



## Antioxidant & Antimicrobial Activities of *Cocos Nucifera* Linn. (Areaceae) Endocarp Extracts

Rajeev K Singla<sup>\*1</sup>, Nitesh Jaiswal<sup>2</sup>, Varadaraj Bhat G<sup>1</sup>, Hitesh Jagani<sup>3</sup>

<sup>1</sup> Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal-576104, Karnataka, India

<sup>2</sup> Department of Medical Microbiology, Kasturba Medical College, Manipal University, Manipal-576104, Karnataka, India

<sup>3</sup> Department of Pharmaceutical Biotechnology, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal-576104, Karnataka, India

Address for Correspondance: [rajeevsingla26@gmail.com](mailto:rajeevsingla26@gmail.com) ; [rajeev.kumar7@learner.manipal.edu](mailto:rajeev.kumar7@learner.manipal.edu)

**ABSTRACT:** *Cocos nucifera* (CN), commonly known as Coconut tree, is used for its several beneficial health effects as antitumor, anthelmintic, antidotal, antiseptic, aperients, aphrodisiac, astringent, bactericidal, depurative, diuretic, hemostat, pediculicide, refrigerant, stomachic, styptic, suppurative and vermifuge. Our objective was to determine the antioxidant activities, DPPH radical scavenging activities and antimicrobial activities of the ethanolic (cold percolation: RNM-01 & hot percolation: RNM-02) extracts, dry distilled extract (RNDS) and aqueous extract (hot percolation: RNA) of endocarp of *cocos nucifera* from Karnataka region in India. Agar disc diffusion method was used for *in vitro* antibacterial and antifungal screening. The overall results provide promising baseline information for the potential use of the crude anti-oxidant extracts from CN in the treatment of bacterial and fungal infection as well as for the reduction of other human diseases. As it has been already reported that there is an inverse relationship between dietary-intake of antioxidant-rich foods and the incidence of a number of human diseases, therefore these results are interesting. © 2011 IGJPS. All rights reserved.

**KEYWORDS:** *Cocos nucifera*; Endocarp; Antioxidant; Antibacterial; Antifungal.

### INTRODUCTION

*Cocos nucifera* L.(Family Areaceae) commonly known as coconut, is considered as an important fruit crop in the tropical countries. The use of natural products with therapeutic properties is as ancient as human civilization. Even though pharmaceutical industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased.

Heating the coconut shells gives an oil that is used against ringworm infections in the popular medicine of India. The alcoholic extract of ripe dried coconut shell has antifungal activity against *Microsporum canis*, *M. gypseum*, *M. audouinii*, *Trichophyton mentagrophytes*, *T. rubrum*, *T. tonsurans* and *T. violaceum*. The activity was mainly attributed to the high content of phenolic compounds [1].

Plant phenols are of interest because they are an important group of natural antioxidants and some of them are potent antimicrobial compounds. Plants can produce antimicrobial compounds to protect themselves from biotic attack that could be essential for microbial infection resistance. [2]

Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils, as well as in tannin [3-5]. By chromatographic methods coupled to mass spectrometry techniques, it has been demonstrated [2] that aqueous *Cocos nucifera* extracts are mainly composed of catechin and epicatechin together with condensed tannins (B-type procyanidins). These classes of molecules have been associated with analgesic and antioxidant activities in several experimental models [6-9]. According to our previous reports, *Cocos nucifera* endocarp found to have significant vasorelaxant and antihypertensive effect when evaluated using isolated rat thoracic aorta and DOCA salt induced hypertensive rats [10]. In continuation to our studies, we are now reporting antimicrobial and antioxidant effect of *cocos nucifera* endocarp extracts.

## **MATERIALS & METHODS**

### **PLANT MATERIALS**

Plant Material (*Cocos nucifera* L. Arecaceae) endocarp was collected from Kota village in Udupi district in the south west part of Karnataka (India). *Cocos nucifera* L. (Arecaceae) was authenticated by Dr. Gopalkrishna Bhat, Emeritus professor, Department of Botany, Poorna Pragna College, Udupi, Karnataka, India. One set of plant material voucher specimen was deposited at Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Karnataka, India and one set was preserved in our laboratory for future reference.

