INT. J. BIOL. BIOTECH., 16 (3): 775-782, 2019.

SCREENING OF *COCOS NUCIFERA* L. AS A SOLUTION TO ANTIMICROBIAL RESISTANCE

Syeda Hira Bukhari[1], Suraiya Jabeen[1*], Nimra Ayub[1], Umme Laila Awan[1], Sundus Fatima[1] and Shagufta Ishteyaque[2]

[1] *Institute of Environmental Studies, University of Karachi, Karachi-75270, Pakistan.*
[2] *Department of Chemical Engineering, University of Karachi, Karachi-75270, Pakistan.*

*Corresponding author: Tel: +923002268537; Email: sujabeen@uok.edu.pk

ABSTRACT

Cocos nucifera L. (family Arecaceae) is known to be helpful in treating physiological disorders. Thin layer chromatography, qualitative phytochemical test and disc diffusion method were used to evaluate the antimicrobial activity of *Cocos nucifera* (L.) extract against *Shigella dysenteriae, Bacillus subtilis, Escherichia coli, Shigella sonnei, Pseudomonas aeruginosa* and *Staphylococcus aureus.* TLC analysis of coarse coconut shell extract showed that fine extract of shell sample found to be more effective for extraction of phytochemicals. Coconut shell is rich in phytochemical (alkaloid, reducing sugar, saponins, tannins, flavonoid, terpenoids, resins and sterol) while phenols quinones and proteins are absent in coconut shell. Reducing sugar, tannins, flavonoid, terpenoid, resins, sterols, phenol and quinones are major phytochemical constituent of coconut leaves while alkaloid, protein, and saponins were absent. Coconut shell extracts showed significant activity against *P. aeruginosa, B. subtilis, E. coli, S. sonnei,* and *S. dysenteriae*.

Key-words: Natural remedies, Phytochemicals. Antimicrobial activity, *Cocos nucifera* L.

INTRODUCTION

Natural products have been used since long as traditional therapeutic agents by medicinal practitioners. These products are the source of synthetic and local herbal remedies (Bhandary *et al.*, 2012). Wide variety of antibiotics and their excessive and immediate use makes infectious species enable to acquire resistance. Resistance of human pathogens has emerged the need of new antimicrobial substances from natural sources (Erdogrul, 2002) which are as effective as antibiotics. World Health Organization reported (WHO, 2004) that for primary healthcare needs, 80% of the world's population relies on traditional medicament. Plants contain several chemical constituents which makes them active against number of infectious diseases (Bhandary *et al.*, 2012; Edeoga *et al.*, 2005).

Cocos nucifera L. belongs to palm family Arecaceae is abundantly found in tropical areas (Shettigar *et al.*, 2014), cultivated in 90 countries which are situated at tropical belt (Solangi and Zafar, 2011). Original habitat of this plant is South East Asia (Philippines, Indonesia, Malaysia, and the islands of Indian and Pacific Oceans) (Elevitch, 2006). In Pakistan it is scattered in the areas near the Sea and cultivated in coastal areas like Karachi (Solangi and Zafar, 2011).

Cocos nucifera and its products are known to have antibacterial, antioxidant, antifungal, antiviral, anti-inflammatory activities. It is also helpful in treating physiological disorders due to the presence of compounds like alkaloids, flavonoids, phenolic compounds and tannins which help as antidepressant, anti-parasitic, anti-diabetic, renal protective, cardioprotective, antimalarial, hepatoprotective, anti-leishmanial anti-hypersensitivity, antineoplastic and analgesic, (Lima *et al.*, 2015) Shell and husk fiber, root, solid albumen and water of coconut plant are used to cure diarrhea, amenorrhea, asthma, kidney inflammation, diuretics, gonorrhea and venereal treatment, relief to rashes caused by HIV-AIDS infections in different parts of the world (Calzada *et al.*, 2007; Hirschhorn, 1983). Each part of coconut plant has active components, leaves have lupeol acetate (Escalante-Erosa *et al.*, 2009; Riedel *et al.*, 2009), oil has a-tocopherol and lauric acid, root has saponin, fiber has catechin, and coconut fiber inflorescence has tannins. So, it can be used in human diet for improving immunity, decreases liver damages and stabilizes the body lipids. It is reported that coconut decreases the viral load of HIV (Shettigar *et al.*, 2014). The aim of the study was to investigate the major phytochemicals of coconut plant and antimicrobial properties of shell, leaves and coconut oil.

