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QUALITATIVE ASSESSMENT OF THE ANTIMICROBIAL, ANTIOXIDANT, PHYTOCHEMICAL PROPERTIES OF THE ETHANOLIC EXTRACTS OF THE ROOTS OF *COCOS NUCIFERA* L.

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ABSTRACT

The roots of *Cocos nucifera* L. have been used in folk medicine to treat several health problems including infections, inflammations, hypertension, wound healing, and others. This study was therefore conducted to evaluate their biological properties, whether they have antimicrobial and antioxidant activities and whether these are reflected in the presence of antimicrobial and antioxidant activities and whether these are reflected in the presence of antimicrobial and antioxidant activities and whether these are reflected in the presence of antimicrobial and antioxidant phytochemicals in the extract. Antimicrobial properties using the agar well-diffusion method showed inhibition against bacterial organisms *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus, and Candida albicans* but no growth inhibition was found in the fungus *Aspergillus niger*. The antioxidant activity evaluated by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) photometric assay and compared with Vitamin C showed that the half maximal inhibitory concentration of the root extract was 24.25 ppm, 14x lower in inhibitory efficiency than the standard Vitamin C. Phytochemicals present in the extract showed the presence of tannins, saponins, alkaloids, steroids, glycosides, and flavonoids which are known antioxidants. Qualitative assessment of the presence of compounds in the extract using Gas Chromatography-Mass Spectrometry identified twenty-one (21) possible bioactive compounds. Of these, eight (8) were found to be known antimicrobials while seven (7) compounds were found to be known antioxidants. This study has shown support to the ethnomedicinal use of the root of *C. nucifera* as an antimicrobial and antioxidant.

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Introduction

For several years, plants have been used as medicine to help maintain human health [1, 2]. In the Philippines, traditional medicine is being practiced especially in rural areas where traditional healers utilize plants to treat many diseases [3]. Very popular is the use of coconut (*Cocos nucifera* L.) for traditional healing [4]. C. *nucifera* called as the "tree of heaven" and "tree of life" in the country is commonly found in coastal areas of the Philippines but also other parts of Southeast Asia, and Melanesia [5], is classified into two major types depending on the plant's height; *Typica* (tall type) (Fig. 1) or *Nana* (dwarf type) [6]. The different parts of the coconut tree have been traditionally used to treat a wide range of diseases. Coconut roots have been ethnomedically used to treat arthritis [7] and lower hypertension; as anthelminthic, anti-inflammatory, antinociceptive, antioxidant, antifungal, antimicrobial, and antitumor, antipyretic, antidiarrheal, hypoglycemic, antidjional medicine to treat various ailments such as stomach disorders and skin diseases. The roots of coconut (*Cocos nucifera* L.) have been traditionally used for its medicinal capabilities. Studies have been carried out to investigate the antimicrobial effect of the crude ethanolic extract of *C. nucifera* L.. [9, 10]. Based on the literature survey of the folkloric uses of *C. nucifera* by the different indigenous people in Mindanao, residents in selected localities of Surigao del Sur and Cotabato used to boil the roots of *C. nucifera* L. in water and orally take to treat Arthritis [7, 11], while the Maranao Muslims in Iligan City drank this to lower hypertension [8]. The traditional healers in North Cotabato extracted the

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endosperm of the coconut fruit, and utilized the extracted juice to treat kidney stones [11] as well as constipation. While the Maranao Muslims drank this to induce urination in a person with a kidney infection [8].

Additionally, they also scraped off the endosperm of the coconut fruit and fried it until the white flesh became brown and produced oil. The oil was then applied directly on the skin to treat dermatitis or eczema [8]. Furthermore, the Higaonon tribe of Rogongon, Iligan City applied the scraped coconut husk directly on the navel to heal the wounds especially on newborns [12]. The folklore medicine consumed by different groups of people living in Mindanao has been observed to treat different kinds of illnesses and improve their health.

In the Philippines and other parts of Southeast Asia, there are many hybrids and varieties of *C. nucifera*, and one of which is the tall variety (var. typica) that is commonly used by the local communities in the treatment of health problems (Fig.1). Several studies have shown the presence of biochemical compounds in the different parts of *C. nucifera* L. from various countries, and they have been argued to be the basis of the different biological properties [13-16]. Many studies have shown that there are a variety of compounds present in many plants used to treat infectious diseases [17, 18]. These include secondary plant metabolites which have been argued to have an extensive activity range based on the species, the topography and the climate of the country or the place the plant originated [19], thus the biological properties of these metabolites may be modified as a result from variations in their chemical compounds which have significant effects on the biological properties of the extracts [20], it was the major objective of the study to evaluate the antimicrobial and antioxidant properties of the extracts from *C. nucifera* var typica using ethanol as a solvent. It was primarily aimed to provide an additional scientific basis for the ethnomedicinal value of the plant variety through the evaluation of its antimicrobial and antioxidant properties, and a qualitative assessment of possible phytochemical compounds present in the extract.



