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Anti-Malaria and Anti-Typhoid Effects of *Cocus nucifera* L Husk Extract

Okwara K. K.[1], Mgbemena I. C.[2], Emeka-Nwabunnia I.[2], Nwoko M. C.[1] and Nkwocha C. J.[1]

[1]Department of Biology, Federal University of Technology, Owerri, Imo State
[2]Department of Biotechnology, Federal University of Technology, Owerri, Imo State

Corresponding Author: Tgod3125@gmail.com

ABSTRACT

The aim of the study is to evaluate the antimalaria and antityphoid effects of ethanolic extracts of *Cocus nucifera* husk. The ethanolic extracts were obtained by maceration of pulverized plant parts in ethanol for 48hours with continual agitation, extracts obtained were evaluated for acute toxicity test (LD_{50}), phytochemical analysis. Antimalarial suppressive and curative test were carried out using (84) albino mice (weighing 20 – 23g) which were infected intraperitoneally with 0.2ml of 10fold dilution of 1 ml of infected blood from malaria infected mice. Packed cell volume (PCV) was measured for both suppressive and curative analysis, percentage parasitemia were also determined. Antimicrobial sensitivity test of the plant samples against *Salmonella typhi* were carried out. The acute toxicity test (LD_{50}) caused no toxicity and death to mice after oral administration even at high doses of 5000 mg/kg of the plant extracts, phytochemical studies reveal the presence of flavonoids, terpenoids and more phenols in the plant extracts, also, proteins and tannins were observed in moderate level. The suppressive effect of the plant extract were statistically significant ($p < 0.05$) with coconut husk extract at 500mg/kg having the highest parasitemia suppression of 58.03%, while chloroquine 25mg/kg (40.05%) suppression. Chloroquine and extract treated groups increased the PCV of *Plasmodium berghei* infected mice when compared to control group, but were not statistically significant ($p > 0.05$). The curative effect of the Chloroquine treated group had a significant ($p < 0.05$) effect with increased PCV while the 250mg/kg and 500mg/kg *C. nucifera* husk extract treated group had a slight increase in their PCVs but it was not statistically significant ($p > 0.05$). Chloroquine statistically reduced the parasitemia load of *P. berghei* ($p < 0.05$), with percentage suppression of 81.25%, the coconut husk 250mg/kg and 500mg/kg treated groups had percentage suppression of 40.60% and 40.00% respectively which is statistically significant ($p < 0.05$). From the study, chloroquine produced the highest curative effect followed by the coconut husk extract. Ethanolic extract of coconut husk had better antibacterial effects on *Salmonella typhi* at 400mg/ml, 200mg/ml and 100mg/ml concentrations with zones of inhibition better than some control antibiotics like Ampicillin, Ceporex but similar with that of Streptomycin, Ofloxacin, Augmentin, Ciprofloxacin. Ethanolic extract of Cocos nucifera husk possesses antimalarial properties at doses of 250 mg/kg and 500 mg/kg. The C. nucifera ethanolic husk extract at 500 mg/kg had better prophylaxis effects and malaria parasite suppression than chloroquine but with a cure rate less than that of chloroquine but statistically significant at both dosages of 250 mg/kg and 500 mg/kg.

Keywords: *Plasmodium berghei, Salmonella typhi*, *Cocos nucifera,* Malaria, Typhoid.

INTRODUCTION

Natural products have long been recognized as valuable sources for drug discovery due to their chemical diversity and potential therapeutic properties (Newman and Cragg, 2020). Among these natural resources, plant extracts have shown immense potential in combating diseases. The coconut husk has gained attention for their pharmacological

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properties and traditional medicinal uses (Ahmed *et al*., 2019; Egunsola *et al.*, 2019).

Malaria and typhoid fever (caused by *Plasmodium berghei*, and *Salmonella typhi* respectively) have remained a significant global health challenge, causing substantial morbidity and mortality worldwide, particularly in developing countries (WHO, 2021). Despite extensive efforts to control and treat these diseases, the emergence of drug resistance and the limited availability of effective therapeutic options necessitate the exploration of alternative approaches. Coconut husk, the fibrous outer shell of the coconut (*Cocos nucifera*), has been utilized for centuries in traditional medicine for its antimicrobial and anti-inflammatory properties (Kurian and Varghese, 2019). Recent studies have focused on its bioactive components, such as polyphenols, flavonoids, and fatty acids, which exhibit potent antibacterial and antimalarial activities (Patra *et al.,* 2019).

