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Phytochemicals and antimicrobial activity of coconut water (*Cocos nucifera*) on microbial pathogens

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Abstract

The phytochemicals and antimicrobial activity of coconut water on microbial pathogens were investigated for its medicinal potential. In this study, coconut water and selected test microbial pathogens; *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella* spp were used. The phytochemical screening and antimicrobial activities of the coconut water against the selected test microbial pathogens were determined using standard method and paper disc diffusion method respectively. The study revealed the presence of flavonoids, tannins, phenols, anthocyanins, terpenoids, and alkaloids as phytochemicals detected. Quantitative phytochemical constituents revealed 0.822±0.11mg of total phenols compound and total flavonoids compound of 25.44±3.68 of the coconut water. With the positve control antibiotic (ciprofloxacin), *Klebsiella* spp exhibited the highest zone of inhibition of 23mm while the least zone of inhibition was observed with *Pseudomonas aeruginosa* and *Escherichia coli* at 20mm respectively. The antimicrobial efficacy of the coconut water was significantly lower when compared with the positive control(ciprofloxacin) at 30mg/ml against the test bacteria but higher at an increased concentration of 60mg/ml with *Staphylococcus aureus* having the highest zone of inhibiton of 28mm while *Pseudomonas aeruginosa* showed the least zone of inhibition at 21mm. Moreover, the inhibitory activity exhibited by coconut water against these bacterial pathogens is an indication of the presence of bioactive compounds that can be harnessed and used for further medical research.

Keywords: Phytochemical screening; Antioxidant properties; Antimicrobial activity; Coconut water; Microbial pathogens

1. Introduction

Coconut (*Cocos nucifera*) is a common fruit in the tropics cultivated in nearly 90 different countries [1]. The endosperm contains water referred to as coconut water, which contains carbohydrates (glucose, fructose, and sucrose), vitamin C, minerals, amino acids, enzymes, hormones, and phytochemicals [2]. Coconut is cultivated for its multipurpose values (nutritional and medicinal). It is a unique source of various natural products for the development of drugs and industrial products [3]. It was reported to have antioxidant properties [4]. Coconut water has been used to treat various ailments, it is used as an antibacterial, antifungal, antiviral, and anti-dermatophyte agents [5]. The antimicrobial property of coconut water is because of its high lauric acid content which has been used as a medication for certain oral infections. Several studies reported that sucrose monolaurate and glycolipid compound present in coconut water have anti-caries properties [6] .

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Phytochemicals are bioactive compounds produced by plants as a result of primary or secondary metabolism [7]. Some phytochemicals have been used as poisons and others as traditional medicines. Phytochemicals present in plants such as alkaloids, phenols, steroids, saponins, cardiac glycosides, flavonoids, etc, have played several roles in disease prevention [8]. Chemical compounds derived from plants play an important role in preventing activities such as anti-inflammatory, anti-diabetic, anti-aging, antimicrobial, anti-parasitic, antidepressant, anticancer, antioxidant, and wound healing [9]. Due to the antimicrobial resistance of microorganisms to antibiotics as a result of broad-spectrum antibiotics and immunosuppressive agents and other factors, the use of plants as an alternative is being studied globally, especially in developing countries like Nigeria, since plants are considered nutritionally safe, biodegradable, and possess antimicrobial phytochemicals [10; 11]. Plants have also been reported to be an excellent source of secondary metabolites that can be used in the production of modern medicines [12]. Many of the secondary metabolites such as tannins, flavonoids, and alkaloids have been demonstrated in many studies to have antimicrobial activities [13]. This study is therefore aimed at evaluating the phytochemicals and antibacterial activity of coconut water on some pathogenic microorganisms.

2. Material and methods

2.1. Sample Collection

Mature coconuts were purchased from a local market in Otuoke, Bayelsa State.

2.2. Sample preparation

The coconut shells were removed and the water in the nut harvested and stored in aseptic container. Aliquots of the harvested coconut water were thereafter used for phytochemical, antioxidant and antibacterial evaluation.