Fig. 1. The native variety of coconut, Cocos nucifera var. typica

Materials and Methods

The mature roots of *C. nucifera* were collected and air dried for three weeks and ground into powder (Fig. 2). Two hundred fifty grams (250.0 grams) of powdered roots were soaked in one and a half liters (1500 ml) of ethanol for seven days in one of the Laboratories of College of Science and Mathematics at Mindanao State University-Iligan Institute of Technology, Iligan City, Philippines. Ethanol was used as a solvent in the extraction since most of the polar compounds are easily extracted by this kind of solvent; thus, it is good for antimicrobial activity evaluation. The supernatant was filtered using Whatman filter paper No. 1. A rotary evaporator was used at 45°C to concentrate the filtrate. The obtained viscous crude extract was stored in storage vials for antimicrobial and antioxidant activities, phytochemical screening, DPPH assay, and GC/MS analysis. The ethanolic extract was qualitatively analyzed using GC-MS at the Chemistry Analytical and Research Laboratory of the Ateneo de Davao University in Davao City, Philippines, while the root extracts were screened for the presence of phytochemicals and antioxidant activity at the Department of Chemistry in MSU-IIT (Mindanao State University-Iligan Institute of Technology), Iligan City, Philippines. The antimicrobial assay was done at the Microbiological Research and Services Laboratory in Natural Sciences Research Institute, University of the Philippines in Diliman.



Fig. 2. The mature roots of C. nucifera used in the study.

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For the antimicrobial properties of the ethanolic extract, agar disk diffusion test was used against the selected test microorganisms. These were the Gram-negative bacteria *Klebsiella pneumoniae* UPCC 1360 and *Salmonella typhimurium* UPCC 1368, the Gram-positive bacteria *Bacillus subtilis* UPCC 1295 and *Staphylococcus aureus* UPCC 1143, and the fungi *Candida albicans* UPCC 2168 and *Aspergillus niger* UPCC 4219. The agar disk diffusion test was used for its ability to detect the antimicrobial activity of the isolates [21]. The qualitative method of the zone of inhibition was used to measure the susceptibility of the bacteria towards the standard antibiotic [22] and of the extracts since it offers many advantages including being simple, inexpensive, being able to test enormous numbers of microorganisms and antimicrobial agents, and the simplicity to interpret the provided results [23]. Chloramphenicol was used as the positive control to compare with the extracts for the gram-negative and gram-positive bacteria which is said to have properties that diffuse efficiently in the body, and does not ionize at physiological conditions [24]. Chloramphenicol is an antibiotic for the treatment of serious and systemic infections [25]. Canesten solution (with 1% Clotrimazole) was used as the positive control to compare the response of the extracts with the fungi. Clotrimazole is an antifungal medication sold under the brand name Canesten. It shows an antifungal activity by targeting the biosynthesis of ergosterol allowing the inhibition of fungal growth [26].

Free radical scavenging activity of root ethanolic extracts of *C. nucifera* L. var. *typica* plant was measured by 2,2-diphenyl-2-picryl hydrazyl (DPPH). DPPH radical scavenging assay is a simple and accurate test used for measuring the ability of different compounds acting as free radical scavengers as well as evaluating the antioxidant activity of medicinal plants [27].

For phytochemical screening, the powdered root ethanolic extract of *C. nucifera* Linn. var. *typica* was subjected to qualitative evaluation for the presence of alkaloids, saponins, flavonoids, tannins, cyanogenic glycosides, steroids and anthraquinones which were recorded using a 3-point scale [+ turbid, ++ moderate and +++ heavy] in scoring based on the Handbook of Philippine Medicinal Plants [28].

GC-MS analysis was performed following the protocol of Chipiti et al. (2015) [29], with modifications to identify the compounds present in the ethanolic extract of *C. nucifera* var. *typica* root. Gas-chromatography mass-spectrometry has been an analytical method used to facilitate, identify and quantify several different metabolites present in a plant extract which would result in a comprehensive coverage of primary metabolic pathways [30].

Results and Discussion

The *in vitro* antimicrobial activities of the ethanolic crude extract of *C. nucifera* against the two species each of Grampositive, Gram-negative bacteria and fungi were assessed using agar well diffusion method, and the results have been shown in Figure 3 and Table 1. The results depicted the extract to have inhibitory effects against most test bacterial microorganisms but not as efficient when compared to the standard antibiotics, chloramphenicol, and Clotrimazole. Inhibition was observed against the Gram-negative bacteria *Klebsiella pneumoniae*, the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and the fungus *Candida albicans*. The extract was not inhibitory to *Salmonella typhimurium* and *Aspergillus niger* (Fig. 3, Table 1).

