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Preliminary Phytochemical Screening and Anti-Bacterial activity of *Cocos nucifera* Linn root

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ABSTRACT

The study was carried out to evaluate the antibacterial activity of *Cocos nucifera* Linn. root. In the study, the following bacteria were used: E. coli, P. aeruginosa, S. aureus and K. pneumonia. The antibacterial effect of ethanolic soluble extract of root of *Cocos nucifera* Linn. at different concentrations were studied by disc-diffusion method in the concentrations of 25µl/disc, 50µl/disc and 100µl/disc. Ciprofloxacin hydrochloride taken at 5µl/disc was selected as the standard drug. The minimum inhibitory concentration (MIC) values of the alcoholic extract against the micro organisms were determined by agar streak dilution method. Phytochemical tests were performed for the identification of various plant constituents. Other tests which were carried out were determination of ash values and fluorescence analysis. Ethanolic extract showed significant activity against all tested microorganisms with maximum inhibition (25mm) against S. aureus and minimum (15mm) against P.aeruginosa. Ethanolic extract showed marked activity against all microorganisms, equating to standard and even exhibited better zone of inhibition in comparison to standard. Cocos nucifera Linn. showed wider spectrum of activity especially against Staphylococcus aureus. Based on this research, it was concluded that broad spectrum antibacterial drugs can be isolated which may result in a great impact in this antibiotic era.

Key words: *Cocos nucifera* Linn. antibacterial, minimum inhibitory concentration, ciprofloxacin hydrochloride, disc diffusion method.

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INTRODUCTION

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 number of prepared young leaves is 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. [6]

This study was conducted to assess the preliminary phytochemical screening and antibacterial activity of *Cocos nucifera* Linn. root.

MATERIALS AND METHODS

Plant collection and authentication

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

Extraction

The type of solvent used for extraction was ethanol. Ethanol is a selective solvent, which dissolves alkaloids, volatile oils, glycosides, resins etc. The coarsely powdered root of Cocos nucifera Linn. was used for the extraction procedure for the preparation of extracts. The shade dried and coarsely powdered root 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.

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

In the present study, the following bacteria were used: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumonia.* The antibacterial effect of ethanolic soluble extract of root of *Cocos nucifera* Linn. at different concentrations was studied by disc-diffusion method in the concentrations of 25µl/disc, 50µl/disc and 100µl/disc. Ciprofloxacin hydrochloride taken at 5µl/disc was selected as the standard drug. It is a broad spectrum antibiotic. The media used is nutrient agar. Its constituents are given below (Table 1):

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Medium preparation and sterilization

The above ingredients were dissolved with aid of heat. The pH was adjusted at 8.0-8.4 with 5M sodium hydroxide and boiled for 10 minutes. It was filtered and sterilized by maintaining at 115^0C for 30 minutes in an autoclave and the pH was adjusted to plus 7.3 or minus 0.1.

Table 1. Nutrient agar medium

S.no	Ingredients	Quantity
1	Beef extract	10gms
2	Peptone	10gms
3	Sodium chloride	5gms
4	Agar	1-2%
5	Water	1000ml

Method

Disc diffusion method- A suspension of the organism was added to sterile nutrient agar medium at 45^0C. The mixture was transferred to sterile petri dishes and allowed to solidify. Sterile discs, 5mm in diameter (made from Whatmann filter paper sterilized in UV lamp) was dipped in solutions of different concentrations of test, standard and blank and were placed on the surface of agar plate. The plates were allowed to stand for 1 hour at room temperature as a period of pre-incubation diffusion to minimize the effect of variation in time between the applications of the different solutions. Then the plates were incubated for 24 hours at 37^0C ± 1^0C and observed for antibacterial activity. The diameter of zone of inhibition was observed.

Minimum inhibitory concentration (MIC)

The MIC values of the alcoholic extract against E. coli, P. aeruginosa, S. aureus, K. pneumonia were determined by agar streak dilution method.

Preparation of plates

10µg/ml stock solution of test compound was prepared using dimethylformamide (DMF) as the solvent. From this stock solution, required quantities of drug solutions were mixed with known quantities of molten sterile agar media aseptically to provide the following concentrations of 12,13,14,15,16,17,18,19,20,21,22,23,24µl/ml. About 20ml of the media containing the drug was dispensed into each sterile petri dish (diameter about 10cms). Then the media was allowed to solidify.

Procedure

Agar streak dilution method- Micro organisms were streaked one by one on the agar plates aseptically. After streaking, all the plates were incubated in the incubator, set at 37^0C for

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24 hours and then observed for growth of microorganisms. The lowest concentration of test compound showing no growth of the given bacteria has been reported as MIC of the test compound against the bacteria.