### **CHEMICALS & REAGENTS**

Folin-Ciocalteu's reagent, Sodium Carbonate, Gallic acid, Hide powder, Mueller Hinton agar medium, Mueller Hinton Broth, Sabourauds dextrose agar medium, DPPH, Sodium hydroxide, DMSO, Sodium nitroprusside, ethanol were procured from the standard companies.

### **PREPARATION OF SAMPLES**

#### **Preparation of RNM-01**

The endocarp of *cocos nucifera* fruit were air dried, pulverized to a coarse powder in a mechanical grinder, passed through a 40-mesh sieve, and extracted using cold maceration method with ethanol for 144 hrs. The extract was decanted, press the marc, mixed both menstruum and filtered with Whatman No. 1 filter paper. Concentrate it at reduced pressure below 40°C through rota vapor to obtain dry **RNM-01** (% w/w). This RNM-01 was taken up for biological screening.

#### **Preparation of RNM-02**

The endocarp of *cocos nucifera* fruit were air dried, pulverized to a coarse powder in a mechanical grinder, passed through a 40-mesh sieve, and extracted in a Soxhlet extractor with ethanol for 72 hrs. The extract was decanted, filtered with Whatman No. 1. Filter paper and concentrated at reduced pressure below 40°C through rota vapor to obtain dry RNM-02 (2.164% w/w). This **RNM-02** was taken up for biological screening.

### **Preparation of RNA**

The residue left after extraction of RNM-02 was successively extracted with distilled water for 72 hrs. The extract was decanted, filtered with Whatman no. 1 filter paper and concentrated at reduced pressure below 70°C through rota vapor to obtain dry RNA(%w/w). This **RNA** was taken up for biological screening.

### **Preparation of RNDS**

The pulverized coarse powder of *cocos nucifera* fruit's endocarp was subjected to dry distillation. Dry distillation is the process of heating the solid materials to produce gaseous products (which may condense into liquids or solids). The condensed product was extracted with dry ether (4×50ml), followed by passed through anhydrous sodium sulfate. The excess of the ether was removed by using rota vapor. The oily liquid (RNDS) collected in the amber colored bottle and kept at cool place. Density: 1.079g/ml. This **RNDS** was taken up for biological screening.

### **DETERMINATION OF TOTAL PHENOLIC CONTENT**

Total phenolic content of the all extracts were determined using Folin-Ciocalteu reagent and gallic acid as standard [1]. 3.33 milligrams of the extracts were weighed into 5ml test tube, dissolved in 2ml of DMSO resulting in 1.665 mg/ml. Two hundred microliters (three replicates) were introduced into screw cap test tubes; 1.0 ml of Folin-Ciocalteu's reagent and 0.8 ml of sodium carbonate (20.25%) were added. The tubes were vortexed and allowed to stand for 2 h. The absorption at 750 nm was measured (Shimadzu UV Visible spectrophotometer) and the total phenolic content was expressed as milligram of gallic acid equivalents per gram dry material.

### **DETERMINATION OF TOTAL FLAVONOID CONTENT**

Total flavonoids were estimated in the plant extracts using a colorimetric method based on the formation of a complex flavonoid-aluminum, having the absorptivity maximum at 430 nm. All determinations were made in triplicate and values were calculated from a calibration curve obtained with rutin. Final results were expressed as milligram of rutin equivalent per gram of dried weight.

### **DETERMINATION OF TOTAL TANNIN CONTENT (TTC)**

About 2 g. of the powdered drug was added to 150 ml water and boiled for 30 min. The solution was filtered, cooled and diluted to 250 ml with water. The total amount of material extractable into water was determined by evaporating 50 ml of the plant material extract and the residue was dried at 105° C for 4 hrs and weighed (T1). The amount of plant material not bound to the hide powder extractable into water was determined by adding 6.0g of hide powder to 80 ml of plant material extract. The mixture was shaken for 60 min and was filtered. 50 ml of the filtrate was evaporated and the residue was dried at 105° C and weighed (T2). The solubility of hide powder was determined by taking 6.0 gm of hide powder. About 80 ml of water was added and the mixture was shaken for 60 min and filtered. 50 ml of clear filtrate was evaporated and the residue was dried at 105° C and weighed (To).