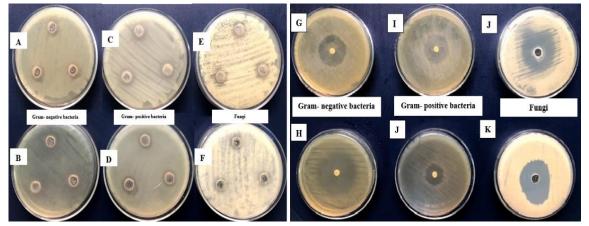


Fig. 3. The Antibacterial Indices of Gram-negative bacteria: S. typhimurium (A) and K. pneumoniae (B); Gram-positive bacteria: S. aureus (C) and B. subtilis (D); and Fungi: C. albicans (E) and A. niger (F); positive control Chloramphenicol used in Gram-negative bacteria (G&H) and Gram-negative bacteria (I&J); Canesten solution in Fungi (K&L) using agar well diffusion method in three replicates of the Cocos nucifera L. root ethanolic extract.

 Table 1. Computed average of Inhibition Zone (mm) and Antimicrobial Index (AI) from the test organisms in the C.

 nucifera root ethanolic extracts.

UPCC Test Organism	Mean	Antimicrobial Index (AI)
Gram-negative bac	teria	
S. typhimurium UPCC 1368	-	0
Chloramphenicol disc ^b	30	4.0

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K. pneumoniae UPCC 1360	13.67	0.4
Chloramphenicol disc	38	5.3
Gram-positiv	ve bacteria	
S. aureus UPCC 1143	13	0.3
Chloramphenicol disc	33	4.5
B. subtilis UPCC 1295	13.67	0.4
Chloramphenicol disc	20	2.3
Fun	gi	
C. albicans UPCC 2168	12	0.2
Canesten solution ^c	32	2.2
A. niger UPCC 4219	-	0
Canesten solution	42	3.2

The sensitivity of the test strains was classified in decreasing order: *K. pneumoniae* and *B. subtilis* > *S. aureus* > *C. albicans*. Negative inhibition against the fungus *A. niger* was observed. It was known that the composition of the outer cell wall of *A. niger* is made of chitin and glucan [31] which may explain the absence of any inhibition by the extract. It was also possible that this fungus just like any other species of fungi have developed mechanisms to counteract the effects of antifungals by reducing the accumulation of the compounds within the fungal cell or lowering the affinity of the compounds in the extract for the target fungus, or there were modifications of metabolism to counterbalance the effect of the compounds. [32]

As for the responses of the bacteria tested, the positive but lower effects of the extracts against the bacterial species tested when compared to the standard antibiotic could be due to the presence of a large impermeable cell wall of the bacteria [33]. Their outer membrane has been composed of large hydrophobic molecules which may explain the slow entry of the compounds in the extracts that can cross [34, 35] and inhibit growth. However, even the results have shown a slow inhibition to the 4 out of the 6 bacterial and fungal microorganisms, it is important to note that the coconut had inhibitory actions, and it thus may explain its use against infections such as *K. pneumoniae*, a lactose- fermenting bacterium that can cause pneumonias, urinary tract infections, and liver abscesses [36], *S. aureus*, a bacterial human pathogen which can be the source of bacteremia, skin diseases (cellulitis and impetigo), urinary tract infections, gastroenteritis, and pneumonia [37], and *B. subtilis*, an aerobic spore-forming rod bacteria that can cause endocarditis, meningitis, osteomyelitis [38] and the fungus *C. albicans*, an opportunistic pathogenic yeast that overgrowth of these fungi can cause problems in the skin, oral cavity, the gastrointestinal tract, and the reproductive tract [39]. These may explain the use of the coconut root against infections earlier reported. The root extract's possession of antimicrobial activities may provide a scientific basis for its use in traditional medicine.

The results of the current study have shown the presence of some phytochemicals in varying concentrations. These phytochemicals detected in the extract including alkaloids, flavonoids, saponins, steroids, and tannins (Table 2) can be considered to be responsible for the biological and pharmacological activity of the plant [10, 40, 41]. Some studies have shown that the roots of *Cocos nucifera L*. contain some phytochemical constituents that are antimicrobial [42, 43]. About 2,000 of these phytochemicals have been argued to be of pharmacological interest, and have been used as anesthetics, analgesics, anticancer drugs, antihypertensive agents, vasodilators, antiarrhythmia, antiasthma therapeutics and antimalarial [44, 45].

Alkaloids, for example, were shown to possess antibacterial [46-48] antibiotic-enhancing and antivirulence activities [49]. Other phytochemicals such as saponins were also observed in the extracts. These compounds were shown in many studies to have biological and pharmacological activities including anti-inflammatory, antifungal/anti-yeast, antibacterial/ antimicrobial, antiparasitic, cytotoxicity and antitumor activity, antiviral and antioxidant [50], cholesterol-lowering and antidiarrheal activities [51-53]. The study, however, showed the failed inhibition of growth of Gram-negative *E. coli*, and this might be due to its effective permeability barrier composed of a thin lipopolysaccharide exterior membrane that could restrict the permeation of plant extract or maybe due to the fact that the Gram-negative bacteria were more resistant to the plant-origin antimicrobials and showed no effect of growth inhibition [54].