MATERIALS AND METHODS

Plant collection and Preparation of extract

Shells of mature Coconut plant were collected from local market of Karachi, washed, dried, cut in small pieces and then grind to get in powdered form. Leaves of plant were collected from Institute of Environmental Studies University of Karachi's (UoK) lawn, washed and dried to make powder. Pre-weighted plant materials were then soaked in absolute methanol for a week and then filtered to make crude extract. Methanol was evaporated at room temperature. Residues were mixed in a small quantity of methanol again to make a high concentration dose.

Coconut Oil Preparation

Oil preparation was done through thermal process (Hot extraction Virgin Coconut oil HVCO) (Narayanankutty *et al.*, 2018)**.** For the extraction of oil, mature *Cocos nucifera* meat was shredded and dipped in water for an hour. After squeezing and filtering, coconut milk was placed in a container for overnight. Two layers of cream and skim were formed, cream layer rich in oil is separated and heated until oil was found.

Bacterial strains and Inoculum preparation

Six bacterial strains two Gram positive (*B. subtilis,* and *S. aureus*) and four pathogenic Gram negative (*E. coli, P. aeruginosa, S. sonnei, S. dysenteriae)* were used. Preserved cultures of mentioned strains were collected from Institute of Environmental Studies (IES), University of Karachi for inoculum preparation. A loopful of preserved cultures was transferred in a nutrient broth tubes and incubated at 37 °C for 24 hr. to activate them.

Antimicrobial Susceptibility test

To check antimicrobial activity of *Cocos nucifera* extract, disk diffusion method was performed (Bauer *et al*., 1966). Lawn of microbial strains were made on nutrient agar plate with the help of sterile swab. Sterilized disc loaded with respective extract were placed on nutrient agar plate and incubated aerobically at 37 °C. Zone of inhibition were measured in millimeters (mm) including 6 mm of disc diameter after 24 hr. to check antimicrobial susceptibility

Thin layer chromatography (TLC)

Diversity of compounds present in *Cocos nucifera* extracts were estimated through TLC. A glass TLC plate coated with silica gel were used for separation technique. All the chemicals and reagent used in analysis were laboratory grade and purchased from Merck suppliers. Solvent system was comprises of Hexane (H), petroleum ether (PE), ethanol (E), methanol (M), water (W) and acetone (A) with different combinations H : PE (4 : 6) E : M (3 : 7) E : M (4 : 6) E : M (7 : 3) E : M : W (4 : 3 : 3) made for coconut shell coarse and fine extract and H : A (6 : 4) H : A (7 : 3) H : A (8 : 2) H : A (9 : 1) for coconut leaves extract then extracts were allowed to run on a TLC plate in a pre-saturated chamber. Plates were developed through iodine crystals and number of bands/spots and solvent front were noted for Retention factor calculation (Hamburger and Cordell, 1987). Rf were calculated by following formula.

$$RF = Zx / (Zf - Zo)$$

Where Zx is distance covered by sample, Zf is distance traveled by solvent front and Zo is he distance from the sample origin to the position used as the origin for the mobile phase (Poole, 2003).

Extract preparation and Phytochemical analysis

Three extracts (alcoholic, aqueous and acidic) were prepared by adding 5 g of powdered sample in a 50 mL of solvents (ethanol, water and Conc. HCl, respectively) and allowed to stand for 30 minutes then filtered. Only aqueous extract was boiled before filtration. These extracts were used for phytochemical analysis. Analysis was carried out to check the presence or absence of alkaloid, protein, reducing sugar, saponins, tannins, flavonoid, terpenoid, resins, sterols, phenols and quinones with the help of standard procedure for phytochemical analysis described by Hamburger and Cordell (Harborne, 1973; Rosenthaler, 1930) (Table 1).

Statistical Analysis

Statistical analysis was done through IBM SPSS 25.0. Data on antimicrobial activity of coconut extracts was subjected to one-way ANOVA at 95% confidence limit. *p-value of* $p < 0.05$ consider as significant.

