Research Article

Phytochemical, antimicrobial and antioxidant studies of *Cocos nucifera* (L.) flowers

Chinnasamy Chitra Vadivu[1]*, Mallipalayam Palanisamy Ambika Devi[1], Veluchamy Balakrishnan[2], Thirumalaisamy Sundari[3]

[1]*PG and Research Department of Botany, Vellalar College for Women (Autonomous), Thindal, Erode- 638012, Tamil Nadu, India*

[2]*PG and Research Department of Botany, Arignar Anna Government Arts College, Namakkal –637 002, Tamil Nadu, India*

[3]*Department of Chemistry, K.S.R. College of Engineering, Tiruchengode-637 215, Tamil Nadu, India*

Received: 3 September 2020 Revised: 13 October 2020 Accepted: 30 October 2020

Abstract

Background: Medicinal plant have various phytochemicals and used for the treatment of different kinds of disease. **Objectives:** To determine the phytochemical, antimicrobial activity and antioxidant analysis of *Cocos nucifera* (L.) flowers. **Materials and Methods:** *Cocous nucifera* flowers were shade dried and extracted with solvents ethanol and water then extract screen for antimicrobial and antioxidant activities. Phytochemical screening such as carbohydrates, alkaloids, steroids, phlobatannins, saponins, tannins, terpenoids, quinones, flavonoids, phenols and glycosides. For antimicrobial studies seven bacterial and two fungal clinical isolates selected for the study. DPPH antioxidant studies also performed based on the phytochemicals present in *Cocos nucifera* flowers. **Results and conclusion:** Phytochemical analysis confirmed that ethanol, chloroform and aqueous extracts were showed the presence of carobohydrates, flavonoids, phenols, terpenoids, quinones, sterols and glycosides. The *Cocous nucifera* flower extract tested seven bacterial clinical isolates and two fungal clinical isolates. The 4mg of plant sample showed the maximum zone of inhibition. The DPPH assay showed maximum 5 mg of plant extracts having better activity in ethanol, chloroform and ascorbic acid. The results showed that phytochemical constituents present in *Cocous nucifera* flower extract showed that the potential antimicrobial and antioxidant activities.

***Keywords*:** Antioxidants, clinical isolates, DPPH assay, *Cocous nucifera,* antimicrobial

Introduction

Free radicals contribute to more than one hundred disorders in humans including arthritis, ischemia, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999; Cook and Samman, 1996). Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), tertiary butylated hydroquinone and Gallic acid ester, have been suspected to cause or prompt negative health effects. Hence, tough restrictions have been placed on their utilization and there is a trend to alternate them with naturally occurring antioxidants. Moreover, those synthetic antioxidants also show low solubility and moderate antioxidant activity (Barlow, 1990).

Recently there has been a boom of importance in the therapeutic potential of medicinal plants as antioxidants in contracting such free radicals, induced injury (Pourmorad et al., 2006) besides well known and conventionally used natural antioxidants from teas, wines, fruits, vegetables and spices. Antioxidant estimated in *Gloriosa superba* by several authors (Gopi, 2016; Jothi et al., 2019; Kavitha and Uduman Mohideen, 2018). Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases (Stary and Hans, 1998).

Cocos nucifera (L.) is a significant member of the family Arecaceae (Palm family) popularly known as Coconut, coco-da-bahia, or Coconut-of-the-beach. The plant is basically from Southeast Asia (Malaysia, Indonesia and the Philippines) and the islands between the Indian and Pacific Oceans (Lima et al., 2015). Every part of it is advantageous to mankind for numerous functions including food, drinks, fibers, building materials and chemicals discovering their way into an immense range of modern day products. The coconut maintain a source of meat, milk, oil, fibers,

Address for Corresponding Author:
Dr. Chinnasamy Chitra Vadivu
PG and Research Department of Botany,
Vellalar College for Women (Autonomous), Thindal, Erode- 638012, Tamil Nadu, India
Email: chitravadivuchinnu@gmail.com

DOI: https://doi.org/10.31024/ajpp.2020.6.5.4

vitamins and minerals provides enormous health assistance beyond its nutritional content.