Quantity of tannins (%) =  $[T1 - (T2 + T0)] \times 500 / w$

Where w, is the weight of powder in grams.

## MICROORGANISMS USED

Methicillin resistant *Staphylococcus aureus*(Clinical isolate), Methicillin sensitive *Staphylococcus aureus*(Clinical isolate), *Pseudomonas aeruginosa*(Clinical isolate), *E. coli* (Clinical isolate), *Klebsiella pneumonia*(Clinical isolate), *Acinetobacter baumannii* (Clinical isolate), *Citrobacter Freundii* (Clinical isolate), *Enterococcus* (Clinical isolate), *Streptococcus pyogenes*(Clinical isolate), *Candida albicans*(Clinical isolate), *Bacillus subtilis*, *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus*, *Micrococcus luteus*.

## ANTIMICROBIAL TESTING

### Kirby-bauer diffusion method:

Antibacterial Activity: The target extracts were tested for the antibacterial activity against variety of the test organism(Mentioned in section 2.7) by the punch well and disc diffusion methods on Mueller hinton agar medium using gentamycin and ciprofloxacin as reference drugs. The antibacterial screening was carried out with four different concentrations using DMSO as solvent. Positive and negative control were also taken so as to nullify any possible errors.

Antifungal Activity: The target extracts were tested for the antifungal activity against clinical strain of *Candida albicans* by the Kirby-bauer disc diffusion method on Sabourads Dextrose Agar Medium using fluconazole as a reference drug. The antifungal screening was carried out with four different concentrations (10, 50, 100, 300 mcg/disc) using DMSO as solvent. Positive and negative control were taken so as to nullify any possible errors.

### Tube dilution assay:

For determining antibacterial activity of RNM-01 and the control drug were dissolved in absolute dimethylsulfoxide (DMSO). Further dilutions were prepared at the required quantities of 1024, 512, 256, 128, 64, 32, 16, 8, 4, 2, and 1  $\mu\text{g mL}^{-1}$  on the *Bacillus subtilis* at the concentrations studied. The stock solutions were prepared in DMSO and DMSO had no effect on the microorganisms in the concentrations studied. Antimicrobial activity of extract was determined using the broth dilution method proposed by the National Committee for Clinical Laboratory Standards (CLSI). MIC, which is the lowest concentration of a compound that completely inhibits microbial growth, was determined by a standard broth dilution technique adapted from the CLSI.

## ANTIOXIDANT TESTING

**Diphenylpicrylhydrazyl radical scavenging method:** Hwang et al, 2001 method was followed for the current work. 10  $\mu\text{l}$  of the drug solution was taken in the 96 well plate. Add 200  $\mu\text{l}$  of the DPPH. Kept it in incubator for 20 min at 37 °C with aluminium foil covered. Sample absorbance was measured at 540 nm using ELISA reader.

**Nitric Oxide radical scavenging method:** Garrat, 1964 and Marcocci et al, 1994 method were followed for the current work. Took 400  $\mu\text{l}$  Sodium Nitroprusside, 100  $\mu\text{l}$  Phosphate buffer, 100  $\mu\text{l}$  of the extract solution in a dry test tube of volume 5 ml. Incubate this reaction mixture at 25 °C for 2.5 hrs in a dark chamber. Then 50  $\mu\text{l}$  of this reaction mixture and 100  $\mu\text{l}$  of the sulfanilic acid were taken in the 96 micro well plate. After five minutes, add 100  $\mu\text{l}$  Naphthyl ethylene diamine dihydrochloride(NEDD). Stand it for 30 min. Sample absorbance was measured at 540 nm using ELISA reader.