Table 1. Qualitative Phytochemical Tests of *Cocos nucifera* extract.

Compound	Test	Extract	Observation	References
Alkaloid	Wagner's test	Acidic	Brown ppt	(Parekh *et al*., 2006)
Protein	Xanthoproteic test	Aqueous	Yellow ppt	(Tiwari *et al*., 2011)
Saponins	Foam test	Aqueous	Foam produced	(Parekh and Chanda, 2007)
Tannins	Ferric chloride test	Alcoholic	Blue-green color	(Kumar *et al*., 2007)
Flavonoid	Alkaline regent test	Aqueous	Yellow color	(Onwukaeme *et al*., 2007)
Terpenoid	Salkowski's test	Acidic	Reddish-brown color	(Edeoga *et al*., 2005)
Resins	Extract + d/w	Alcoholic	Turbidity	(Borkar *et al*., 2016)
Steroids	Salkowski's test	Alcoholic	Red ring form	(Onwukaeme *et al*., 2007)
Phenols	Ferric chloride test	Acidic	Blue-black color	(Tiwari *et al*., 2011)
Reducing sugar	Fehling's test	Acidic	Red ppt on heating	(Akinyemi *et al*., 2005)

RESULTS

Thin Layer Chromatography

The TLC analysis of coarse coconut shell extract shows multiple bands. Hexane: Petroleum ether gives two band with Rf values 0.31 and 0.69. Ether: Methanol with different ratios 3: 7, 4: 6 and 7: 3 gives three, two and four, respectively. Two bands were spotted when samples were treated with Ether: Methanol: Water. Fine extract of same shell sample when treated with same eluent system gives more bands then coarse extract. H : PE (4 : 6) E : M (7 : 3) E : M (3 : 7) E : M (4 : 6) E : M : W (4 : 3 : 3) gives three, four, four, three and one bands respectively. The Rf values of sample are shown in Table 2.

Cocos nucifera leaves extract when run with Hexane and acetone with different ratios 6 : 4, 7 : 3 and 8 : 2 gives three bands having Rf values 0.88, 0.16, 0.33 for first ratio, Rf 0.21, 0.23, 0.40 for second ratio and Rf 0.10, 0.33, 0.50 for third ratio.

Qualitative Phytochemical analysis

Phytochemical analysis of Coconut shell and leaves includes in Table 3. The results show that coconut shell is rich in Phytochemicals. Since to date no complete study was conducted for phytochemical screening of coconut plant to compare results however presence and absence of flavonoid, reducing sugar, tannins, quinones alkaloid and saponin in coconut leaves were also confirmed from (Manalo *et al*., 2017). Phenols were absent in our shell extract, but studies show that phenol is a major constituent of coconut shell. Due to the hard structure, extraction of phenolic compounds are time and temperature dependent (Rodrigues and Pinto, 2007) may be this is the reason phenols were not found in shell extract however it is present in leaves extract.

Table 2. Rf (Retention factor) values of numbers of *Cocos nucifera* L. shell and leaves bands by TLC method.

Sample	Solvent system	No. of bands	Rf No.1	Rf No.2	Rf No.3	Rf No.4
C.S (coarse)	H : PE (4 : 6)	2	0.31	0.69		
	E : M (3 : 7)	3	0.28	0.52	0.64	
	E : M (4 : 6)	2	0.45	0.68		
	E : M (7 : 3)	4	0.20	0.33	0.65	0.97
	E : M : W (4 : 3 : 3)	2	0.23	0.27		
C.S (fine)	H : PE (4 : 6)	3	0.29	0.44	0.76	
	E : M (7 : 3)	4	0.15	0.32	0.52	0.92
	E : M (3 : 7)	4	0.15	0.28	0.51	0.85
	E : M (4 : 6)	3	0.32	0.44	0.68	
	E : M : W (4 : 3 : 3)	1	0.30			
C.L	H : A (6 : 4)	3	0.09	0.16	0.33	
	H : A (7 : 3)	3	0.21	0.23	0.40	
	H : A (8 : 2)	3	0.10	0.33	0.50	

C.S (c): Coconut Shell (coarse); C.S (f): Coconut Shell (fine); C.L: Coconut Leaves

Table 3. Qualitative analysis of *Cocos nucifera* L. leaves and shell extract.