Table 2. Phytochemical screening determined in <i>Cocos nucliera</i> L. root extracts.							
Alkaloids	Saponins	Flavonoids	Steroids	Tannins	Glycosides	Anthraquinones	
+ ++ +++ +++ +							
(+) indicates present: +turbid, ++moderate, +++ heavy; (-) indicates absent							

Table 2. Phytochemical screening determined in Cocos nucifera L. root extracts

The flavonoid compounds found to be abundant in the *C. nucifera L.* root extract have been argued to possess great biological and pharmacological activities which have made it a good source as antimicrobial aside from being found to be effective as hepatoprotective, anti-inflammatory, and anticancer [55]. Known antimicrobial mechanisms associated with flavonoids may explain the effectiveness of its antimicrobial activity of these compounds from the crude extract [56]. This has been said to be due to their ability to complex with the bacterial cell wall which allows the inhibition of the microbial growth. Moreover, antibacterial mechanisms of action of various flavonoids include the inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function and inhibition of energy metabolism [57]. The presence of flavonoids, in *C. nucifera* L. roots, can be a potential source to treat bacterial infections, cardiovascular diseases, and inflammation that

correlates to the ethnomedicinal uses of the plant. Flavonoids also possess anti-aging, antidiabetic and cardioprotective activities [58], the source for anti-allergy, cytotoxicity, osteogenic activity, and estrogenic activity [59]. They have also been found to be a good source of antioxidant activities as they can prevent injury caused by free radicals through direct scavenging of reactive oxygen species (ROS), activation of antioxidant enzymes, and inhibition of oxidases [60] similar to that of vitamins C in fruits [61].

Steroids have been found in numerous plant families, and they included the roots of *C. nucifera* as shown by the results of this study. These compounds have been known to possess antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia. coli* [62] aside from being an effective anti-inflammatory, anti-cancer, cytotoxic, and antiproliferative [63], and also being effective against rheumatoid arthritis [64].

The current study showed abundant tannins in the extract (Table 2). This group of compounds were found to be effective antimicrobials [65] specifically against *Staphylococcus aureus, Shigella boydii, Shigella flexneri, Escherichia coli*, and *Pseudomonas aeruginosa* [66], especially in wound healing by its ability to form a protective layer over the exposed tissue keeping the wound from getting more infected [67]. Epidemiological data also showed that the intake of tannins might cause a prevention against chronic diseases [68] being an effective antioxidant, enzyme-inhibitor, and antimutagenic [65]. This group of compounds has also been known to have antidiarrheal, haemostatic, anti-inflammatory and antihemorrhoidal properties [67]. This information might also explain the ethnomedicinal uses of *C. nucifera* L. roots to treat wounds and skin inflammation.

The *C. nucifera* L.extract was also observed to have small amounts of glycosides which are also widely distributed in other plants. These compounds are known to have a variety of action, effect, and medicinal application [69, 70]. The glycosidic residue has been found to possess antimicrobial properties that inhibited the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* [71]. However, it is important to consider that Glycosides in certain cases have toxic effects, and though plant glycosides are not normally toxic but when ingested orally, they are known to inhibit chloride transport in the stomach [72].

Table 3 shows the result of the antioxidant activity evaluation of the *C. nucifera* L. root ethanolic extract. The ethanolic extract has shown a potential source for antioxidants. When compared to Vitamin C, however, the results showed Vitamin C (IC50=1.74ppm) to be 14x more effective than the extract as an antioxidant (IC50=24.25ppm). This meant that the plant extract needed higher concentration to have higher inhibition against free radicals that caused oxidative stress and more damage to cells when compared to Vitamin C. Nevertheless, the result of this study indicated that the root extract had antioxidant properties showing the inhibition of free radicals even at lower concentrations. Many studies on coconut showed that other parts also exhibited good antioxidant activities [73-75]. The tender coconut water (TWC), coconut inflorescence, and coconut husk have been known to be good a source of antioxidants which are rich in Vitamin C and other phenolic compounds [76-78] known to help prevent or delay products from oxidization through scavenging free radicals and lowering oxidative stress [79-87], thus may slow down the aging process and progression of various diseases such as cardiovascular diseases, cancers, neurodegenerative diseases and inflammatory diseases, arthritis, eczema, diabetes, and gastrointestinal inflammatory diseases.