2.3. Collection of isolates

The microbial isolates were collected from the Federal Medical Center (FMC) Yenagoa Bayelsa stock culture. They were cultured on nutrient agar and incubated at 37^{o}C for 24 hours before usage.

2.4. Confirmation of isolates

The identification of isolate of *Escherichia coli* was on Eosin Methylene blue agar, *Staphylococcus aureus* on Mannitol salt agar, *Klebsiella* spp, and *Pseudomonas aeruginosa* on MacConkey agar and was confirmed after incubation using morphological, cultural, and biochemical characteristics which include Gram staining, catalase, citrate, oxidase, indole and coagulase tests [14].

2.5. Antimicrobial susceptibility test

Antimicrobial susceptibility test was done using Kirby-Bauer disc diffusion method [15]. They were measured and recorded in millimeters.

2.6. Qualitative phytochemical analysis

Tests for flavonoids, tannins, carbohydrates, glycosides, saponins, resins, terpenoids and alkaloids were carried out using standard methods [16; 17].

2.7. Quantitative phytochemical analysis

Total phenolics were determined using Folin-Ciocalteau Reagent (FCR) as described by [18], with slight modifications.

- Tannin content in each sample was determined using insoluble polyvinyl-polypirrolidone (PVPP), which binds tannins as described by [19].
- The flavonoids and flavonols content were determined according to the method described by [20]) with slight modifications. Monomeric anthocyanin contents of the plant extracts were measured using a spectrophotometric pH differential protocol described by [21].

2.8. Quantitative DPPH radical-scavenging assay

Scavenging activity on DPPH free radicals by the extract was assessed according to the method reported by [22] with slight modifications.

2.8.1. Hydroxyl radical (·OH)-scavenging assay

The 2-deoxyribose assay was used to determine the scavenging effect of the extract on the hydroxyl (·OH) radical, as reported by [23].

2.8.2. Superoxide radical ($O_2^{\cdot-}$)-scavenging assay

This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) [24] and the method used by [25] to determine superoxide dismutase with slight modifications.

2.8.3. Lipid peroxidation assay

A modified thiobarbituric acid-reactive species (TBARS) assay was used to measure the lipid peroxide formed, using egg yolk homogenates as lipid-rich media [26].

2.8.4. Nitric oxide radical (NO·) scavenging assay

Nitric oxide (NO·) generated from Sodium Nitroprusside (SNP) was measured according to the method of [27].

2.9. Statistical analysis

Data was expressed as mean standard deviation. The data obtained was subjected to Analysis of Variance (ANOVA) test to determine the significant difference at 95% confidence limit.

3. Results

The results of the present study showed that coconut water had a growth inhibitory effect on *Klebsiella* spp, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.* There was no zones of inhibition recorded against all the four isolates when tested with the negative control; 2.5% of dimethylsofloxide (DMSO). The positive control is as shown in **Table 1**. Using ciprofloxacin, *Klebsiella* spp exhibited the highest zone of inhibition of 23mm while the least zone of inhibition was exhibited by *Pseudomonas aeruginosa* and *Escherichia coli* at 20mm respectively.

Table 1 Zones of inhibition of ciprofloxacin (positive control) against test bacteria

Organism	**Concentration (µl)**	**Ciprofloxacin(mm)**
Klebsiella spp.	30.0	23.00
Pseudomonas aeruginosa	30.0	20.00
Escherichia coli	30.0	20.00
Staphylococcus aureus	30.0	21.00

Key: µl = microliter, mm = millimeter

The results in **Table 2** revealed that the antimicrobial efficacy of coconut water was significantly lower when compared to the positive control (ciprofloxacin) at 30mg/ml which was used as a standard against the test bacteria but higher at an increased concentration of 60mg/ml with *Staphylococcus aureus* having the highest zone of inhibiton of 28mm while *Pseudomonas aeruginosa* showed the least zone of inhibition at 21mm.