These compounds have demonstrated antimicrobial properties against a wide range of pathogens, including bacteria and protozoa (Tadesse *et al.,* 2018). Several studies have highlighted the antimicrobial properties of coconut husk extracts. Patra *et al*. (2019) reported that coconut husk extract exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacteria, including drug-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA). Furthermore, Pereira *et al.* (2020) demonstrated that coconut husk-derived polyphenols showed potent antimalarial activity against *Plasmodium falciparum*, another species of malaria parasite. These findings suggest the potential of coconut husk extracts as a source of antimicrobial and antimalarial compounds. However, their specific effects on *Plasmodium berghei* and *Salmonella typhi*, remain largely unexplored. This research intends to address these knowledge gaps by investigating the potential therapeutic effects of coconut husk extracts against *Plasmodium berghei* and *Salmonella typhi.*

MATERIALS AND METHODS

Plant Collection

Cocus nucifera husk were collected in June 2023 at Ihiagwa, Owerri west L.G.A Imo State Nigeria. These plants were identified (Tadesse *et al.,* 2018) by Dr. C.M. Duru of the Department of Biology at Federal University of Technology, Owerri, Imo State.

Bacteria collection for Antimicrobial test

The bacteria used were chloroquine sensitive *Plasmodium berghei* which was collected from the Faculty of Veterinary Medicine University of Nigeria Nsukka. *Salmonella typhi* was collected from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences Nnamdi Azikiwe University, Awka. The bacteria samples were further authenticated by sub-culturing and subjecting pure isolates to systematic culture identification protocol.

Animal collection for toxicity test

A total number of Eighty-four (84) albino mice which comprised of both genders, weighing between 20-25g were procured from the faculty of Veterinary Medicine, University of Nigeria, Nsukka. These animals were acclimatized and fed for two (2) weeks with standard feed of pellets and water.

Preparation of Plant Extract

The *C. nucifera* husks were washed in clean water and air dried at room temperature (27^{o}C) for seven days. The *C. nucifera* husks were further cut into pieces and oven dried at a controlled temperature between 40^{o}C. The *C. nucifera* husks were oven dried for 3hours due to the recalcitrant nature of the husk. The dried

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C. nucifera was further pulverized into tiny particles. A measurement of 1400g of the pulverized *C. nucifera* husk was mixed or macerated in 1500ml of ethanol for 48 hours; the mixture was constantly agitated at intervals to aid extraction. After 48hours, the mixture was filtered using muslin clothe and the recovered filtrate was further concentrated to paste using a water bath at 50^{o}C. The concentrated husk extract obtained was 111.72g with percentage yield of 7.98%, which was used for the study.

Qualitative Phytochemical Analysis

The plant extract was screened for the presence of flavonoids, saponins, glycosides, steroids, terpenoids, phenol, alkaloids, and tannins using Sofowora (1999), Harborne's method as described by Yadav and Agarwala (2011).

Preparation of plant extract for antimicrobial test

stock concentrations of each of *Cocus nucifera* extract was made by weighing 400 mg of crude extract into sterile tubes. Then 2 ml of Dimethyl sulfoxide (organic diluent) is added into the sample and reconstituted properly. This gave a stock concentration of 200 mg/mL of the extract, thereafter two fold serial dilutions was made from the stock concentration to get graded concentrations (100, 50, 25, 12.5 mg/mL) of each of the crude extract.

Determination of antimicrobial activity

The antibacterial assay for the crude extracts was carried out using the agar well diffusion assay as described by Okezie *et al*. (2021) with slight modifications. The microbial suspensions were adjusted to 0.5 McFarland turbidity standards and inoculated onto previously sterilized Mueller-Hinton Agar plates (diameter: 90 mm). A sterile cork-borer was used to make five (5) wells (8 mm in diameter) on each of the MHA plates. Aliquots of 80 µl of each extract dilutions were applied in each of the wells in the culture plates previously seeded with the test organisms. Streptomycin (S), Ampicillin (PN), Ceporex (CEP), Tarivid (OFX), Nalidixic Acid (NA), Peflaxin (PEF), Gentamycin (CN), Augmentin (AU), Ciprofloxacin (CPX), and Streptomycin (SXT) served as the positive controls. The cultures were incubated at 37 ^{o}C for 24 h. The antimicrobial potential for each extract was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). For each of the crude extract, three replicates were conducted against each organism. Each of the samples was tested against all the test isolates.