It is used to be antiblenorrhagic, antibronchitis, febrinfugal and antigingivitic, antibacterial activity. In ayurvedic medicine, the milk, oil, cream, water of the coconut is all used to treat hair loss, burns and heart problems (Manisha and Shyamapada, 2011). The present work to study about the phytochemical constituents, antimicrobial and antioxidant activity of *Cocos nucifera* flower extract**.**

Materials and Methods

Collection and identification of plant material

The flowers of *Cocos nucifera was* collected from Mallipalayam, Gobichettipalayam area, Tamil Nadu, India. These flowers were shade dried and powered and then stored in air lock covers or bottles. It is stored for further uses. Medicinally important plant species of *Cocos nucifera* flowers are selected for this study. The botanical identification of the plant samples was carried out by Botanical Survey of India, Coimbatore, India. Certificate No: BSI/SRC/5/23/2018/TECH./563.

Preparation of extracts

The collected plant parts were shade dried to remove the water content from the plants to get dried powder. The dried plant flowers were extracted with solvents like ethanol and water for antimicrobial and antioxidants activities. The powered extract was extracted by taking 5g of sample in 100ml of solvent. The mixture was kept in shaking condition for about 24 to 48 hours by closing it tightly. This is because some of the solvent gets evaporated quickly. Then they were taken and filtered using Whatmann No.1 filter paper. These filtered extracts were dried by pouring it in petridishes and allow them for dry up to one week. The dried plates were than scarped completely using sterile blades. The collected powder was taken and stored in proper containers and then sealed using parafilm.

Test pathogens

The clinical isolates of *E. coli* (Mm306), *P. aeruginosa* (MM502), *S. aureus* (MM1002), *Salmonella* sp (MM104), *Bcillus* sp (MM919), *E. feacalis* (MM611), *K. pneumoniae* (MM218), *A. niger* (MM316), *Fusarium* sp (MM308) were procured from Microtech, Microbiology Laboratory, Coimbatore which were used for the present study. All isolates were inoculated onto Chromogenic agar media for confirmation. After 24 hrs, observed pigment and colony morphology of each isolates. According to color and colony morphology, isolates were confirmed.

Determination antimicrobial activity of plant extract

This test was carried out according to the method of Jahir et al. (2011). The plates were inoculated with freshly prepared over night inoculums which were swabbed over the entire surface of the medium, rotating the plate 60 degrees after each application by using a sterile cotton swab, to ensure the spread of the tested microbes on the surface of the plate completely. Inoculums were 10^8 CFU/ml of bacteria. The 6mm diameter of well was made with borer on the agar plates. Different concentrations of plant extracts were filled in well with the help of micropipette and one well filled with plant extract. The Ampicillin (10µg/ml) was added in one well and added 100µl of extract in another well. Incubate the plate at 37°C for 24hrs, after observed the zone of inhibition. In case of fungal isolates, Itracanazole was added as control and incubated at room temperature for 48 hrs.

Collection of clinical isolates

In this study, 7 bacterial genera and 2 fungal genera were procured from Microtech Microbiology laboratory, Coimbatore. All isolates were inoculated into chromogenic media for confirmation. According to colony morphology isolates were confirmed as *E. coli, K. pneumoniae, E.feacalis, P. aeruginosa, Salmonella sp, Bacillus spp.* and *P. mirabils.* in case of fungal isolates, *A. niger* and *Fusarium* sp were collected and confirmed with colony morphology. In this study, totally 9 isolates were obtained and subjected for this investigation.

Antioxidant activity - DPPH Assay

Aliquot 3.7 ml of absolute methanol in all test tubes along with blank was taken. Then, added 100µl of absolute methanol to blank.100 µl of Ascorbic acid to tube marked as standard and 100 µl of respective samples to all other tubes marked as tests. Finally, added 200µl of DPPH reagent to all the test tubes including blank. Then, all test tubes were incubated at room temperature and dark condition for minimum of 30 minutes of duration. The absorbance of all samples was taken in Spectrophotometer at 517nm (Naznin and Hasan, 2009).

