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Formulation and Evaluation of Herbal Syrup containing *Cocos nucifera* Linn. Root

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ABSTRACT

The study was carried out to evaluate the antibacterial activity of herbal syrup formulation containing *Cocos nucifera* Linn. In the study, the following bacteria were used: *E. coli*, *P. aeruginosa*, *S. aureus* and *K. pneumonia*. The antibacterial effect of aqueous 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. 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. Increased antibacterial activity was observed with increase in concentration. Maximum activity was observed for *S. aureus* as a test microorganism. Hence *S. aureus* alone was chosen for the formulation. It was observed that the anti bacterial activity of formulated herbal syrup against *S. aureus* was found to be similar to that of 100µl /disc concentration of water soluble extract. The study concluded that the additives present in the formulation were not affecting the antibacterial activity.

Key words: *Cocos nucifera* Linn, antibacterial, *S. aureus*, 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 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. [6]

This study was conducted to evaluate antibacterial effect of herbal syrup containing *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 water. 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 water 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 pneumoniae*. The antibacterial effect of water 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. It is a broad spectrum antibiotic. The media used is nutrient agar. Its constituents are given below (Table 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

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^oC for 30 minutes in an autoclave and the pH was adjusted to plus 7.3 or minus 0.1.

Method: Disc diffusion method- A suspension of the organism was added to sterile nutrient agar medium at 45^oC. 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^oC ± 1^oC and observed for antibacterial activity. The diameter of zone of inhibition was observed.

Preparation of simple syrup (USP): 66.7gm of sucrose was weighed and added to purified water and heated until it dissolved with occasional stirring. Sufficient boiling water was added to produce 100ml.

Preparation of herbal syrup: Required quantity of the drug extract was weighed and added to the prepared simple syrup. Following that, the required quantity of methyl paraben and peppermint oil was added to the above mixture and the final volume was adjusted with simple syrup.

The herbal syrup was evaluated for various parameters such as physical appearance (colour, odour, taste), pH, weight/ml, and viscosity. Phytochemical constituent screening and invitro antibacterial activity was also carried out by disc diffusion method using ciprofloxacin hydrochloride as standard.

RESULTS

Extraction of the plant material *Cocos nucifera* roots using water by cold maceration method gave a yield value of 3.3% (Table 2).

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

Extract	Colour	Consistency	Yield (%w/w)
Aqueous	Brown	Crystal	3.3%

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 aqueous extract and drug powder is shown in Tables 4 and 5 respectively.

Table 4: Fluorescence analysis of aqueous extract

Extract	Day light	UV light	
		Short 254 nm	Long 365 nm
Aqueous extract	Dark brown	Dark green	Light 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 + 1N HCl	Dark brown	Brown	Light green
3	Powder + water	Brown	Brown	Green
4	Powder + 1N HNO ₃	Brown	Dark brown	Brown
5	Powder + 1N H ₂ SO ₄	Colorless	Light brown	Colorless
6	Powder + 1N NaOH	Dark brown	Dark brown	Green
7	Powder + Alc. NaOH	Dark brown	Dark brown	Light green
8	Powder + 1N 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

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	-

Evaluation of the organoleptic parameters of the herbal syrup revealed that the syrup was dark brown in colour and had a pleasant odour and sweet taste (Table 7).

Table 7: Organoleptic parameters of the herbal syrup

Parameter	Result
Colour	Dark brown
Odour	Pleasant
Taste	Sweet

Evaluation of the physicochemical parameters of the herbal syrup of *Cocos nucifera* Linn. root revealed that the syrup had a weight/ml of 2.17 g/ml and viscosity and pH of 1.9034 poise and 8.3 respectively (Table 8).

Table 8: Physicochemical parameter of the herbal syrup

Parameter	Result
Weight/ml	2.17g/ml
Viscosity	1.9034 poise
pH	8.3

Table 9 and 10 show the in vitro antibacterial activity of aqueous extract of root of *Cocos nucifera* Linn. and the antibacterial effect of the herbal syrup formulation containing *Cocos nucifera* Linn. (Figure 1 and 2).

Table 9: In-vitro antibacterial activity of aqueous extract of root of *Cocos nucifera* Linn.

Organisms used	Zone of inhibition (mm)			
	Standard	25µl	50µl	100µl
Gram negative organisms				
<i>E. coli</i>	39	14	17	21
<i>K.pneumoniae</i>	38	13	18	20
<i>P.aeruginosa</i>	38	12	16	21
Gram positive organisms				
<i>S.aureus</i>	39	15	19	23

Table 10: Antibacterial effect of herbal syrup formulation containing *Cocos nucifera* Linn.

Organism	Zone of inhibition (mm)			
	Standard	25µl	50µl	100µl
<i>S. aureus</i>	39	17	19	23

Figure 3 indicates the comparison of aqueous extract and herbal syrup formulation of *Cocos nucifera* Linn. with the standard drug most effective against micro organisms.