Preparation of Stock Solution for Administration to Animals:

A: Extract: A total of 1000 mg (1 g) of both leaf and husk extract were weighed and dissolved in 20 ml of distilled water respectively, which served as high dose (5000mg/kg) stock (50 mg/ml). A double-fold dilution of high dose stock was made to have lower concentration (25 mg/ml) for low dose (250 mg/kg). Animals were administered various doses of the extract based on their body weight calculation using the formula below;

$$\text{Dose} = \frac{\text{Weight of mice (g)}}{1000\ g} \text{ x dose}$$

B: Chloroquine: 1 tablet = 250 mg

Dose = 25 mg/kg

Stock preparation =1 tablet (250 mg) was dissolved in 100 ml of distilled water, yielding a stock of 250 mg/100 ml = 2.5 mg/ml

Suppressive Test

A total of thirty (30) albino mice were grouped into 5 of six mice in each group. Group one which is the control was given clean, non-infected water. The other groups were

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inoculated intraperitoneally with 0.2ml suspension of blood from the *P. berghei* infected mice. This infected blood was diluted with normal saline using 10-fold dilution. Of the infected groups, group 2 which is the negative control was not given any treatment. Group 3 which is the positive control were treated with 25mg/kg /day of chloroquine given orally using a disposable syringe. Group 4 received 250mg/kg/day of *C. nucifera* husk extract orally using a disposable syringe. Group 5 received 500mg/kg/day of *C. nucifera* husk extract orally using a disposable syringe. Treatment started immediately after infection with the *P. berghei* parasite and continued for three (3) consecutive days. On the fourth day, blood samples were collected for determination of packed cell volume and parasitemia percentage calculation. The suppressive activity of the plant samplewas evaluated using the method described by Birru *et al.* (2017) with little modification.

Curative Test

A total of thirty (30) albino mice were grouped into five groups of six mice each. Group one is the normal control containing mice given clean, non-infected water. The other groups of 2 to 5 were all infected intraperitoneally with 0.2ml of blood from the *P. berghei* infected mice. The infected blood was diluted in normal saline using 10 fold dilutions. Group 2 which is the negative control was not given any treatment, group 3: the positive control was treated with chloroquine, 25mg/kg orally. Group 4 were treated with 250mg/kg of *C. nucifera* husk extract orally using disposable syringe. Group 5 received 500mg/kg of *C. nucifera* husk extract orally using disposable syringe. After infection, the infected groups were left for 3 days for infection to be established before treatment. The infected groups with exception of group two were treated for 5 consecutive days on the sixth day, blood samples were collected for determination of packed cell volume (PCV) and percentage parasitemia. The curative activities of the plant extracts were evaluated using the method described by Birru *et al.* (2017).

At the end of the study, blood samples were collected from the inferior vena cava and were delivered into lithium herparinized tube and mixed gently to avoid clotting.

Determination of packed cell volume: Heparinized capillary tubes were used to collect blood samples, the capillary tubes were filled with blood up to ¾ of the volume and the opening of the tube was sealed tight with sealing clay. The tubes were placed in a haematocrit centrifuge with the sealed end outwards and centrifuged for 5 minutes at 11000rpm. The tubes were then taken out of the centrifuge, the packed cell volume were determined using a graduated metre rule and the formula:

$$\frac{Volume\ of\ erythrocytes\ in\ given\ volume\ of\ blood}{Total\ blood\ volume} \times 100$$

The essence of packed cell volume measurement is to determine the effectiveness of the test plant extract in preventing haemolysis resulting from increased parasitemia, hence relating to the effectiveness of the test extracts.

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Determination of percentage parasitemia

Blood samples were collected from the herparinized tube, thick blood smear on the slide were prepared and allowed to air dry and then stained using 20% giemsa. The percentage parasitemia were determined by counting the number of infected erythrocytes using ×100 objective of the electron microscope, average percentage parasitemia was calculated using the formula:

$$A - \frac{B}{B} \times 100$$

Where A is the average percentage parasitemia of the negative control group and B is the average parasitemia in the test group.

Biological studies

Identification of Test Organism: the bacteria isolates were identified using selected confirmatory biochemical tests which include catalase test, oxidase test, indole test, and citrate test (Kumar *et al.,* 2022).

Data analysis

Data generated in the course of this study were statistically analyzed using One-way Analysis of Variance (ANOVA) and mean separation was carried out by Duncan Multiple range test (DMRT) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Phytochemical properties of *C. nucifera* husk extract

The phytochemical analysis of *C. nucifera* husk extract were determined and the result as is shown in Table 1.The Table showed the presence of proteins, carbohydrate, phenols, tannin, flavonoids, and terpenoids in the husk extract. Saponins, glycosides, steroids, and alkaloids were not detected in the extract. The level of phenol was higher compared to the other phytochemicals present. Proteins, tannins, and flavonoids were observed in moderate level. Trace concentrations of carbohydrates and terpenoids were observed (**Table 1**). According to Oboh *et al.* (2018) there are diverse group of secondary metabolites with antioxidant and other bioactive properties. They can scavenge free radicals, inhibit oxidative stress, and protect cells from damage (Oboh *et al.,* 2018). Alkaloids, flavonoids, terpenoids, and phenolic compounds possess chemical constituents which contribute to their therapeutic properties and exhibit antimicrobial, anti-inflammatory, antioxidant, anticancer, and immune-modulatory effects (Kendeson *et al.,* 2019; Newman and Cragg *et al*., 2020).