**Alkaline DMSO method/ Nitroblue tetrazolium Assay:** Elizabeth K and Rao MNA, 1990 method was followed for the current study. Took 1ml of alkaline DMSO , 0.1 ml of Nitroblue tetrazolium(NBT) and 0.3 ml of drug solution in 3 ml test tube. Sample absorbance was measured at 540 nm using double beam UV spectrophotometer.

$$\% \text{ Scavenging} = \frac{(\text{Test-blank}) - (\text{Control-blank})}{(\text{Test-blank})} \times 100$$

**STATISTICAL ANALYSIS:** All the experiments were done in triplicate. The triplicate data were subjected to an analysis of variance for a complete random design using commercially available software (Prism version 4.0; Graph Pad Software, San Diego, CA, USA).

## RESULTS & DISCUSSION

RNM-01, RNM-02, RNDS and RNA-01 were successfully extracted from endocarp of cocos nucifera. **Table 1** reports the results of total phenolics and total flavonoid analysis. The amount of total flavonoids varied widely in the different analysed extracts and ranged from 33 to 326.8 mg/g of the extract. The variation can be expected for the plant extracts due to different methods and/or due to the presence of other constituents. Among the extracts the RNM-01 contained the highest amount of flavonoids(326.8 mg/g) followed by RNA(276.6 mg/g) whereas the lowest level was found in RNDS(33 mg/gm)

Extracts of cocos nucifera	Total Phenolic content *(mg/g)	Total flavonoid Content ** (mg/g)
RNM-01	143.77	326.8
RNM-02	145.77	208.8
RNA	125.2	276.6
RNDS	108	33

**Table 1 Total Phenolic and Total Flavanoid Content of Endocarp Extracts**

Total tannin content of RNM-02 was found to be 6.02 %. All the extracts were exhibited no sensitivity towards the clinical strains of the microorganisms(Bacterial and fungal) except RNM-01 having insignificant activity towards MRSA, MSSA. RNM-01 was exhibiting potent activity towards B. Subtilis, P. Aeruginosa, S. Aureus, M. Luteus even at 10 mcg/disc, whereas showing resistance for the E. coli. By tube dilution method, MIC value of RNM-01 was found out to be 300-350 µg mL<sup>-1</sup> against B. subtilis.(Refer Table 2,3,4,5,6)

Code	MRSA <sup>A</sup>				MSSA <sup>A</sup>				E.COLI <sup>A</sup>			
	10µg/disc	50µg/disc	100µg/disc	300µg/disc	10µg/disc	50µg/disc	100µg/disc	300µg/disc	10µg/disc	50µg/disc	100µg/disc	300µg/disc
RNM-01	R	R	R	S	R	R	R	10	R	R	R	R
RNM-02	R	R	R	S	R	R	R	9	R	R	R	R
RNA-01	R	R	R	R	R	R	R	R	R	R	R	R
RNDS	R	R	R	R	R	R	R	R	R	R	R	R
GENT. <sup>c</sup>		19				20				20		

**Table 2. Antibacterial Screening of the Cocos Nucifera Endocarp Extracts**  
A Clinical strains ; R= Resistance; c at 10mcg/disc

Code	P. AERUGINOSA <sup>A</sup>				ENTEROCOCCUS FAECALIS <sup>A</sup>				KLEBSIELLA PNEUMONIAE <sup>A</sup>			
	10µg/disc	50µg/disc	100µg/disc	300µg/disc	10µg/disc	50µg/disc	100µg/disc	300µg/disc	10µg/disc	50µg/disc	100µg/disc	300µg/disc
RNM-01	R	R	R	R	R	R	R	11	R	R	R	R
RNM-02	R	R	R	R	R	R	R	12	R	R	R	R
RNA-01	R	R	R	R	R	R	R	R	R	R	R	R
RNDS	R	R	R	R	R	R	R	R	R	R	R	R
GENT. <sup>c</sup>		16					18			16		