	Shell	Leaves		Shell	Leaves
Alkaloids	+	-	Terpenoids	+	+
Proteins	-	-	Resins	+	+
Reducing sugars	+	+	Sterols	+	+
Saponins	+	-	Phenols	-	+
Tannins	+	+	Quinones	-	+
Flavonoids	+	+			

Antimicrobial activity of *Cocos nucifera* L.

Using Disc diffusion method six microbial strain were investigated against coconut plant extracts. According to ANOVA table the inhibition property of all extract of Coconut plant is significantly different (Table 4). The higher F-value of coconut oil, coconut shell fine and coconut shell Coarse extract interpreting the extracts are significantly different at desired significance level. Shell extracts (fine and coarse) were found more effective against all strain as compare to other ones while coconut oil only suppresses the growth of single microbial strain. Coarse and fine extract inhibit the growth of *S. dysenteriae* with zone of inhibition 14.5mm and 11.3mm respectively. Coconut oil and coconut leaves do not have significant inhibition against *S. dysenteriae.* Coconut shell extracts also have notable activity against *B. subtilis.* Coconut leave extract has insignificant while coconut oil does not have efficiency against *B. subtilis.*

E. coli the pollution indicator and the most resistant one is inhibited when treated with coconut shell extracts. Significant zone of inhibition 13.6mm and 17mm measured against coconut shell coarse and fine extract respectively. Zone of inhibition noted against *S. sonnei (*coconut shell coarse and fine extract) 17.6mm and 11.6mm and *P. aeruginosa* 11mm and 16.6mm shown in (Table 5) supported the significance of antimicrobial activity of coconut shell. Similar results of Coconut shell extracts against *E.coli* and *S. aureus* were also reported in previous studies (Sinsinwar *et al.*, 2018). Coconut leaves do not have any significant effectiveness against any microbial strain however a very little zone of inhibition shown against 2 Gram negative *(S. sonnei and P. aeruginosa)* and Gram positive *(S. aureus). S. aureus* the most common flora of human skin and one of the most common pathogenic specie were significantly inhibited by coconut oil only. Pure coconut oil shows remarkable effect on *S. aureus* with 16.3mm zone of inhibition (Table 5). Out of six *S. aureus* was found the most struggling specie.

Table 4. ANOVA of Zone of Inhibition at significance level $p < 0.05$.

Extracts		SS	df	MS	F	Sig.
Coconut leaves	Between Groups	33.778	5	6.756	4.677	0.013
	Within Groups	17.333	12	1.444		
	Total	51.111	17			
Coconut oil	Between Groups	232.667	5	46.533	49.271	<0.001
	Within Groups	11.333	12	.944		
	Total	244.000	17			
Coconut Shell Coarse	Between Groups	171.778	5	34.356	24.736	<0.001
	Within Groups	16.667	12	1.389		
	Total	188.444	17			
Coconut Shell fine	Between Groups	153.111	5	30.622	32.424	. <0.001
	Within Groups	11.333	12	.944		
	Total	164.444	17			

SS: sum of square; Df: degree of freedom; MS: mean squares

Table 5. Antimicrobial activity of *Cocos nucifera* extract against six microbial strains.

	Zone of inhibition (mm) n = 3 ± SE				
Extracts	C.L	C.O	C.S (c)	C.S (f)	Control
S.dysenteriae	6.6 ± 0.3	7.3 ± 0.6	14.5 ± 0.6	11.3 ± 0.3	6 ± 0.0
B.subtilis	7.3 ± 0.3	7.0 ± 0.5	10.6 ± 0.8	15.3 ± 0.6	6 ± 0.0
E.coli	9.0 ± 0.0	6.6 ± 0.3	13.6 ± 0.6	17.0 ± 0.5	6 ± 0.0
S.sonnei	9.3 ± 0.6	6.3 ± 0.3	17.6 ± 0.4	11.6 ± 0.8	6 ± 0.0
P.aeruginosa	10.6 ± 1.2	6.3 ± 0.4	11.0 ± 0.5	16.6 ± 0.3	6 ± 0.0
S.aureus	9.6 ± 0.8	16.3 ± 0.8	8.0 ± 0.5	9.3 ± 0.3	6 ± 0.0