Table 1: Phytochemical analysis of *C. nucifera* husk extract.

S/N	Phytochemical	Test	Husk extract
1	Proteins	Millon's test	++
2	Carbohydrates	Iodine test	+
3	Phenols	Litmus test	+++
		Ferric chloride test	+++
4	Tannins	Geletin test	++
5	Flavonoids	Alkaline reagent test	++
6	Saponins	Frothing test	-
7	Glycosides	Liebermann's test	-
		Keller-kilani test	-
8	Steroids	Liebermann-Burchard's test	-
9	Terpenoids	Salkowski's test	+
10	Alkaloids	Wagner's test	-

Key: "-'' (Absent), "+" (Present in trace), "++" (Present in moderate level), "+++" (Present in abundant level.

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Antimicrobial activity of *C. nucifera* husk extract

The Table 2 showed that *C. nucifera* husk exhibited antibacterial potential at 400 mg/mL, 200 mg/mL and 100 mg/mL concentrations. There was no antimicrobial activity observed for concentrations 50 mg/mL and 25 mg/mL. The zone of inhibition observed for the husk extract decreased as the concentration of the extract decreases; 400 mg/mL showed zone of inhibition of 8.0 mm, 200 mg/mL showed 7.5 mm inhibition zone, and 100 mg/mL showed 4.0 mm inhibition zone (Table 2). All of the antibiotic discs used as control showed antimicrobial activity, exhibiting zones of inhibition ranging from 3.0 mm to 10.0 mm. The lowest inhibition zone was observed for Ceporex antibiotic (3.0 mm). Coconut husk extracts have been widely used for treatment of Urinary tract Infections (UTIs) (Uy *et al.,* 2019). Antimicrobial activity was observed to increase (wider zone of inhibition) at higher concentration of the plant extract. This result corresponds with other researchers (Parvathy *et al.,* 2020; Kumar *et al.,* 2018; Onyishi *et al.,* 2018). According to Onyishi *et al.* (2022) the higher the concentration of *C. nucifera* husk extract for the treatment, the better its effect on the host. The inhibitory ability of *C. nucifera* husk extract against *Salmonella typhi* indicates the wide spectrum antimicrobial potential of *C. nucifera*. The phytochemicals present in the husk extract (flavonoids, terpenoids, and phenolic compounds) contribute to the therapeutic properties of the extract (Gebrehiwot *et al.,* 2029).

Table 2: Antimicrobial activity of *C. nuifera* husk extract highlighting the zones of inhibition.

Treatment	Concentration (mg/mL)	Zone of inhibition
Husk extract	400	8.0
	200	7.5
	100	4.0
	50	0.0
	25	0.0
Control	Streptomycin	10.0
	Ampicillin	5.0
	Ceporex	3.0
	Tarivid	10.0
	Nalidixic Acid	10.0
	Peflaxin	10.0
	Gentamycin	10.0
	Augmentin	7.0
	Ciprofloxacin	10.0
	SXT	10.0

Toxicity of *C. nucifera*

Single admininstration of the 500 mg/kg using the Up and Down procedure did not result to any death within 24 hours, and 7 days period of observation. Thus, the LD_{50} was estimated to be above 5000 mg/kg (Table 3).The result for the acute toxicity assay showed that *C. nucifera* husk extract presented no signs of toxicity at concentration used (5000 mg/kg). No significant physical body decrease was observed with the test animals in the different groups of both plant extract. The LD_{50} value is >5000 mg/kg b.w. This suggests that toxicity will be observed beyond the used concentration 5000 mg/kg. The absence of toxicity and death suggests that at the used concentration, the plant extracts are safe for use.

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Table 3: Toxicity of *C. nufera* husk extract.