Results

The results of phytochemicals screening of *Cocos nucifera* flowers showed the presence of various phytochemicals (Table 1). Three solvents such as ethanol, chloroform and Aqueous showed positive results for the presence of flavonoids, phenols, tannins and carbohydrate, sterols and terpinoids. In addition, the ethanol extract showed the presence of saponins and quinones. The alkaloids observed only in chloroform extract. The proteins are absent in all of the extracts and ethanol extract had highest number of phytochemicals and followed by chloroform extract.

Collection of clinical isolates

According to colony morphology isolates were confirmed as *E. coli, K. pneumoniae, E. feacalis, P. aeruginosa, Salmonella* sp, *Bacillus* spp. and *P. mirabils.* in case of

Table 1. Preliminary phytochemicals analysis of *Cocos nucifera flowers*

S. No.	Phytochemicals test	Chloroform Extract	Ethanol Extract	Water extract
1.	Alkaloids	+	-	-
2.	Carbohydrate	+	+	+
3.	Flavonoids	+	+	+
4.	Phenols	+	+	+
5.	Saponins	-	+	-
6.	Tannin	+	-	+
7.	Terpenoids	+	+	+
8.	Quinones	+	+	+
9.	Sterols	+	+	+
10.	Proteins	-	-	-
11.	Glycosides	+	+	+

+, Present; - Absent

Table 2. Morphological characterization of bacterial isolates

S. No.	Name of the Microorganisms	Morphology/Colour
1.	*E. coli*	Pink colony chromogenic media
2.	*Pseudomonas aeruginosa*	Colour less colony chromogenic media
3.	*Enterococcus faecalis*	Small blue colony chromogenic media
4.	*Klebsiella pneumonia*	Blue colony with mucoid chromogenic media
5.	*Bacillus*	milky, large, convex, opaque, dry colonies
6.	*Salmonella* sp.	Black colour on SS agar
7.	*S. aureus*	Yellow colour on chromogenic media
8.	*A. niger*	Black colony on SDA media
9.	*Fusarium* sp.	Pale colored; whitish to yellow

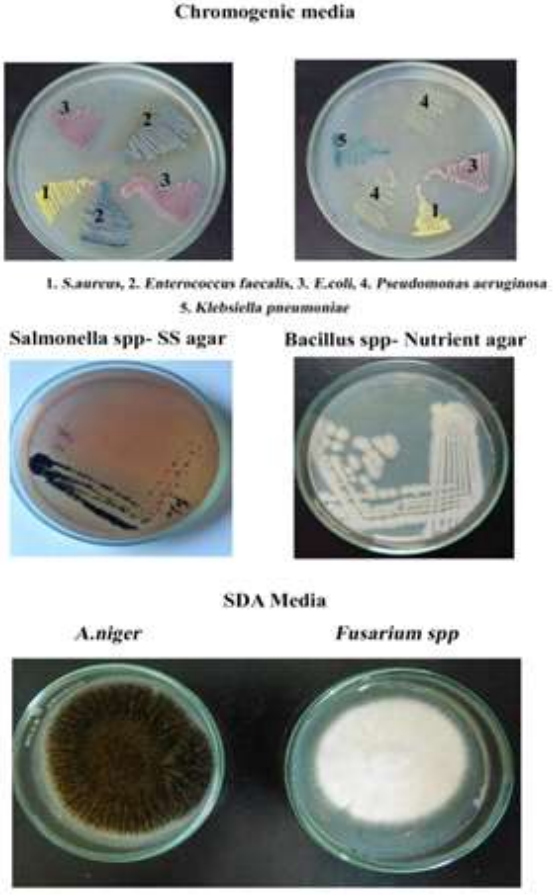

Figure 1. Confirmation of bacterial and fungal isolates

fungal isolates, *A. niger* and *Fusarium* sp were collected and confirmed with colony morphology. The results were tabulated in table 2 and figure 1. In this study, totally 9 isolates were obtained and subjected for this investigation.

Antibacterial and antifungal activities of ethanol extract of *Cocos nucifera* L.