RESULTS

Extraction of the plant material Cocos nucifera roots using ethanol by cold maceration method gave a yield value of 4.1% (Table 2).

Table 2: Yield of *Cocos nucifera* roots after extraction by cold maceration method

Extract	Colour	Consistency	Yield (%w/w)
Ethanol	Brown	Crystal	4.1%

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

The fluorescence analysis of the alcoholic extract and drug powder is shown in Tables 4 and 5 respectively.

Table 4: Fluorescence analysis of alcoholic extract

Extract	Day light	UV light	
		Short 254 nm	Long 365 nm
Alcoholic extract	Dark brown	Dark green	Fluorescent green

Table 5: Fluorescence analysis of drug powder

S.No.	Drug powder	Day light	UV light	
			Short 254 nm	Long 365 nm
1	Drug powder as such	Dark brown	Dark brown	Light green
2	Powder + 1 N HCl	Dark brown	Brown	Light green
3	Powder + water	Brown	Brown	Green
4	Powder + 1 N HNO_3	Brown	Dark brown	Brown
5	Powder + 1 N H_2SO_4	Colorless	Light brown	Colorless
6	Powder + 1 N NaOH	Dark brown	Dark brown	Green
7	Powder + Alc. NaOH	Dark brown	Dark brown	Light green
8	Powder + 1 N KOH	Dark brown	Dark brown	Dark brown
9	Powder + Alc. KOH	Dark brown	Dark green	Fluorescent green
10	Powder + NH_3	Dark brown	Dark brown	Fluorescent green

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

Table 6: Phytochemical screening of plant constituents

Constituents	Aqueous extract
Flavonoids	+
Steroids	-
Glycosides	+
Carbohydrates	+
Proteins	-
Alkaloids	-
Phenols	-
Tannin	+
Saponins	+
Quinones	-

+ indicates presence, - indicates absence

Table 7 shows the in vitro antibacterial activity of ethanolic extract of root of Cocos nucifera Linn. (Figure 1).

Table 7: In-vitro antibacterial activity of ethanolic extract of root of *Cocos nucifera* Linn.

Organisms used	Zone of inhibition (mm)			
Gram negative organisms	**Standard**	**25µl**	**50µl**	**100µl**
E. coli	36	18	21	24
K.pneumoniae	37	16	20	22
P.aeruginosa	36	15	19	23
Gram positive organisms S.aureus	38	17	20	25

Table 8 demonstrates the minimum inhibitory concentration values of ethanolic extract of roots of Cocos nucifera Linn. (Figure 2).

Table 8: Minimum inhibitory concentration values of ethanolic extract of root of *Cocos nucifera*

Organisms	MIC values (µl)
E.coli	13
K.pneumoniae	16
P.aeruginosa	17
S.aureus	14

Figure 3 indicates the comparison of *Cocos nucifera* Linn. root extract with the standard drug most effective against micro organisms.

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Figure 1: Anti bacterial effect of root of *Cocos nucifera* Linn. against *E. coli, K. pneumoniae, P. aeruginosa* and *S. aureus*