Extract Concentration (ppm)	Percent Inhibition (%)	Vitamin C Concentration (ppm)	Percent Inhibition (%)
Control	0	Control	0
5	9.58	2	60.07
10	15.97	3	83.91
20	40.00	4	87.73
30	59.58	5	91.55
50	80.69	10	94.56
100	94.72	20	94.44
200	94.86		
300	95.14		
IC ₅₀ ^a =24.25 ppm		IC ₅₀ = 1.74 ppm	

Table 3. Antioxidant properties of the extract of C. nucifera I

The results of the qualitative assessment of the compounds detected by GCMS showed that a total of 8 compounds were having antimicrobial properties (Table 4, Figs. 4 and 5). These compounds such as *n*-Decane [88], *n*-Tridecane [89], *n*-Hexadecane [90, 91]; Antifungal [92, 93], 2,4-Di-*tert*-butylphenol [94-96], *n*- Eicosane [88, 97], Hexadecanoic acid, ethyl ester [91, 98], 3,7,11,15-Tetramethyl-2-hexadecen-1-ol [99, 100]. The exact mechanisms which these active components of the plant extract contributed to the antibacterial activity has not been known yet, but could be due to the hydrophobic activity of the membrane which enables thermo partition of the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and allowing them to be more permeable [101]. The results, however, still indicated that the roots of *C. nucifera L.* can be a great potential source for antimicrobials [102-104].

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	Name of Compound	Formula	\mathbf{SI}^{a}	Mol. Wt.	Reported Biological Properties
1	n-Decane	C ₁₀ H ₂₂	92	142	Antifungal Antibacterial [88]
2	3,7-dimethylundecane	C10H28	80	184	No reported functional property
3	1,1-Diethoxyhexane	$C_{10}H_{22}O_2$	74	174	Flavoring agent [105] Perfumery [106]
4	2,3,3-Trimethyloctane	C11H24	78	156	Antioxidant [107-109]
5	<i>n</i> -Tridecane	C10H28	96	184	Antimicrobial [89]
6	3,8-Dimethylundecane	C13H28	89	184	No reported functional property
7	n-Hexadecane	C ₁₆ H ₃₄	90	226	Antibacterial [90, 91] Antifungal [92, 93] Antioxidant [90, 93]
8	2,4-Di-tert-butylphenol	C ₁₄ H ₂₄ O	94	206	Antioxidant [94, 110, 111] Antifungal [94-96] Cytotoxic effects [94, 112] Antibiotic [113]
9	Propanoic acid, 2-methyl-, 1- (1,1-dimethylethyl)2-methyl-1,3- propanediyl ester	C ₁₆ H ₃₀ O ₄	89	286	Volatile biomarker of lung cancer [114]
10	2,6,10, 14- Tetramethylpentadecane	$C_{19}H_{40}$	91	268	Anti-inflammatory [115]
11	2,6,10- Trimethyl,14-ethylene- 14-pentadecene	C ₂₀ H ₃₈	90	278	Antiproliferative [116]
12	Phthalic acid, butyl undecyl ester	C ₂₃ H ₃₆ O ₄	79	376	No reported functional property
13	Octadecanoic acid, ethenyl ester	$C_{20}H_{38}O_2$	69	310	No reported functional property
14	n- Eicosane	C ₂₀ H ₄₂	89	282	Antioxidant [117] Antibacterial and cytotoxic effects [88] Antifungal [97] Antitumor [88]
15	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	94	284	Antioxidant [117, 118] Antibacterial [91] Antifungal Antitumor [98] Anticancer [117] Hypocholesterolemic [118]
16	2,6,10,14- Tetramethylhexadecane	C ₂₀ H ₄₂	84	282	No reported functional property
17	3,7,11,15-Tetramethyl-2- hexadecen-1-ol	$C_{20}H_{40}O$	96	296	Antimicrobial [99, 100] Anti-inflammatory [100] Anti-diuretic/Anticancer [119] Antioxidant [119]
18	n- Eicosane	C ₂₀ H ₄₂	85	282	Antioxidant [117] Antibacterial and cytotoxic effects [88] Antifungal [97] Antitumor [88]
19	9,12- Octadecadienoic acid (Z,Z)-, ethyl ester	$C_{20}H_{36}O_2$	94	308	Dermatitigenic, Choleretic, Hypocholesterolemic, Antiarthritic, Hepatoprotective, Antiandrogenic, Antihistaminic, Anticoronary, Antieczemic, Antiacne [100]

Table 4. Bioactive compounds qualitatively identified from the ethanolic extracts of <i>C. nucifera</i> L. using GC-MS analysis
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20	Ethyl Linoleolate	$C_{20}H_{36}O_2$	88	308	Perfumery flavoring agent [120] Antiacne [121] Antioxidant [122]
21	Ethyl <i>n</i> -heptadecanoate	$C_{19}H_{38}O_2$	87	298	Antimicrobial [123]

^aSimilarity Index; ^bMass Peak; ^cBase Peak (m/z); ^dRetention Time (mins); ^eNeeds further study