A Clinical strains ; R= Resistance; c at 10mcg/disc

Table 3. Antibacterial Screening of the Cocos Nucifera Endocarp Extracts

Code	ACINETOBACTER BAUMINI <sup>A</sup>				CITROBACTER FREUNDII <sup>A</sup>				STREPTOCOCCUS PYOGENES <sup>A</sup>			
	10µg/disc	50µg/disc	100µg/disc	300µg/disc	10µg/disc	50µg/disc	100µg/disc	300µg/disc	10µg/disc	50µg/disc	100µg/disc	300µg/disc
RNM-01	R	R	R	R	R	R	R	R	R	R	R	R
RNM-02	R	R	R	R	R	R	R	R	R	R	R	R
RNA-01	R	R	R	R	R	R	R	R	R	R	R	R
RNDS	R	R	R	R	R	R	R	R	R	R	R	R
GENT. <sup>c</sup>			19			19				17		

A Clinical strains ; R= Resistance; c at 10mcg/disc

Table 4. Antibacterial Screening of the Cocos Nucifera Endocarp Extracts

Code	RNM-01				CIPROFLOXACIN
	10µg/disc	50µg/disc	500µg/disc	1000µg/disc	5µg/disc
B. SUBTILIS	11	13	14	16	28
E. COLI	R	R	13	16	37
P. AERUGINOSA	11	13	13	14	36
S. AUREUS	11	12	13	14	22
M. LUTEUS	12	12	14	16	28

b- at 5µg/disc; R= Resistance

Table 5. Antibacterial Screening of the Cocos Nucifera Endocarp Extracts

Code	CALBICANS <sup>A</sup>			
	10µg/disc	50µg/disc	100µg/disc	300µg/disc
RNM-01	R	R	R	R
RNM-02	R	R	R	R
RNA-01	R	R	R	R
RNDS	R	R	R	R
FLUCONAZOLE	21			

A Clinical strains; R= Resistance

Table 6. Antifungal Screening of the Cocos Nucifera Endocarp Extracts

After the DPPH radical scavenging analysis, All extracts RNM-01, RNM-02, RNA-01 and RNDS were found to be significant antioxidant activity 4.1828, 3.31, 20.83, 1.0179 µg/mL<sup>-1</sup> respectively comparable to standard ascorbic acid (Table 7). RNM-01 showed significant results after nitric oxide assay and alkaline DMSO method, activity was similar to that of the standard drug, ascorbic acid (Table 8).

Code	DPPH Radical Scavenging Method(IC50 (µg/ml))
RNM-01	4.1828
RNM-02	3.31
RNA-01	20.83
RNDS	1.0179
Ascorbic acid	2.98

Table 7. DPPH radical scavenging method of the Encocarp extracts of cocos nucifera

Code	IC50 (µg/ml)	
	Nitric Oxide Method	Alkaline DMSO method
RNM-01	538.44	31.25
Ascorbic acid	89.9	22

Table 8. Result of antioxidant potential of RNM-01

Crude methanolic extracts(RNM-01 & RNM-02) may contain thousands of compounds including flavones, proanthocyanidin and even soluble polysaccharide which might be responsible for the above activities. The antioxidant property of the phenolics mainly depends on the number and position of the hydroxyl group. The antioxidant property of the extracts were verified by the DPPH scavenging method. DPPH test confirms the radical scavenging potentiality of the endocarp extracts by measuring the inactivation potential of the radical in an aqueous medium. Free radical scavenging property of RNM-01 was further verified by the nitric oxide assay and alkaline DMSO method.

Antimicrobial activity of the endocarp extracts shows strong activity against *B. Subtilis*, *P. Aeruginosa*, *S. Aureus*, *M. Luteus*. However, they shows no response against *E. coli* and clinical strains of bacterial and fungal microbes. In this respect further investigation is going on.

## **CONCLUSION**

To our knowledge, for the first time we have reported about the strong antioxidant and antimicrobial properties of the endocarp extracts of cocos nucifera. The endocarps of cocos nucifera are discarded as waste and it is considered as one of the major agro wastes of the tropical countries. Therefore our study will definitely open up a scope for future utilization of these agro wastes for therapeutic purposes.

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