Qualitative analysis on coconut water revealed the presence of important phytochemical constituents including phenolic compounds (tannins and flavonoids), saponins, alkaloids, glycosides as bioactive compounds as shown on **Table 3.**

Table 2 Zones of inhibition (mm) of coconut water against test bacteria

Organism	**Concentration (mg/ml)**		
	60	**40**	**20**
Klebsiella spp	23.00	17.00	11.00
Pseudomonas aeruginosa	21.00	15.00	7.00
Escherichia coli	25.00	22.00	12.00
Staphylococcus aureus	28.00	24.00	15.00

Key: µl = microliter, mm = millimeter

Table 3 Phytochemical constituents in coconut water

S/N	**Phytochemicals**	**Results**
1.	Flavonoids	++
2.	Tannins	++
3.	Phenols	+
4.	Anthocyanins	+
5.	Alkaloids	+
6.	Terpenoids	-
7.	Carbohydrates	+
8.	Glycosides	+
9.	Saponins	+
10.	Steroids	-
11.	Resin	-
12.	Protein	-

Phenolic compounds were found to be the major class of bioactive components in the coconut water. The amount of total phenolics was 0.885 ± 0.008 mg GAE/mg of aliquot of coconut water extract as shown in **Table 4**.

Table 4 Quantitative phytochemical constituent

Extract	**Phenolic contents ***			**Total anthocyanin †**	**Total flavanols ‡**	**Total flavonoids ‡**
	Total Phenols	**Non-tannins**	**Tannins**			
Coconut water	0.885 ± 0.008	0.511 ± 0.004	0.374 ± 0.008	0.45 ± 0.02	38.85 ± 0.32	25.44 ± 3.68

Fig. 1, showed coconut water inhibited 92.26± 1.30 % of DPPH at a concentration of 125 µg/ml compared to ascorbic acid which inhibited 94.18.± 3.22 % at the same concentration.

Aliquots of coconut water aliquots scavenged ·OH radical in a concentration dependent manner. It inhibited 2-deoxyribose degradation above 30% with maximal inhibition of 50.36 ± 1.20% at concentration of 500 µg/ml. The scavenging ability of coconut water was significant at all tested concentrations. **(Fig. 2)**

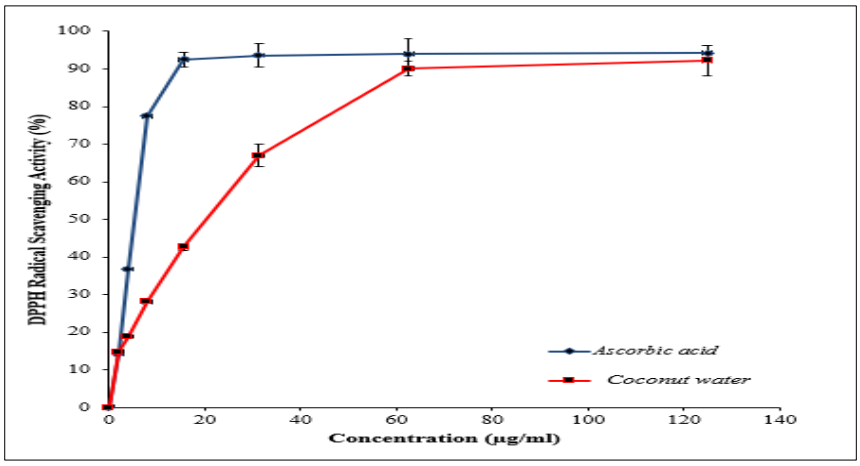

Figure 1 DPPH radical scavenging activity of coconut water Data represented as mean ± SEM (n = 3)