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The evaluation of the results from the GCMS analysis showed polyphenolic compounds present. Polyphenols have been argued to act as strong antioxidants as they can prevent oxidative damage and reduce inflammation [107]. The GCMS result showed the phenol-containing compounds present in the extracts of the roots of *C. nucifera* L. including 2,4-Di-*tert*-butylphenol [94, 110, 111] and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol [119]. These compounds could delay the autoxidation by inhibiting the formation of free radicals and other reactive oxygen species (ROS) or through interrupting the proliferation of the free radical by scavenging species that initiated peroxidation, breaking the autoxidative chain reaction, and reducing localized O₂ concentrations [124]. They could also scavenge free radicals through the hydrogen atom transfer mechanism as there were higher energies involved in the single electron transfer process [125]. Acting as antioxidants, they affected enzyme activities, plasma, membranes, transcription factors *in vivo* [126] enhancing the total oxidant-scavenging capacities of human blood by binding to red blood cells [127]. Aside from polyphenolic compounds, the results also showed the presence of other bioactive compounds known to have antioxidant activities. These compounds included 2,3,3-Trimethyloctane [107-109], n-Hexadecane [90, 93], *n*-Eicosane [117], Hexadecanoic acid [117, 118], Ethyl Linoleolate [122] (Table 4).

It could be seen from this study that the root extract from the typica variety of the coconut had phytochemicals such as flavonoids and phenolic acids that have been said to be good sources for antioxidants enabling the reduction of oxidative stress and allowing the protection from degenerative disease [128]. Tannins have been observed to be primary antioxidants or free radical scavengers [129]. Steroids have also been identified to be a good source of antioxidant [130]. Also, Saponins were reported to have exhibited good antioxidant activity [131]. Thus looking at the folkloric uses of the coconut root as a treatment for selected diseases, there has been a basis as shown by the results of the study.

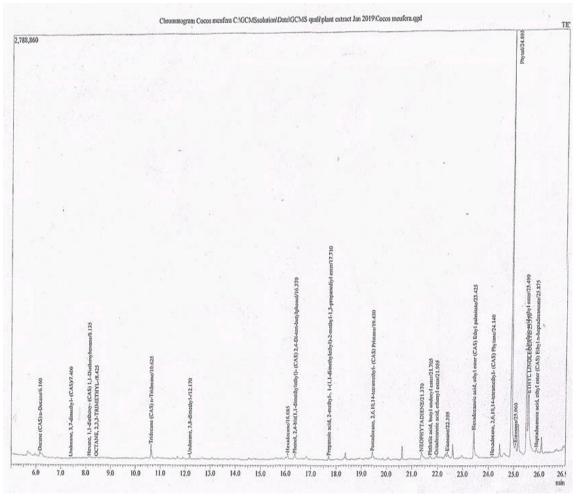


Fig. 4. The mass peak of the bioactive compounds against its retention time

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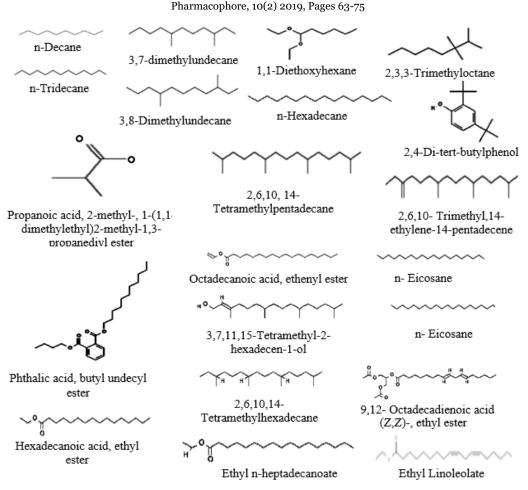


Fig. 5. The compounds identified in the roots of Cocos nucifera L. var. typica

Conclusion

The study was conducted to provide a scientific basis for the ethnomedicinal properties of the use of the coconut root (*Cocos nucifera* L. var. *typica*) by qualitatively evaluating the presence of antimicrobial, antioxidant properties and phytochemical compounds in the ethanolic extract. The growth of four out of six test microorganisms was inhibited by the extract which showed antioxidant activities but not as strong as Vitamin C. Phytochemical screening showed the presence of alkaloids, flavonoids, tannins, saponins, steroids, and glycosides which were known to have both antimicrobial and antioxidant properties. The GC-MS analysis revealed that out of the identified twenty-one (21) bioactive compounds, 8 were antimicrobials, while seven were known antioxidants. The study, therefore, showed that the folkloric use of the root to treat some health concerns might have a scientific basis.