According to results of phytochemicals analysis, highest phytocompounds containing ethanol and chloroform extracts were selected for the antimicrobial activity. In the present study, 7 bacterial genera were subjected to antimicrobial activity test with both solvents extracts. In case of ethanol extract, highly suppressed to *S. aureus* and followed by *Bacillus* spp, *E. coli*. Among the 7 genera, *Salmonella* was highly resistance to ethanol extract. The zone of inhibition was ranged from 10mm to 22mm. In this study, most of the isolates were suppressed while using 3mg concentration of extract (Table 3 and Figure 2). In case of fungal genera, *A. niger* was highly suppressed, it exhibiting zone of inhibition was 14 mm to 19 mm, and zone of inhibition was stated with 1mg concentration of extract. The *Fusarium* sp. was not suppressed with ethanol extract (Table 4; Figure 3).

Antibacterial and antifungal activities of chloroform extract of *Cocos nucifera* L.

In this study, chloroform extract of *Cocos nucifera* also utilized for the antimicrobial activity. The zone of inhibition

Table 3. Antibacterial activity of ethanol extract of *Cocos nucifera* Linn.

S. No.	Name of the Microorganisms	Different Concentration of plant extract (mg) (Zone of inhibition in mm)					
		1	2	3	4	5	6
1.	*E. coli*	14	19	17	19	-	-
2.	*K. pneumoniae*	-	-	14	17	-	-
3.	*P. aeruginosa*	-	-	14	15	-	-
4.	*Salmonella* sp.	-	-	-	-	-	-
5.	*S. aureus*	10	14	19	22	-	-
6.	*E. faecalis*	-	10	15	17	-	-
7.	*Bacillus* sp.	15	17	19	21	-	-

Table 4. Antifungal activity of ethanol extract of *Cocos nucifera* L.

S. No.	Name of the Microorganisms	Different Concentration of plant extract (mg) (Zone of inhibition in mm)					
		1	2	3	4	5	6
1.	*A. niger*	10	12	14	16	-	-
2.	*Fusarium* sp.	-	-	-	-	-	-

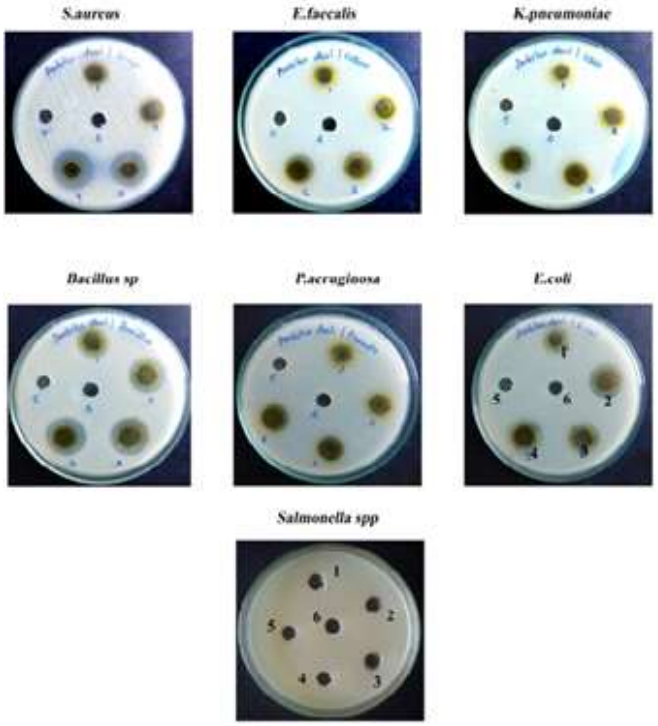

Figure 2. Antibacterial activity of ethanol extract of *Cocos nucifera* L.

Table 5. Antibacterial activity of chloroform extract of *Cocos nucifera* L.

S. No.	Name of the Microorganisms	Different Concentration of plant extract (mg) (Zone of inhibition in mm)					
		1	2	3	4	5	6
1.	*E. coli*	12	13	15	17	-	-
2.	*K. pneumoniae*	-	-	12	14	-	-
3.	*P. aeruginosa*	-	-	-	12	-	-
4.	*Salmonella* sp.	-	-	-	12	-	-
5.	*S. aureus*	-	10	11	14	-	-
6.	*E. faecalis*	-	10	13	14	-	-
7.	*Bacillus* sp.	-	10	11	14	-	-

was ranged from 10mm to 17mm. among the 7 bacterial genera; *E. coli* was highly suppressed and followed by *E. faecalis*. The *Salmonella* spp. was not suppressed with chloroform extract. In this study, 5 isolates were suppressed while using 3mg of concentration and all isolates were suppressed while using 4mg concentration of plant extract (Table 5 and Figure 4). In case of fungal genera, both isolates were minimal suppressed with chloroform extract (Table 6 and Figure 5). In this investigation, relevant solvents and standard antibiotic of ampicillin were used for control. These were not produced the zone of inhibition against bacterial and fungal genera.