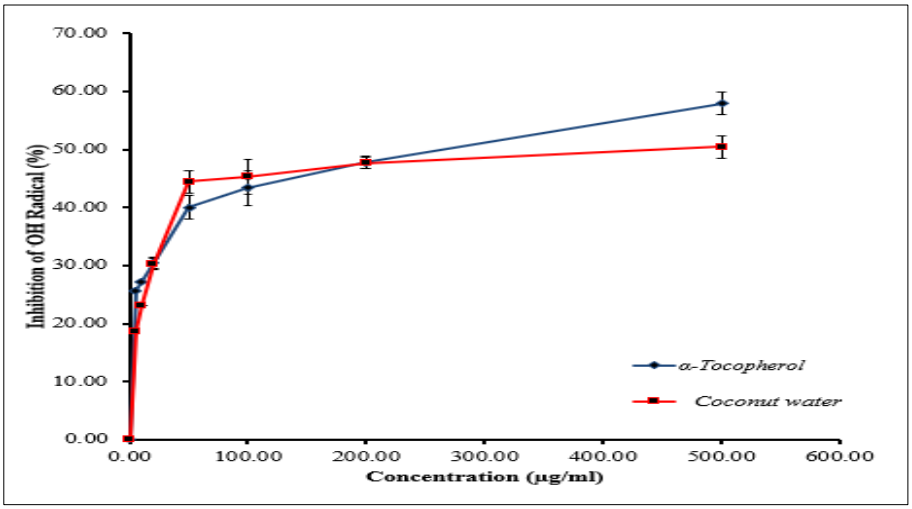

Figure 2 Hydroxyl (·OH) radical inhibitory activity of coconut water aliquots Data represented as mean ± SEM (n = 3)

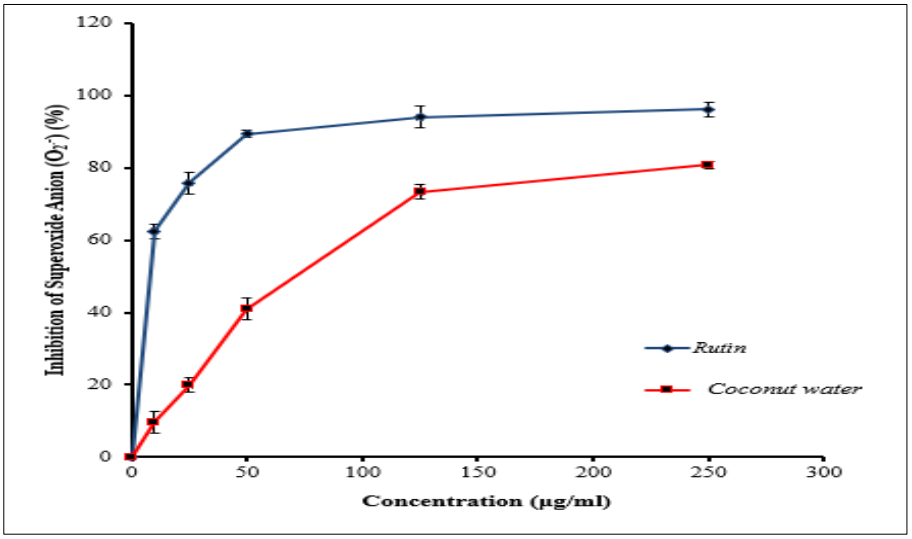

Figure 3 Superoxide anion radical ($O_2^{\cdot-}$) inhibition by Coconut water aliquots Data represented as mean ± SEM (n = 3)

Effect of Coconut water on superoxide ($O_2^{\cdot-}$) anion radical revealed that aliquot of coconut water inhibited the formation of reduced NBT in a dose-related manner. As shown in **Fig. 3,** Coconut water showed the maximal $O_2^{\cdot-}$ anion inhibitory activity of 80.74 ± 1.22 %, at the concentration of 250 µg/ml compared to rutin (95.96 ± 2.2 %, at 250 µg/ml). The $O_2^{\cdot-}$ scavenging effect of the extract could culminate therefore in the prevention of ·OH radical formation since $O_2^{\cdot-}$ and H_2O_2 are required for ·OH radical generation.