C.L: Coconut Leaves; C.O: Coconut Oil; C.S (c): Coconut Shell (coarse); C.S (f): Coconut Shell (fine)

DISCUSSION

TLC of *Cocos nucifera* L. plant extract

Currently TLC technique is being used frequently for quantification of phytochemicals because it is convenient, basic, accurate and cost-effective practice (Sasidharan *et al.*, 2011). Therefor to check the diversity of phytochemicals in alcoholic extract of *Cocos nucifera,* TLC technique is preferred. TLC analysis of extracts shows that *Cocos nucifera* plant has numerous phytochemicals that's the way multiple bands were visualizes when treated through iodine chamber. Spots or bands on plate indicate the presence of natural phytochemicals in sample (Jagessar, 2017). Fine sample of Coconut shell has more bands then course coconut shell sample indicating extraction is maximum when the shell was crushed finely, and surface area increases. TLC of coconut leaves with different solvent system gives band but not as maximum as coconut shell. However, qualitative analysis of leaves shows positive results for different phytochemicals. For the identification of phytochemicals present in coconut leaves and shell qualitative phytochemical analysis were performed.

Qualitative Phytochemical analysis

Phytochemical compounds are responsible for various therapeutic, antimicrobial and antifungal activity as well as ailment treatment. *Cocos nucifera* extracts were subjected to selective phytochemical analysis (Table 3). Manalo *et al*. (2017) reported the effective activity of coconut leaves extract against Alzheimer disease. Tannins, flavonoid and phenols are important bioactive compound also present in *Cocos nucifera (*Levy, 1994). Flavonoid present in extracts also known for its chelating and antioxidant abilities (Heim *et al.*, 2002; Korkina and Afanas' Ev, 1996). Studies also proved the anti-inflammatory, antimycotic, antiviral, antibacterial, antioxidant, enzyme inhibitor, immune modulator and mutagenic properties of flavonoid (Havsteen, 2002). Terpenoids are also under study for antineoplastic, antibacterial, and other therapeutic activities (Yamunadevi *et al.*, 2011) present in both samples. Tannins found in coconut shell and leaves are effective against antimicrobial activity. It is also capable to antiviral, antifungal (Lima *et al.*, 2015) and anti-helminthic activity (Hoste *et al.*, 2006). Saponins present in coconut shell extract are known for cardio-depressant and hypotensive abilities (Olaleye, 2007).

Another product of Coconut, coconut haustorium is rich in antioxidants, dietary fibre, phenolics and iron (Manivannan *et al.*, 2018). Evidence suggests that diets rich in phenolic compounds can significantly enhance human health because of the effects of phenolic antioxidants (Naczk and Shahidi, 2004). Plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Many parts of South Indian regions use coconut oil for cooking purpose (Narayanankutty *et al.*, 2018)

Antimicrobial activity of *Cocos nucifera* L.

Phytochemical analysis and disc diffusion method (Table 5) confirms the *Cocos nucifera* effectiveness for physiological disorders as well as for infections treatment. Efficacy of plant products against multiple microbes could heal the infection which are difficult to treat through synthetic drugs. Researchers try their best to investigate the plant material with significant antimicrobial and anti-viral activity (Hoffmann *et al.*, 1993; Kirszberg *et al.*, 2003) because use of natural products especially derived from plants as an alternative of therapies, is a growing interest now-a-day (Rates, 2001). Though, they required in high concentrations to get desirable microbial inhibition (Samant *et al.*, 2015).

Due to the presence of different phytochemicals including polyphenols, flavonoids and tannin identified through qualitative phytochemical test in different parts of *Cocos nucifera,* significant antimicrobial activity is noted against 6 microbial strains. The alcoholic extract of fine and coarse shell found more effective against all pathogens. Coconut shell has good antimicrobial activity against diverse group of microorganisms indicating that we can use

shell against multiple disease-causing bacteria. Moreover, consumers are also preferring natural antimicrobials because of allopathic medicines side effects (Espina *et al.,* 2011; Negi, 2012).