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



Figure 2: In vitro antibacterial effect of herbal syrup formulation containing *Cocos nucifera*

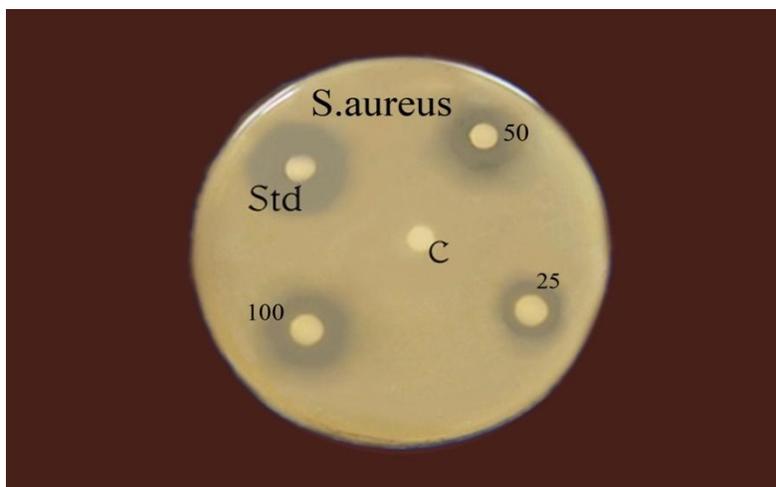
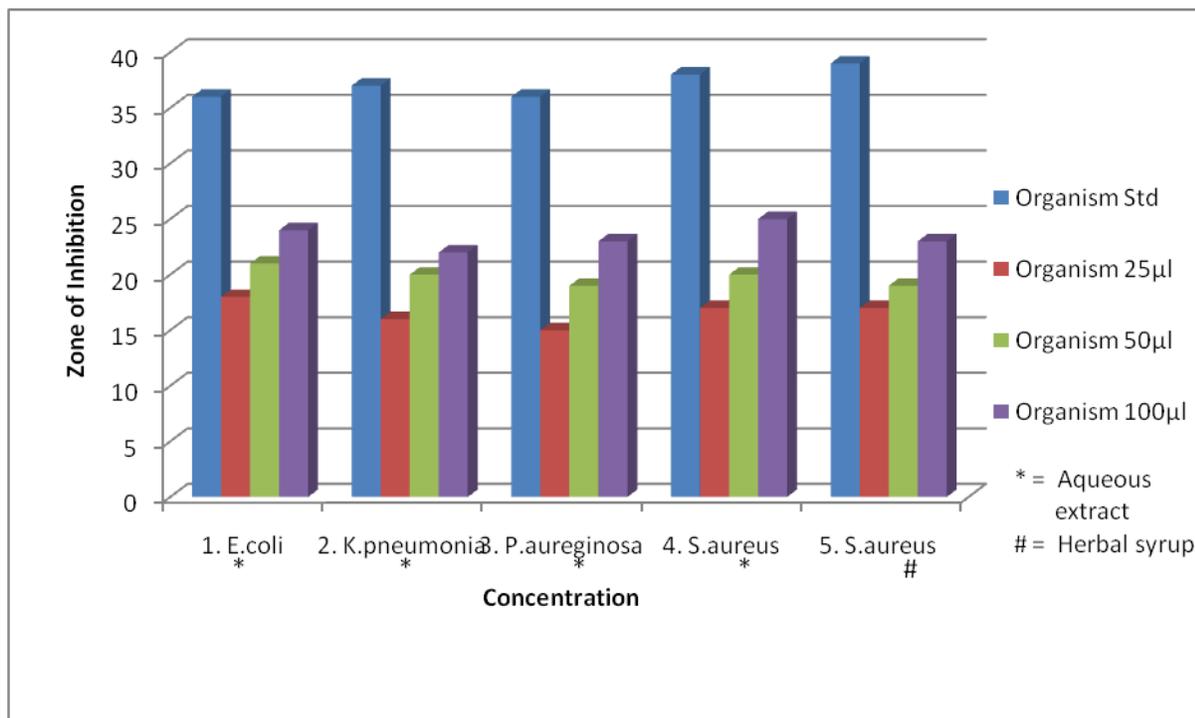


Figure 3: Comparison of aqueous extract and herbal syrup formulation of *Cocos nucifera* Linn. with the standard drug most effective against micro organisms



DISCUSSION

The yield of extract obtained after extraction of the plant material *Cocos nucifera* root using cold maceration method with water was found to be 3.3%. 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 aqueous extract revealed dark brown appearance in day light and dark green and light 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.

The water soluble extract of *Cocos nucifera* Linn. root had been used for this study and investigated in a systemic way for antibacterial studies in an attempt to rationalize their use as a drug of therapeutic importance. In order to prove the claim that water soluble extract of root of *Cocos nucifera* Linn root has anti bacterial property, microbiological studies were performed and the water soluble extract was formulated as herbal syrup. No work had been previously



done in connection with this, hence a formulation was developed and the antimicrobial study was carried out.

Screening of anti-bacterial activity was carried out by disc diffusion method. Increased antibacterial activity was observed with increase in concentration. Maximum activity was observed for *S.aureus* as a test microorganism. Hence *S.aureus* alone was chosen for formulation. It was observed that the anti bacterial activity of formulated herbal syrup against *S. aureus* was found to be similar to that of 100µl /disc concentration of water soluble extract.

CONCLUSION

The report of in-vitro antibacterial activity revealed that there was no significant difference in activity between the water soluble extract and the formulation containing water soluble drug. The study concluded that the additives present in the formulation were not affecting the antibacterial activity. A further complete Pharmacognistical and pharmacological screening is yet to be done.

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