Effect of Coconut water aliquots on Fe^{2+}-induced lipid peroxidation is as shown in **Fig. 4**. Coconut water aliquots showed a dose-dependent inhibition. It was most efficient with inhibitory activities of 67.75 ± 1.20 % and at concentration of 1000 µg/ml compared to butylated hydroxytoluene 66.28 ± 1.92.

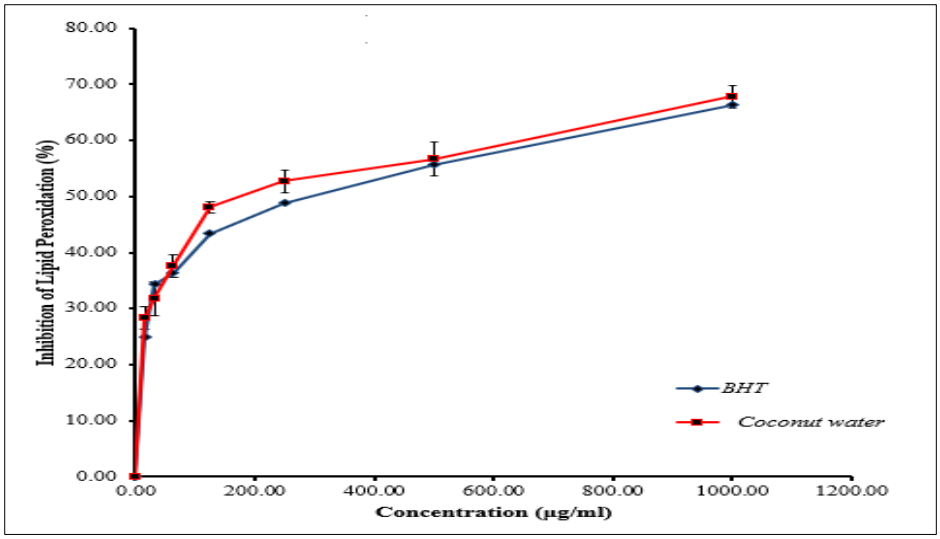

Figure 4 Lipid peroxidation inhibitory potential of Coconut water Data represented as mean ± SEM (n = 3)

The concentration of coconut water aliquots that inhibited 50% of the free radicals and lipid peroxidation (IC_{50}) was used to determine the potency of the extract. The lower the IC_{50} value the better the extract potency. As shown in Table 7 below, coconut water was efficient inhibitors of different free radicals compared to standard anti-oxidants. The IC_{50} values for DPPH radical inhibition was 9.6 ± 10 µg/ml; ·OH radical inhibition was 99.4 ± 1.7 µg/ml; $O_2^{\cdot-}$ anion inhibition was 64.6 ± 1.5 µg/ml; while lipid peroxidation inhibition was ranged from 282.9 ± 9.3 µg/ml **(Table 5).**

Nitric oxide (NO·) released from sodium nitroprusside (SNP) has a strong NO^+ character which can alter the structure and function of many cellular components. The effect of coconut water aliquots on nitric oxide (NO·) radical production showed that the phenol rich aliquots of coconut water in SNP solution decreased levels of nitrite, a stable oxidation product of NO· Liberated from SNP **(Fig. 5)**. The aliquots exhibited strong NO· Radical scavenging activity leading to the reduction of the nitrite concentration in the assay medium, a possible protective effect against oxidative damage. The NO· Scavenging capacity was concentration dependent with 250µg/ml of the extracts scavenging most efficiently compared to α-tocopherol.