Antifungal activity of dried coconut shell against *Microsporum canis*, *M. gypseum*, *M. audouinii*, *Trichophyton mentagrophytes*, *T. rubrum T. tonsurans* and *T. violaceum* is also reported. (Venkataraman *et al.,* 1980). The activity was also due to the high content of phenolic compounds. Coarse and fine extract inhibited the growth of all organisms at high rate having maximum zone of inhibition 17.6mm against (*S. sonnei)* and 16.6mm against (*P. aeruginosa* and *E. coli)* respectively but still efficiency of fine extract is much better than the coarse one. However, both extracts were unsuccessful to suppress the growth of *S.aureus* the common pathogen.

Coconut oil is healthy and do not cause problems if consuming as a diet have been proved through scientific studies (Chinwong *et al.*, 2017; Khaw *et al.*, 2018). Due to presence of lauric acid (Tangwatcharin and Khopaibool, 2012) coconut oil shows significant inhibition against *S. aureus*. Among different coconut oil biological effects of virgin coconut oil VOC were studied in detail by Narayanankutty *et al.* (2018). It is also reported as an antibacterial agent capable to react against *Candida gingivitis* responsible for oral diseases (Ogbolu *et al.*, 2007). From the above discussion it appears that *Cocos nucifera* is a good choice as far as human health is concerned.

Conclusion

The study concluded that rich in phytochemical compounds and strong antimicrobial activity against pathogenic cultures makes coconut plant the most suitable substitute in contrast to various drugs. Common pathogens get resistant day by day against over-the-counter medicines. The medicines available for treating physiological disorders have too many side effects and not affordable for everyone, therefore that need to be replaced by products having pure diverse phytochemical components like coconut shell and leaves.

REFERENCES

Akinyemi, K. O., O. Oladapo, C. E. Okwara, C. C. Ibe and K. A. Fasure (2005). Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. *BMC Complement Altern Med,,* 5: 6. doi:https://doi.org/10.1186/1472-6882-5-6.

Bauer, A. W., W. M. M. Kirby, J. C. Sherris and M. Turck (1966). Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *Am J Clin Path.,* 45(4): 493-496.

Bhandary, S. K., S. Kumari, V. S. Bhat, K. Sharmila and M. P. Bekal (2012). Preliminary phytochemical screening of various extracts of Punica granatum peel, whole fruit and seeds. *J Health Sci., 2*(4): 35-38.

Borkar, S. D., A. R. Sawarkar, S. G. Jadhao and P. Thakare (2016). Phytochemical Analysis of Aqueous and Alcohlic Extract Of Some Medicinal Plants. *Int. j. sci. res. Manag.*, 4(9). doi: 10.18535/ijsrm/v4i9.17

Calzada, F., L. Yepez-Mulia and A. Tapia-Contreras (2007). Effect of Mexican medicinal plant used to treat trichomoniasis on Trichomonas vaginalis trophozoites. *J Ethnopharmacol,* 113(2): 248-251.

Chinwong, S., D. Chinwong and A. Mangklabruks (2017). *Daily Consumption of Virgin Coconut Oil Increases High-Density Lipoprotein Cholesterol Levels in Healthy Volunteers: A Randomized Crossover Trial.* Vol. 2017.

Edeoga, H. O., D. E. Okwu and B. O. Mbaebie (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.,* 4(7): 685-688.

Elevitch, C. (2006). *Species profiles for Pacific Island agroforestry*. Hawaii: Permanent Agriculture Resources (PAR).

Erdogrul, Ö. T. (2002). Antibacterial Activities of Some Plant Extracts Used in Folk Medicine. *Pharm. Biol.,* 40(4): 269-273.

Escalante-Erosa, F., G. C. Fernandez-Concha and L. M. Pena-Rodriguez (2009). Skimmiwallinin, an additional skimmiwallinol derivative from the cuticular wax of *Cocos nucifera*. *Nat. Prod. Res.,* 23(10): 948-952.

Espina, L., M. Somolinos, S. Lorán, P. Conchello, D. García and R. Pagán (2011). Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. *Food Control,* 22(6): 896-902.

Hamburger, M. O. and G. A. Cordell (1987). A direct bioautographic tlc assay for compounds possessing antibacterial activity. *J. Nat. Prod.,* 50(1): 19-22.