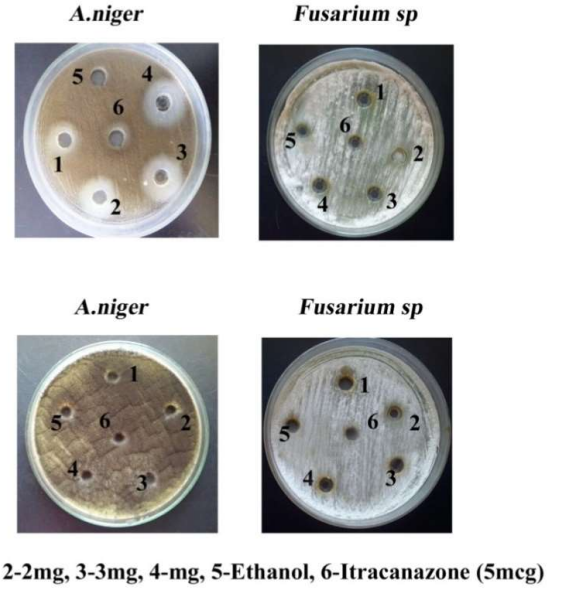

Figure 3. Antifungal activity of ethanol extract of *Cocos nucifera* L.

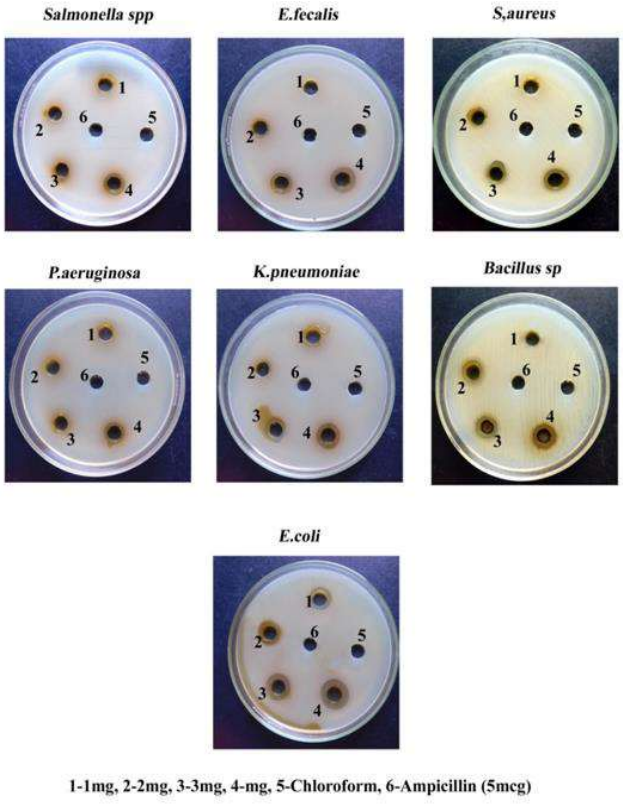

Figure 4. Antibacterial activity of chloroform extract of *Cocos nucifera* L.

Antioxidant activity of ethanol extract of *Cocos nucifera* Linn.

According to phytochemicals constituents, ethanol and chloroform extracts was utilized for the antioxidant activity through DPPH free radical scavenging assay. The antioxidant capacities of the extracts were compared with

Table 6. Antifungal activity of chloroform extract of *Cocos nucifera* L.

