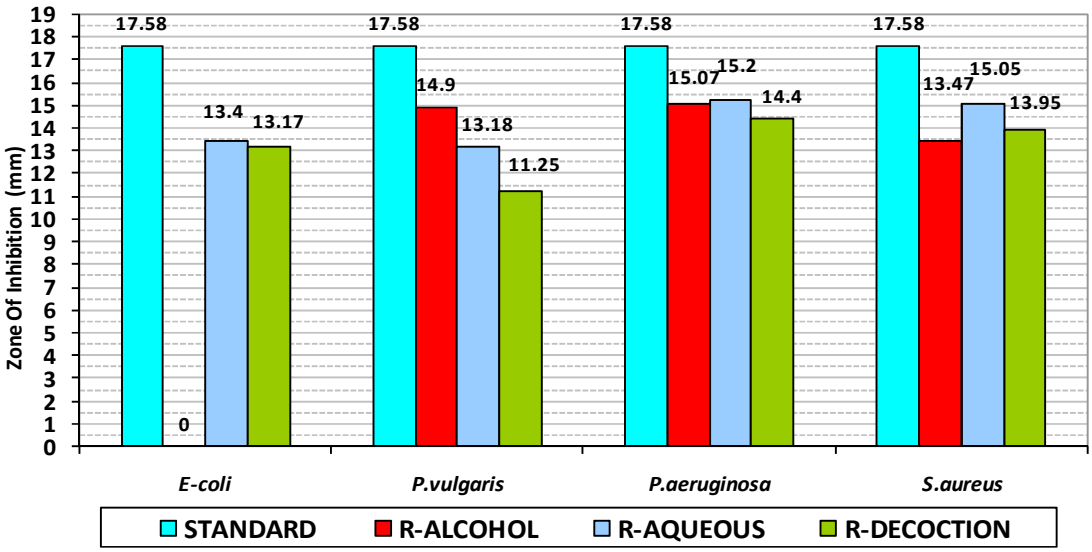


Table 10
Invitro screening for antibacterial activity of bark extracts of C. nucifera Linn. against UTI pathogens

Organisms	Standard	B-aqueous	B-decoction
E.coli	17.58 ± 1.021 ***	11.13 ± 0.8756 ***	12.10 ± 0.7239 ***
P.vulgaris	17.58 ± 1.021 ***	13.13 ± 0.8140 ***	12.25 ± 0.8939 ***
P.aeruginosa	17.58 ± 1.021 ***	13.37 ± 0.7118 ***	14.15 ± 0.9915 ***
S.aureus	17.58 ± 1.021 ***	12.13 ± 0.7474 ***	13.42 ± 0.8542 ***

Figure 2

Bar graph showing comparison of antibacterial sensitivity of bark of Cocos nucifera Linn. against UTI bacterial pathogens

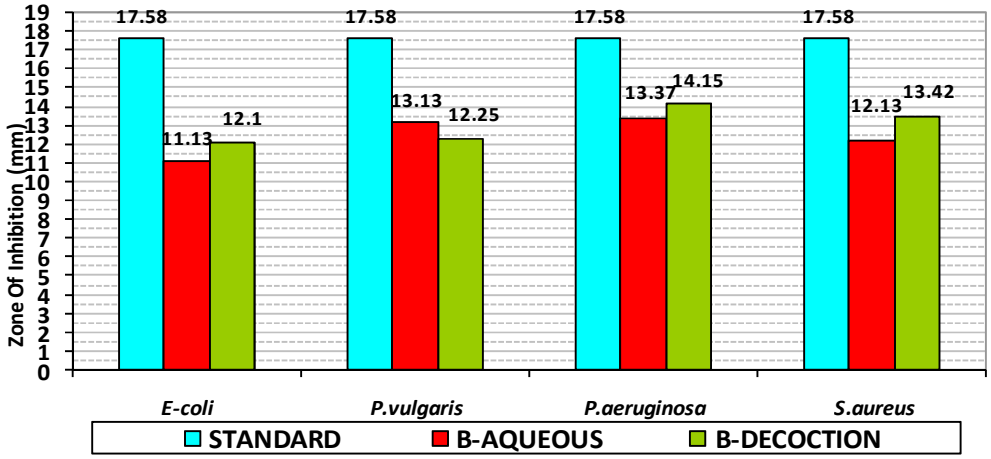
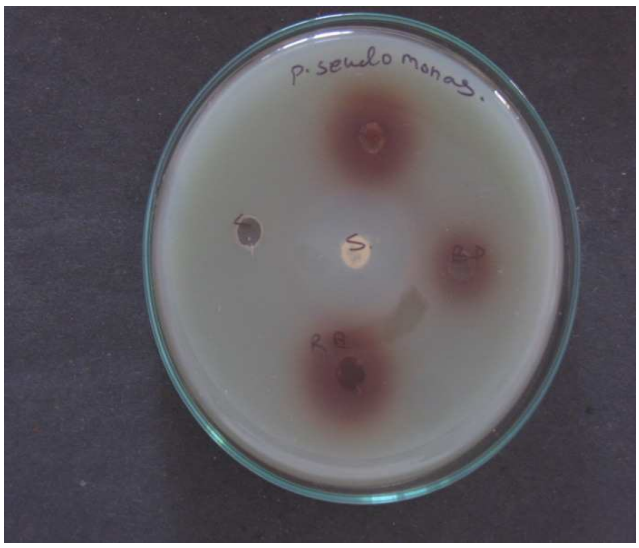