Harborne, J. B. (1973). Phytochemical method. In: *Methods of Plant Analysis*: Springer Netherlands.

Havsteen, B. H. (2002). The biochemistry and medical significance of the flavonoids. *Pharmacology & therapeutics,* 96(2-3): 67-202.

Heim, K. E., A. R. Tagliaferro and D. J. Bobilya (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nutr Biochem.,* 13(10): 572-584.

Hirschhorn, H. H. (1983). Botanical remedies of the former Dutch East Indies (Indonesia). Part I: Eumycetes, Pteridophyta, Gymnospermae, Angiospermae (Monocotyledones only). *J Ethnopharmacol.,* 7(2): 123-156.

Hoffmann, J. J., B. N. Timmermann, S. P. McLaughlin and H. Punnapayak (1993). Potential Antimicrobial Activity of Plants from the Southwestern United States. *Pharm. Biol.,* 31(2): 101-115.

Hoste, H., F. Jackson, S. Athanasiadou, S. M. Thamsborg and S. O. Hoskin (2006). The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends Parasit.,* 22(6): 253-261.

Jagessar, R. (2017). Phytochemical Screening and TLC Profile of *Montricardia arborescens*. *Am J Res Commun,* 5(1): 129-142.

Khaw, K.-T., S. J. Sharp, L. Finikarides, I. Afzal, M. Lentjes, R. Luben and N. G. Forouhi (2018). Randomised trial of coconut oil, olive oil or butter on blood lipids and other cardiovascular risk factors in healthy men and women. 8(3): e020167.

Kirszberg, C., D. Esquenazi, C. S. Alviano and V. M. Rumjanek (2003). The effect of a catechin-rich extract of *Cocos nucifera* on lymphocytes proliferation. *Phytother. Res,* 17(9): 1054-1058.

Korkina, L. G. and I. B. Afanas' Ev (1996). Antioxidant and chelating properties of flavonoids. *Adv Pharmacol.,* 38: 151-163.

Kumar, G., K. Jayaveera, C. Kumar, U. P. Sanjay, B. Swamy and D. Kumar (2007). Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. Trop. J. Pharm. Res, 6(2): 717-723.

Levy, S. (1994). Drug resistance: the new apocalypse (special issue). *Trends in Microbiology,* 2(10): 341-425.

Lima, E. B., C. N. Sousa, L. N. Meneses, N. C. Ximenes, M. A. Santos Junior, G. S. Vasconcelos, N. B. Lima, M. C. Patrocinio, D. Macedo and S. M. Vasconcelos (2015). *Cocos nucifera (L.)* (Arecaceae): A phytochemical and pharmacological review. *Braz J Med Biol Res.,* 48(11): 953-964.

Linnaeus, C. V. (1753). *Cocos nucifera L.* Available from Missouri Botanical Garden, Tropicos® Retrieved 1 june 2018, from Tropicos.org http://www.tropicos.org/Name/2400007

Manalo, R. V., M. A. Silvestre, A. L. A. Barbosa and P. M. Medina (2017). Coconut *(Cocos nucifera*) ethanolic leaf extract reduces Amyloid-β (1-42) aggregation and paralysis prevalence in transgenic Caenorhabditis elegans independently of free radical scavenging and acetylcholinesterase inhibition. *J Biomed.,* 5(2): 17.

Manivannan, A., R. Bhardwaj, S. Padmanabhan, P. Suneja, K. B. Hebbar and S. R. Kanade (2018). Biochemical and nutritional characterization of coconut *(Cocos nucifera L.)* haustorium. *J. Food Chem.,* 238: 153-159.

Naczk, M. and F. Shahidi (2004). Extraction and analysis of phenolics in food. *J Chromatogr. A,* 1054(1-2): 95-111.

Narayanankutty, A., S. P. Illam and A. C. Raghavamenon (2018). Health impacts of different edible oils prepared from coconut (Cocos nucifera): A comprehensive review. *Trends Food Sci Technol.,* 80: 1-7.

Negi, P. S. (2012). Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. *Int J Food Microbiol,* 156(1): 7-17.