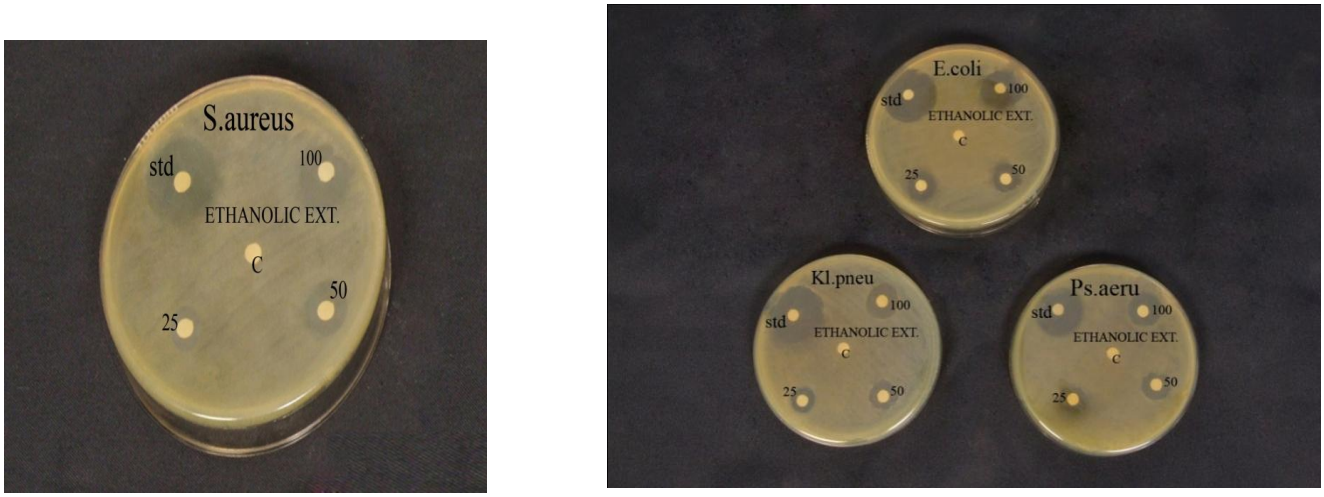

Figure 2: MIC of ethanolic extract of *Cocos nucifera* Linn. root against E. *coli, K. pneumoniae, P. aeruginosa* and *S. aureus*

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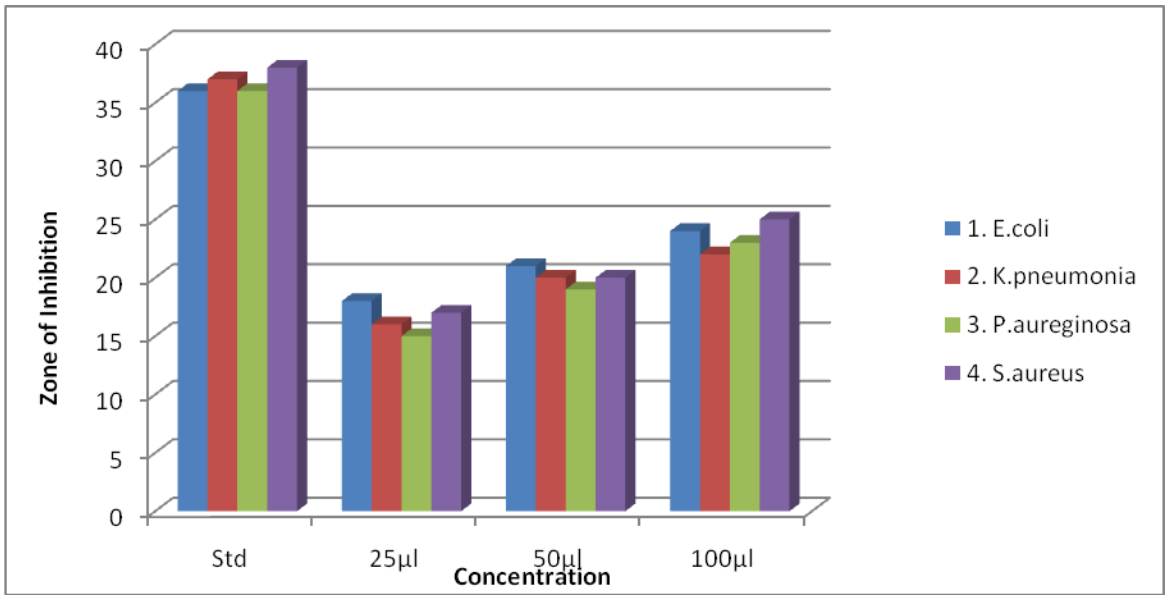
Figure 3: Comparison of *Cocos nucifera* Linn. root extract with the standard drug most effective against micro organisms



DISCUSSION

The yield of extract obtained after extraction of the plant material *Cocos nucifera* root using cold maceration method with ethanol was found to be 4.1%. The colour and consistency of the extract was found to be brown and crystalline in nature respectively.

Determination of ash values like total ash, acid insoluble ash and water insoluble ash gave mean values of 0.07% w/w, 0.03% w/w and 0.19% w/w respectively. The fluorescence analysis of the alcoholic extract revealed dark brown appearance in day light and dark green and fluorescent green colour in UV light of wavelengths 254 nm and 365 nm respectively.

Qualitative chemical analysis for the presence of phytoconstituents in aqueous extracts of root of *Cocos nucifera* Linn. revealed that flavonoids, glycosides, carbohydrates, tannins and saponins were found to be present whereas steroids, proteins, alkaloids, phenols and quinines were found to be absent.

Ethanolic extract showed significant activity against all tested microorganisms with maximum inhibition (25mm) against *S. aureus* and minimum (15mm) against *P.aeruginosa*. Ethanolic extract showed marked activity against all microorganisms, equating to standard and even exhibited better zone of inhibition in comparison to standard.

CONCLUSION

The preliminary research work was carried out to find the anti-bacterial activity of *Cocos nucifera* Linn. root. *Cocos nucifera* Linn. showed wider spectrum of activity especially against

Staphylococcus aureus. Based on this research, it was concluded that broad spectrum antibacterial drugs can be isolated which may result in a great impact in this antibiotic era.

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