Table 5 Free radical and lipid peroxidation inhibitory potency (IC_{50})

Sample	**IC_{50} value for inhibitory potential (µg/ml)**			
	DPPH radical	**Hydroxyl radical (·OH)**	**Superoxide anion ($O_2^{\cdot-}$)**	**Lipid peroxidation**
Coconut water	9.6 ± 1.0	99.4 ± 1.7	64.6 ± 1.5	282.9 ± 9.3
Standard anti-oxidant	4.1 ± 0.3 *	38.9 ± 2.8 #	3.3 ± 0.2 β	24.3 ± 1.4 £

Data represented as mean ± SEM (n = 3), * compared to ascorbic acid, # compared to α-Tocopherol β compared to rutin, £ compared to butylated hydroxyltoluene

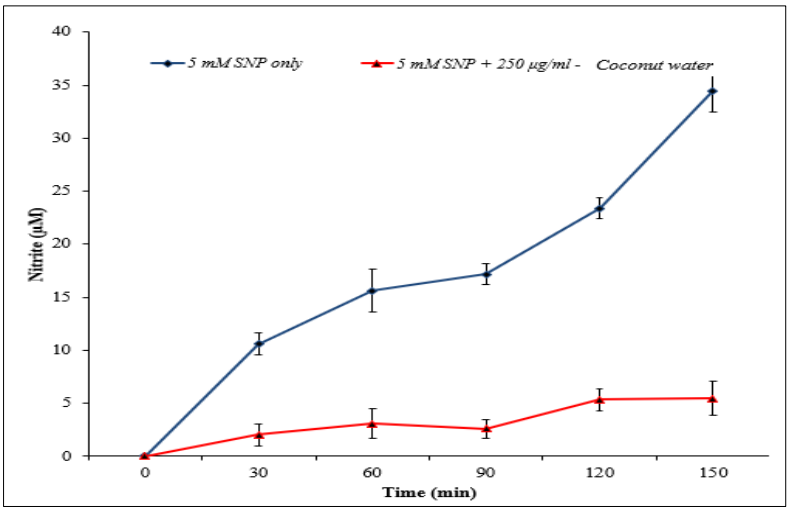

Figure 5 Effect of Coconut water aliquots on the accumulation of nitrite upon decomposition of SNP; (5 mM) at 25°C
Each plot represents the mean ± SEM (n = 3)

4. Discussion

The results of the present study showed that coconut water had a growth inhibitory effect on *Klebsiella* spp, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.* There were no zones of inhibition recorded against all the four test isolates when tested with the negative control; 2.5% of dimethylsofloxide (DMSO). Using ciprofloxacin as the positive control, *Klebsiella* spp exhibited the highest zone of inhibition of 23mm. The least zone of inhibition was exhibited by *Pseudomonas aeruginosa* and *Escherichia coli* at 20mm respectively. *Staphylococcus aureus* was slightly higher at 21mm.

The coconut water exerted significant antimicrobial effect on the test isolates at a concentration of 60mg/ml in our study as compared with previous work done by [28] which observed inhibition at 100% concentration. The inhibition efficacy of coconut water can be attributed to the presence of phytochemicals, such as saponins, cardiac glycosides, and alkaloids. This is in line with the report of [29] that fermented tender coconut water has an antimicrobial effect on *E. coli* and the report of [30] that tender coconut water exhibited an antimicrobial effect on *S. aureus, E. coli, B. cereus, and P. mirabilis.* [31] investigated the antimicrobial activity of coconut water and oil on Gram-positive and Gram-negative bacteria including *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa.* The results showed that coconut water had antibacterial properties.

The quantitative analysis on phytochemical constituents revealed the phenolic compounds found to be the major class of bioactive components in the coconut water. The amount of total phenolics was 0.885 ± 0.008 mg GAE/mg of aliquot of coconut water extract and total flavonoid of 25.44 ± 3.68 rutin equivalents /g dry weight extract of coconut water. Over the years, coconut has been extensively explored for its use in different fields. Results from this study also revealed high phenol and flavonoid contents contributes to inhibitory effect of the coconut water, consistent with a study on the phytochemical analysis of *Cocos nucifera* endosperm by [32], and [33]; conferring its antioxidant ability to significantly lower cellular oxidative stress [34]. Flavonoids also function as signaling molecules andexerting a positive effect on cognitive function [35]. Phenolic compounds derived mostly from plants possess an antioxidant potential to manage oxidative stress-associated diseases like Alzheimer's and other neurodegenerative diseases [36].