Ogbolu, D. O., A. A. Oni, O. A. Daini and A. P. Oloko (2007). *In vitro* antimicrobial properties of coconut oil on *Candida* species in Ibadan, Nigeria. *J Med Food,* 10(2): 384-387.

Olaleye, M. (2007). Cytotoxicity and antibacterial activity of methanolic extract of Hibiscus sabdariffa. *J Med Plant Res,* 1(1): 9-13.

Onwukaeme, D., T. Ikuegbvweha and C. Asonye (2007). Evaluation of phytochemical constituents, antibacterial activities and effect of exudate of *Pycanthus Angolensis* weld warb (Myristicaceae) on corneal ulcers in rabbits. *Trop. J. Pharm. Res.,* 6(2): 725-730.

Parekh, J. and S. Chanda (2007). In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turk J Biol.,* 31(1), 53-58.

Parekh, J., N. Karathia and S. Chanda (2006). Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata L*. bark. *Afr J Biomed Res.,* 9(1), 53-56.

Poole, C. F. (2003). Chapter 6 - Thin-Layer Chromatography. In: C. F. Poole (Ed.), *The Essence of Chromatography* (pp. 499-567). Amsterdam: Elsevier Science.

Rates, S. (2001). Plants as source of drugs. *Toxicon.,* 39(5): 603-613.

Riedel, M., M. Riederer, D. Becker, A. Herran, A. Kullaya, G. Arana-López, L. Peña-Rodríguez, N. Billotte, V. Sniady and W. Rohde (2009). Cuticular wax composition in *Cocos nucifera L.*: physicochemical analysis of wax components and mapping of their QTLs onto the coconut molecular linkage map. *Tree genetics & genomes,* 5(1): 53.

Rodrigues, S. and G. A. Pinto (2007). Ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder. *Journal of food engineering* 869-872.

Rosenthaler, L. L., B. (1930.). *Botanical chemistry.* London, G. Bell and sons, ltd.

Samant, H., A. K. Jha, M. A. H. Ansari and M. J. Akhtar (2015, 13-15 April 2015). *Design of CPW fed IDC resonator for non invasive testing of chemical solvents.* Paper presented at the 2015 IEEE 16th Annual Wireless and Microwave Technology Conference (WAMICON).

Sasidharan, S., Y. Chen, D. Saravanan, K. Sundram and L. Y. Latha (2011). Extraction, isolation and characterization of bioactive compounds from plants extracts. *Afr J Tradit Complement Altern Med,* 8(1): 1-10.

Shettigar, R., R. Lala and N. Ny (2014). Evaluation of Antimicrobial Activity of Coconut Husk Extract. *Ann Appl Biosci.,* 1: 23-27.

Sinsinwar, S., M. K. Sarkar, K. R. Suriya, P. Nithyanand and V. Vadivel (2018). Use of agricultural waste (coconut shell) for the synthesis of silver nanoparticles and evaluation of their antibacterial activity against selected human pathogens. *Microb Pathog.,* 124: 30-37.

Solangi, A. H. and I. M. Zafar (2011). Chemical composition of meat (kernel) and nut water of major coconut *(Cocos nucifera L.)* cultivars at coastal area of Pakistan. *Pak J Bot.,* 43(1): 357-363.

Tangwatcharin, P. and P. Khopaibool (2012). Activity of virgin coconut oil, lauric acid or monolaurin in combination with lactic acid against *Staphylococcus aureus*. *Southeast Asian J Trop Med Public Health,* 43(4): 969-985.

Tiwari, P., B. Kumar, M. Kaur, G. Kaur and H. Kaur (2011). Phytochemical screening and extraction: a review. *Int. Pharma. Sci.,* 1(1): 98-106.

Venkataraman, S., T. R. Ramanujam and V. S. Venkatasubbu (1980). Antifungal activity of the alcoholic extract of coconut shell (*Cocos nucifera* Linn*). J Ethnopharmacol,* 2(3): 291-293.

WHO. (2004). *WHO Guide lines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems*. Geneva: World Health Organization. .

Yamunadevi, M., E. G. Wesely and M. Johnson (2011). Phytochemical studies on the terpenoids of medicinally important plant *Aerva lanata* L. using HPTLC. *Asian Pac J Trop Biomed.,* 1(2): 220-225.

(*Accepted for publication May 2019*)