Figure 3 shows the invitro antibacterial activity of root and bark extracts of *C. nucifera* Linn. against UTI pathogens.

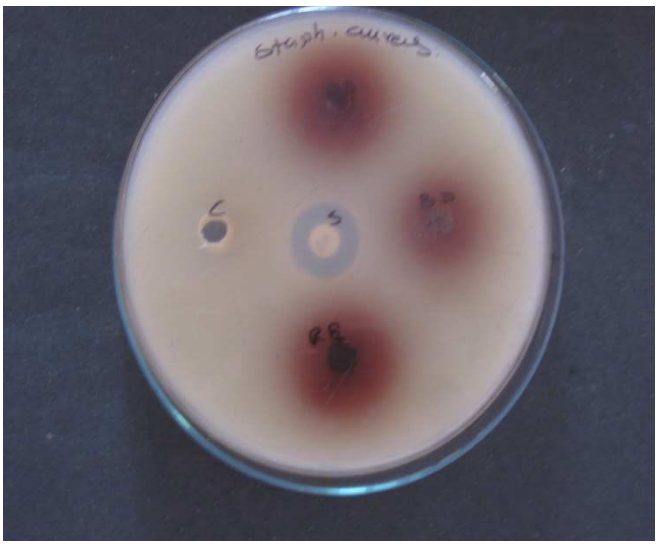
Figure 3

Invitro antibacterial activity of root and bark extracts of C. nucifera Linn. against UTI pathogens

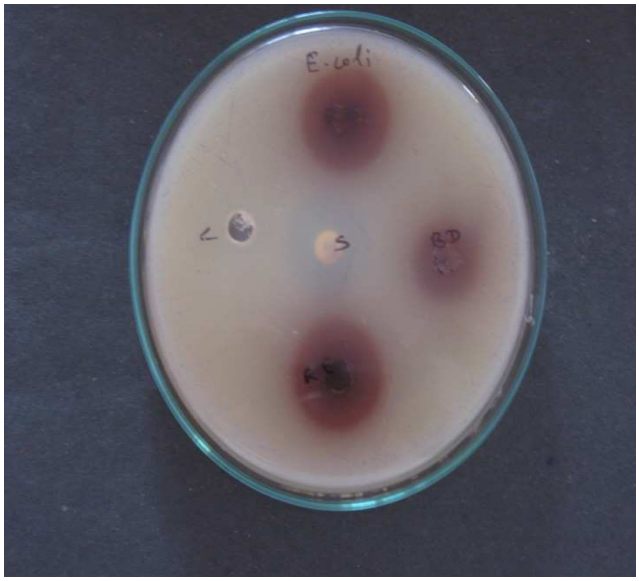
Control



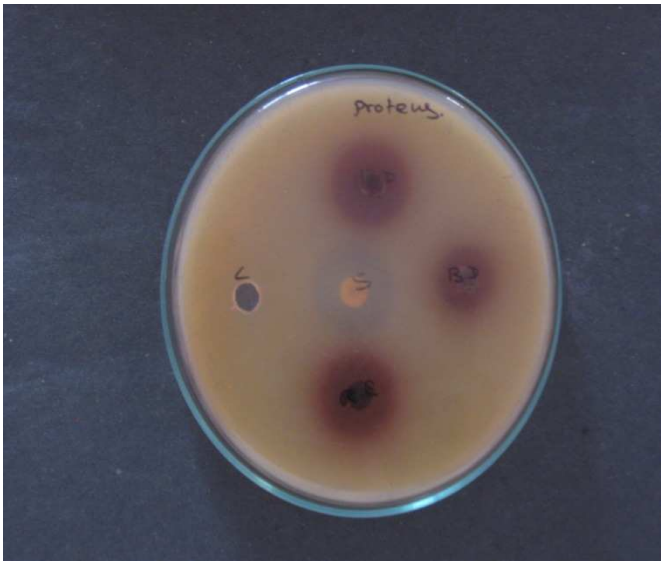
Pseudomonas aeruginosa



Staphylococcus aureus



Escherichia coli



Proteus vulgaris

DISCUSSION

The aqueous, ethanolic and decoction of *Cocos nucifera* Linn. root and bark was prepared and the yield in percentage was calculated. Qualitative chemical analysis for the presence of phytoconstituents revealed that proteins and phenols were present in all the chosen parts. Other phytoconstituents like sugar, alkaloids, triterpenoids, flavonoids and tannins were present in minute amounts.