References

- 1. Karadi, R.V., Shah, A., Parekh, P. and Azmi, P., (2011). Antimicrobial activities of Musaparadisiaca and Cocos nucifera. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2(1), 264-267.
- 2. World Health Organization. (2004). WHO guidelines on developing consumer information on proper use of traditional, complementary and alternative medicine.
- Fiscal, R. R. (2018, July 10). Ethnomedicinal Plants Used by Traditional Healers in Laguna, Philippines. Asia Pacific Journal of Multidisciplinary Research, 5(4), 132-137.
- 4. Berdon, Z. J. S., Ragosta, E. L., Inocian, R. B., Manalag, C. A., & Lozano, E. B. (2016). Unveiling CebuanoTraditional Healing Practices. Asia Pacific Journal of Multidisciplinary Research, 4(1), 51-59.
- Chan, E., & Elevitch, C. R. (2006). Cocos nucifera (coconut). Species profiles for Pacific Island agroforestry, 2(1), 1-27.
- Perera, P. I. P., Wickremasinghe, I. P., & Fernando, W. M. U. (2008). Morphological, Cytogenetic and Genotypic differences between spicata and ordinary tall coconut (Cocos nucifera L.). Journal of the National Science Foundation of Sri Lanka, 36(1), pp:103–108.
- Gruyal, G. A., del Roasario, R., & Palmes, N. D. (2014). Ethnomedicinal plants used by residents in Northern Surigao del Sur, Philippines. Natural Products Chemistry & Research. 2(5), 83. DOI: 10.4172/2329-6836.S1.002

- 8. Olowa, L., & Demayo, C. G. (2015). Ethnobotanical uses of medicinal plants among the Muslim Maranaos inIligan City, Mindanao, Philippines. Advances in Environmental Biology, 9(27), 204-215.
- Singh, M., Srivastava, S., & Rawat, A. K. S. (2007). Antimicrobial activities of Indian Berberis species. Fitoterapia, 78(7-8), 574-576.
- Lima, E.B.C., Sousa, C.N.S., Meneses, L.N., Ximenes, N.C., Júnior, S., Vasconcelos, G.S., Lima, N.B.C., Patrocínio, M.C.A., Macedo, D. and Vasconcelos, S.M.M., (2015). Cocos nucifera (L.)(Arecaceae): A phytochemical and pharmacological review. Brazilian Journal of Medical and Biological Research, 48(11), pp.953-964.
- Rubio M.M., & Naïve M.A. (2018). Ethnomedicinal plants used by traditional healers in North Cotabato, Mindanao, Philippines. J. Bio. Env. Sci. 13(6), 74-82.
- 12. Olowa, L. F., Torres, M. A. J., Aranico, E. C., & Demayo, C. G. (2012). Medicinal plants used by the Higaonon tribe of Rogongon, Iligan City, Mindanao, Philippines. Advances in Environmental Biology, 6(4), 1442-1450.
- 13. Akinyele, T. A., Okoh, O. O., Akinpelu, D. A., & Okoh, A. I. (2011). In-vitro antibacterialproperties of crude aqueous and n-hexane extracts of the husk of Cocos nucifera. Molecules, 16 (3), 2135-2145.
- 14. Baheti, A.M., Rathi, B.S., Khandelwal, K.R. and Bodhankar, S.L., (2006). Diuretic activity of Cocos nucifera husk in rats. Journal of Natural Remedies, 6(1), 35-37.
- Costa, C. T. C., Bevilaqua, C. M. L., Morais, S. M., Camurça-Vasconcelos, A. L. F., Maciel, M. V., Braga, R. R., & Oliveira, L. M. B. (2010). Anthelmintic activity of Cocos nucifera L. on intestinal nematodes of mice. Research in Veterinary Science, 88 (1), 101–103.
- Mantena, S. K., Badduri, S. R., Siripurapu, K. B., & Unnikrishnan, M. K. (2003). In vitroevaluation of antioxidant properties of Cocos nucifera Linn. water. Food/Nahrung, 47(2), 126-131.
- 17. Sumathi, P., & Parvathi, A. (2010). Antimicrobial activity of some traditional medicinal plants. Journal of Medicinal plants research, 4(4), 316-321.
- Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi Journal of Biological Sciences, 25(2), 361-366.
- Savoia, D. (2012). Plant-derived antimicrobial compounds: alternatives to antibiotics. Future microbiology, 7(8), 979-990.
- 20. Hasan, H. A., Raauf, A. M. R., Razik, B. M. A., & Hassan, B. A. R. (2012). Chemical composition and antimicrobial activity of the crude extracts isolated from Zingiber officinale by differentsolvents. Pharmaceut Anal Acta, 3(9), 1-5.
- 21. Jorgensen, J. H., & Turnidge, J. D. (2015). Susceptibility test methods: dilution and disk diffusion methods. In Manual of Clinical Microbiology, Eleventh Edition, 1253-1273. American Society of Microbiology.
- Bhargav, H. S., Shastri, S. D., Poornav, S. P., Darshan, K. M., & Nayak, M. M. (2016). Measurement of the Zone of Inhibition of an Antibiotic. In Advanced Computing (IACC), 2016 IEEE 6th International Conference on (pp. 409-414). IEEE.
- 23. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis, 6(2), 71-79.
- 24. Schwarz, S., Kehrenberg, C., Doublet, B., & Cloeckaert, A. (2004). Molecular basis of bacterialresistance to chloramphenicol and florfenicol. FEMS microbiology reviews, 28(5), 519-542.
- 25. Tian, F., Wang, C., Tang, M., Li, J., Cheng, X., Zhang, S., Ji, D., Huang, Y. and Li, H. (2016). The antibiotic chloramphenicol may be an effective new agent for inhibiting the growth of multiple myeloma. Oncotarget, 7(32), 51934.
- 26. Crowley, P. D., & Gallagher, H. C. (2014). Clotrimazole as a pharmaceutical: past, present and future. Journal of applied microbiology, 117(3), 611-617.
- 27. Marinova, G., & Batchvarov, V. (2011). Evaluation of the methods for determination of the free radical scavenging activity by DPPH. Bulgarian Journal of Agricultural Science, 17(1), 11-24.
- De Padua, L.S., Lugod, G.C., and Pancho J.V. (2005). Handbook of Philippine Medicinal Plants. University of the Philippines, Los Baños 1981; 1-4: 66.
- 29. Chipiti, T. Ibrahim, MA. Singh, M. & Islam, MS. In vitro α-amylase and α-glucosidase inhibitory effects and cytotoxic activity of Albizia antunesiana extracts. Pharmacogn Mag. 2015.11(Suppl 2):S231-6.
- 30. Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., & Fernie, A. R. (2006). Gas chromatography mass spectrometry based metabolite profiling in plants. Nature protocols, 1(1), 387.
- Russell, A. D. (2003). Similarities and differences in the responses of microorganisms to biocides. Journal of antimicrobial chemotherapy, 52(5), 750-763.
- Vandeputte, P., Ferrari, S., & Coste, A. T. (2011). Antifungal resistance and new strategies to control fungal infections. International journal of microbiology, 2012. Article ID 713687, 26 pageshttp://dx.doi.org/10.1155/2012/713687
- 33. Livermore, D. M. (2012). Current epidemiology and growing resistance of gram-negative pathogens. The Korean journal of internal medicine, 27(2), 128.
- 34. Li, X. Z., & Nikaido, H. (2009). Efflux-mediated drug resistance in bacteria: an update. Drugs, 69(12), 1555.