Group	Dose (mg/kg)	Observation
Control	10	No toxicity and death
Husk extract	5000	No toxicity and death

Suppressive effect of *C. nucifera* extract

Chloroquine and extract treated groups decreased the PCV of *P.berghei* infected mice when compared to control group. Chloroquine, 500 mg/kg leaf extract, 250 and 500 mg/kg husk extract statistically significantly ($p<0.05$) reduced the parasitemia load of *P. berghei* infected mice when compared to the induced control group. The 500 mg/kg husk extract produced the highest suppression of 58.03% followed by the 250 mg/kg husk extract, while the chloroquine treatment showed the least suppression (Figure 1). The suppressive test was used to determine the percentage of parasitaemia as it is standard test used for anti-malaria screening (Koffi *et al.,* 2020). An average suppression of parasitemia greater than or equal to 30% in suppressive tests indicates the presence of active antimalarial compound effective for antiplasmodial activity (Koffi *et al.,* 2020). The highest suppression in the extract was observed at the maximum dose (500 mg/kg). This is in line with other researchers that observed highest suppression at the maximum dose (Maximus *et al.,* 2021).

Table 3: Suppressive test: Effects of *Cocus nucifera* husk extract on packed cell volume (PCV) and parasitemia count of *P. berghei* infected mice.

Group	PCV (%)	Parasite count	Parasitemia suppression (%)
Normal control	49.14 ± 4.98	-	
Induced control	41.15 ± 12.91	83.40 ± 18.72	
Chloroquine, 25 mg/kg	48.42 ± 9.16^{ns}	50.00 ± 8.19*	40.05
Husk extract, 250 mg/kg	49.67 ± 9.53^{ns}	49.00 ± 8.04*	41.25
Husk extract, 500 mg/kg	43.34 ± 12.54^{ns}	35.00 ± 8.41*	58.03

Values are expressed as mean ± Standard deviation. $^{ns}P>0.05$: Not statistically significantly different from induced control. *$P<0.05$: Statistically significantly different from induced control.

Curative effect of *C. nucifera* extract

Chloroquine significantly ($p<0.05$) increased the PCV of *P. berghei* infected mice when compared to control group. The 250 mg/kg husk extract caused a slight reduction in PCV compared to chloroquine treatment, but were higher than the PCV observed in the control. Chloroquine statistically significantly ($p<0.05$) reduced the parasitemia load of *P. berghei* infected mice when compared to control group. However, 250 and 500 mg/kg husk extract caused a significant reduction in parasitemia load when compared to control group ($p<0.05$) (Figure 2).Chloroquine produced the highest suppression of 81.25%, followed by the husk extracts, 250 and 500 mg/kg. In curative potential evaluation of *C. nucifera* husk extract, 250 mg/kg and 500 mg/kg of the plant extract did not significantly reduced ($P < 0.05$) parasitaemia level. The test groups treated with *C. nucifera* husk extract all had lower parasite count compared to the untreated group. This same occurrence was observed by Gebrehiwot *et al.* (2019); Maximus *et al.* (2021).

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Table 1: Curative test: Effects of *Cocus nucifera* husk extract on packed cell volume (PCV) and parasitemia count of *P. berghei* infected mice.

Group	PCV (%)	Parasite count	Parasitemia suppression (%)
Induced control	28.73 ± 8.15	367.00 ± 20.49	
Chloroquine, 25 mg/kg	42.95 ± 8.33*	68.80 ± 17.17*	81.25
Husk extract, 250 mg/kg	34.51 ±0.47^{ns}	218.00 ± 68.51*	40.60
Husk extract, 500 mg/kg	31.10 ± 5.30^{ns}	220.20 ± 91.06*	40.00

Values are expressed as mean ± Standard deviation. ^{ns}P>0.05: Not statistically significantly different from induced control. *P<0.05: Statistically significantly different from induced control. PCV (Packed cell volume).

CONCLUSION

Coconut husk extracts contains substantial phytochemicals with high phenolic contents. There was no sign of acute toxicity after oral administration of extracts up to high doses of 5000mg/kg, hence the extracts may be safe for consumption. The antiplasmodial analysis further implies that the plant extracts possess antimalarial properties at doses of 250mg/kg, and more particularly at 500mg/kg dosage. The coconut husk at 500mg/kg had better prophylaxis effects and parasite suppression than chloroquine with a cure rate less than that of chloroquine but statistically significant at both dosages of 250mg/kg and 500mg/kg, hence the coconut husk would make for a better preventive treatment for malaria than chloroquine. The coconut husk had better antibacterial effects against *Salmonella typhi* of which the zone of inhibition is better than some of the control antibiotics like, Ampicilin, Ceporex but similar to other control antibiotics like, streptomycin, ofloxacin, augmetin, ciprofloxacin. Further research recommended into herbal remedies that can be used as a single treatment for malaria, fever and typhoid fever given that coconut husk extracts exhibits such potential.

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