Antimicrobial susceptibility tests revealed that bacterial isolates like *S.aureus* was susceptible to Ofloxacin, Amikacin, Ampisulbactam and Moxifloxacin, intermediate to Cephotaxime, Ciprofloxacin, Methicillin and resistant to Gentamycin, Linezolid, Ceftazidime and Cefuroxime.

E.coli was susceptible to Ofloxacin and Amikacin, intermediate to Cephotaxime and

resistant to Ampisulbactam, Ciprofloxacin, Gentamycin, Cefuroxime, Ceftazidime, Norfloxacin, Ceftriaxone and Nalidixic acid.

P.aeruginosa was susceptible to Ofloxacin, Amikacin and Norfloxacin, intermediate to Cephotaxime, Ciprofloxacin, Ceftazidime and resistant to Ampisulbactam, Gentamycin, Cefuroxime, Ceftriaxone and Nalidixic Acid.

P.vulgaris was susceptible to Amikacin, Ampisulbactam and Cefuroxime, intermediate to Ofloxacin, Cephotaxime, Ceftazidime, Norfloxacin and resistant to Ciprofloxacin, Gentamycin, Cefriaxone, Nalidixic acid and Cefepime.

Out of five extracts of different plant parts screened for antibacterial activity against major UTI pathogens, root and bark of *Cocos nucifera* Linn. showed inhibitory activity against all the isolated microorganisms. Aqueous and decoction of bark and root extracts showed consistent antimicrobial activity compared to alcoholic extract.

Alcoholic extract of *Cocos nucifera* Linn. root showed maximum zone of inhibition against *P.aeruginosa* and *P.vulgaris* (15mm) followed by *S.aureus* (13mm) and it was not found to be active against *E.coli.*

Aqueous extract of root of *Cocos nucifera* Linn. showed maximum zone of inhibition against *P.aeruginosa* and *S.aureus* (15mm) followed by *E.coli* and *P.vulgaris* (13mm).

Decoction of root of *Cocos nucifera* Linn. showed maximum zone of inhibition against *P.aeruginosa* and *S.aureus* (14mm) followed by *E.coli* and *P.vulgaris* (13mm & 11mm respectively).

Aqueous extract of bark of *Cocos nucifera* Linn. showed maximum zone of inhibition against *P.aeruginosa and P.vulgaris* (13mm) followed by *S.aureus* (12mm) and *E.coli* (11mm).

Decoction of bark of *Cocos nucifera* Linn. showed maximum zone of inhibition against *P.aeruginosa* (14mm) followed by *S.aureus* (13mm), *P.vulgaris* (12mm) and *E.coli* (12mm).

CONCLUSION

The study revealed that the root and bark of the plant *Cocos nucifera* Linn. showed antimicrobial activity against UTI isolates. Both the aqueous extract and decoction of *Cocos nucifera* Linn. were found to have almost the same efficacy. The study concluded that the aqueous extract of root of *Cocos nucifera* Linn. was found to be more effective in inhibiting the growth of UTI pathogens than the ethanolic extract and decoction

.REFERENCES

1. Amrita V, Sonal D and Shalini R, Antibacterial effect of herbs and spices extract on *Escherichia coli*. Electronic J Biology, 5(2): 40-44, (2009).
2. Guidelines on the conservation of medicinal plants. www.who.int/medicinedocs/en/d/Js7150e/. (1986).
3. Esquenazi D, Wigg M.D, Miranda M.M.F. et al., Antimicrobial and antiviral activities of polyphenolics from *Cocos nucifera* Linn. (Palmae) husk fiber extract. Res Microbiology, 153(10): 647-652, (2002).
4. The coconut palm. www.dipbot.unict.it/palms/descrol.html.
5. *Cocos nucifera Linn*. www.bpi.da.gov.ph/Publications/mp/pdf/n/niyog.pdf.
6. Saraswathy A, Sunilkumar K.N, Shakila R and Ariyanathan S, Pharmacognostic evaluation of roots of *Cocos nucifera* Linn. Phcog J, 2(12): 498-501, (2010).