S. No.	Name of the Microorganisms	Different Concentration of plant extract (mg) (Zone of inhibition in mm)					
		1	2	3	4	5	6
1.	*A. niger*	-	-	-	10	-	-
2.	*Fusarium* sp.	-	-	-	-	11	-

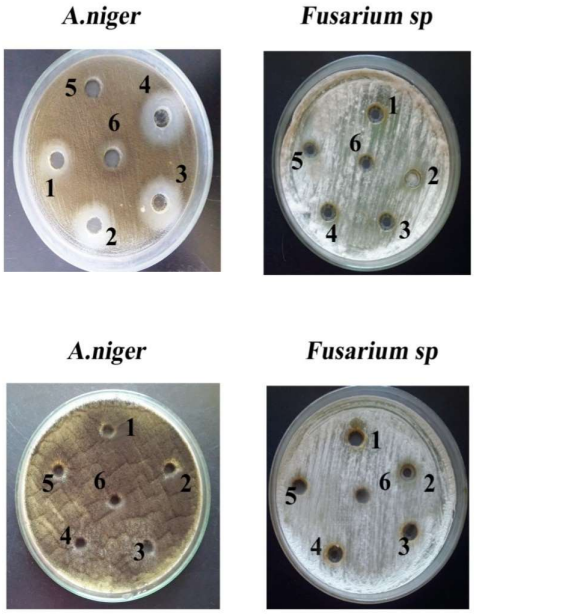

Figure 5. Antifungal activity of chloroform extract of *Cocos nucifera* L.: (1) 1mg; (2) 2mg; (3) 3mg; (4) 4mg; (5) Chloroform; (6) Itracanazole (5mcg)

ascorbic acid as standard antioxidant. The graphical representation shows the increase in DPPH activity with response to the increase in drug concentration (Fig 6). Ethanol extract of *Cocos nucifera* exhibited a greater antioxidant effect at 5mg/ml (78.66%) compared with the effect of ascorbic acid at the same concentration (89.48%). In case of chloroform extract, highest scavenging rate was occurred on 5mg with 71.58%. The IC_{50} value of ethanol, chloroform and ascorbic acid was found to

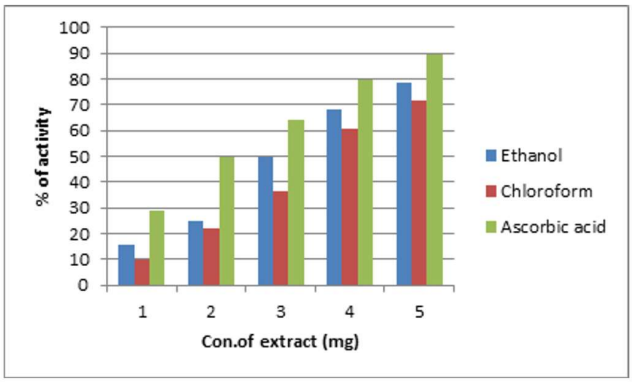

Figure 6. Percentage of antioxidant activity of *Cocos nucifera* L. extract

be 3mg/ml, 3.6mg and 2mg/ml respectively (Table 7).

Discussion

In this study, ethanol and chloroform extracts were used for the antimicrobial activity. Among them, ethanol showed better activity then chloroform extract. In case of ethanol extract, highly suppressed to *S. aureus* and followed by *Bacillus* sp, *E. coli*. Among the 7 genera, *Salmonella* was highly resistance to ethanol extract. Similarly Cristiane et al. (2016) were determined the antibacterial activity of flowers of *Cocos nucifera* against *S. aureus* and *P. aeruginosa*. In case of chloroform extract, *E. coli* was highly suppressed and followed by *E. faecalis*. The antimicrobial activity of ethanol extract of *Cocos nucifera* against bacterial isolates. In *Allium* species antimicrobial studies carried out (Semerci et al., 2020).

Several investigations on medicinal plants indicate that organic solvents such as methanol are extensively used for crude extraction before being re-extracted to obtain purified active compounds using some other organic solvents. Present study also agreed with previous studies, which was inhibited by most of the isolates. The present study was first investigation, in our literature knowledge, this is the first study, and no one inhibited the biofilm and betalactamase and MDR isolates with *Cocos nucifera*.

Table 7. Antioxidant activity of *Cocos nucifera* L.