The effect of coconut water aliquots on DPPH radicals showed significant dose-dependent DPPH radical scavenging capacity. The hydrogen or electron donation abilities of the compounds were measured from the bleaching of the purple-colored ethanol solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). This spectrophotometer assay uses the stable radical DPPH as a reagent [37]. The scavenging ability and the reducing power of coconut water could be attributed to the total flavonoid and total phenol content. The increase in the scavenging ability and the reducing power of coconut water in a dose-dependent manner could related to the total phenolic concentration [38; 33].

DPPH is a stable nitrogen-centered free radical donor that is stabilized by accepting an electron or hydrogen [39]. The ABTS and DPPH radical scavenging ability of coconut water results revealed that the extract can prevent radical-induced oxidative damage because phenolic compounds possess hydrogen-donating abilities to function as an antioxidant [40]. The coconut water also scavenged ·OH radical in a concentration dependent manner. It inhibited 2-deoxyribose degradation above 30 % with maximal inhibition of 50.36 ± 1.20% at concentration of 500 µg/ml. The scavenging ability of coconut water was significant at all tested concentrations. Given that it may be crucial to their antioxidative effects, the strong radical scavenging activity of the coconut water may be closely connected with their overall phenolic content. The effect of coconut water aliquots on Fe^{2+}-induced lipid peroxidation showed a dose-dependent inhibition. It was most efficient with inhibitory activities of 67.75 ± 1.20 % and at concentration of 1000 µg/ml compared to butylated hydroxytoluene 66.28 ± 1.92. The antioxidants present in the extract can reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}), which forms a blue complex that is absorbed at 700 nm [41]. Accordingly, several reports have established a correlation between the health benefit of polyphenolic-rich food and its antioxidant effects [42]. Additionally, current findings show that phenolic compounds exhibit health-promoting effects ranging from radicals scavenging to metal chelation responsible for lipid peroxidation [43].

The concentration of coconut water aliquots that inhibited 50% of the free radicals and lipid peroxidation (IC_{50}) was used to determine the potency of the extract. The lower the IC_{50} value the better the extract potency. The coconut water was efficient inhibitors of different free radicals compared to standard anti-oxidants. The IC_{50} values for DPPH radical inhibition was 9.6 ± 10 µg/ml; ·OH radical inhibition was 99.4 ± 1.7 µg/ml; O_2^{-} anion inhibition was 64.6 ± 1.5 µg/ml; while lipid peroxidation inhibition was ranged from 282.9 ± 9.3 µg/ml. The amount of antioxidant present in the sample was able to decrease the initial DPPH and since phenolic compouds have been shown to be major contributors of antioxidant activity, their presence may be responsible for the observed antibacterial effect of coconut water.

This study showed that the phenol rich aliquots of coconut water in SNP solution decreased levels of nitrite, a stable oxidation product of NO· liberated from SNP. The aliquots exhibited strong NO· Radical scavenging activity leading to the reduction of the nitrite concentration in the assay medium, a possible protective effect against oxidative damage. The NO· Scavenging capacity was concentration dependent with 250µg/ml of the extracts scavenging most efficiently compared to α-tocopherol.

5. Conclusion

Coconut water was proved to be a valuable antibacterial agent against *Klebsiella* spp, *Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli.* Therefore the antioxidant activities of coconut water is of interest as a potential natural food antioxidant additive, nutraceutical, and requires further evaluation. *C. nucifera* has significant inhibitory action against microbial pathogens indicating the presence of highly effective active compounds, which can be identified and incorporated into modern medical systems for controlling various diseases. Further studies should be done to isolate and characterize bioactive compounds present in coconut water.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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