S. No.	Concentration (mg/ml)	DPPH free radical scavenging effect (%)		
		Ethanol extract	Chloroform extract	Ascorbic acid
1.	1	15.62	10.20	29.23
2.	2	25.14	22.13	49.79
3.	3	49.50	36.44	64.35
4.	4	68.12	60.42	79.91
5.	5	78.66	71.58	89.48

It has observed that the natural compounds in higher plants have antioxidant activity. Several mechanisms have been proposed to be involved in the antioxidant activity such as hydrogen donation, termination of free radical mediated chain reaction, prevention of hydrogen abstraction, chelation of catalytic ions, and elimination of peroxides (Gordon, 1990). The unsettle to the complex reactive nature of phytochemicals, the antioxidant activities of plant extracts can be evaluated by DPPH method, authenticity (Schlesier et al., 2002; Chanda and Dave, 2009). Therefore in the present study, the antioxidant activities of *Cocos nucifera* extract were analyzed by DPPH free radical scavenging activity. Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants have scavenging activity and it's significantly contributed for the treatment of diseases. DPPH stable free radical method and which is useful for the determination of antioxidants in plants (Koleva et al., 2002; Suresh et al., 2010).

In this study, two solvents of plant extracts were utilized for the antioxidant activity, among them, ethanol showed better activity than other extracts. The IC_{50} value of ethanol, chloroform and ascorbic acid was found to be 3mg/ml, 3.6mg and 2mg/ml respectively. This activity result was resembled to ascorbic acid activity. Similarly, *Cocos nucifera* contain antioxidants having a different chemical nature from ç-Sitosterol, Stigmast, quercetin content was found to be high in *Cocos nucifera* flowers, and capable to confer a high antioxidant power to this species (Singla et al., 2011). Since DPPH assay is easily reproducible and linearly related to molar concentration of the antioxidants present, thus it can be reported that quercetin may act as a free radical scavenger, capable of transforming reactive free radical species into stable non radical products (Zhang et al., 2011: Jagtap and Satpute , 2014) .

Esquenazi et al. (2002) made an *in vitro* study in *C. nucifera* and have stated that it has antimicrobial activity. They tested the organisms like *Salmonella* sp*, Escherichia coli, Enterococcus faecalis, Streptococcus faecalis, Klebsiella pneumoniae, Proteus vulgaris, Serratia marscens, Staphylocus epidermidis, Micrococcus luteus, Vibrio vulnificus, V. fluvialis, Vibrio* sp*, S. aureus, P. aeruginosa* and *V. parahaemolyticus*. He also demonstrated that human herpes simplex virus is susceptible to the crude extract that was resistant to acyclovir.

Naznin and Hasan (2009) studied the antioxidant activity of Coconut shell extract. It varied from 92.32% to 94.20%. They also found the presence of phenolic content at the range of 5.33 ± 0.02mg/g. the extract was more effective in human fungal pathogens like *M. canis, M. gypseum, A. fumigates, A. niger, A. flavus, T. rubrum, , T. mentagrophyte* and *T. vercossum*. Most of the dermal mycosis was susceptible for the crude shell extract. The MIC results ranged from 62 to 90mm.

Conclusion

The antimicrobial activity study revealed that ethanol extract was highly suppressed to the *S. aureus, Bacillus* and *E. coli* but *Salmonella* sp. was resistant. In case of fungi, minimal inhibition zone was observed from *A. niger* and *Fusarium* .In case of Chloroform extract, this was highly effective against *E. coli* followed by *E. faecalis*. The *Salmonela* sp. and the fungal isolates of *A. niger* and *Fusarium* were lesser zone inhibition than *E. coli* and *E. faecalis*. Ethanol extract of *Cocos nucifera* exhibited a greater antioxidant effect and highest scavenging rate was occurred in chloroform extract.

Acknowledgement

The authors are thankful to the Management, Principal and Head of the Department of Botany, Vellalar College for Women *(Autonomous)* Thindal, Erode for providing necessary laboratory facilities to carry out the work successfully.

Conflicts of interest

We declare that we have no conflicts of interests

Ethical clearance

Not necessary for this work.

Source of funding

This work did not received and fund from any funding agency

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