- 35. Holmes, N. E., & Howden, B. P. (2011). The rise of antimicrobial resistance: a clear and present danger. Expert review of anti-infective therapy, 9(6), 645-648.
- Paczosa, M. K., & Mecsas, J. (2016). Klebsiella pneumoniae: going on the offense with a strong defense. Microbiology and Molecular Biology Reviews, 80(3), 629-661.
- 37. Taylor, T. A., & Unakal, C. G. (2019). Staphylococcus Aureus.
- Turnbull, P., Kramer, J., & Melling, J. (1990). Bacillus In: Topley and Wilson Principles of Bacteriology. Virology and Immunity 8th ed Edward Arnold, London, 185-210.
- 39. Nobile, C. J., & Johnson, A. D. (2015). Candida albicans biofilms and human disease. Annual review of microbiology, 69, 71-92.
- Savithramma, N., Rao, M. L., & Suhrulatha, D. (2011). Screening of medicinal plants for secondary metabolites. Middle-East Journal of Scientific, 8 (3): 579-584.
- Yadav, R. N. S., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. Journal of phytology. 3(12): 10-14.
- Sivakumar, M., Dhanapal, C., Moideen, M., Sheik, B., Sivakumar, M., & Varghese, R. (2011). Preliminary Phytochemical Screening and Anti-Bacterial activity of Cocos nucifera Linn root. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2(4), 468-477.
- 43. Wintola, O. A., & Afolayan, A. J. (2015). The antibacterial, phytochemicals and antioxidants evaluation of the root extracts of Hydnora africana Thunb. used as antidysenteric in Eastern Cape Province, South Africa. BMC complementary and alternative medicine, 15(1), 307.
- 44. Richard, T., Temsamani, H., Cantos-Villar, E., & Monti, J. P. (2013). Application of LC–MS and LC–NMR techniques for Secondary Metabolite Identification. In Advances in Botanical Research, 67, 67-98. Academic Press.
- 45. Pallardy, S. G. (2010). Physiology of woody plants. Academic Press.
- Roberts, M. F. (Ed.). (2013). Alkaloids: biochemistry, ecology, and medicinal applications. Springer Science & Business Media.
- 47. Manosalva, L., Mutis, A., Urzúa, A., Fajardo, V., & Quiroz, A. (2016). Antibacterial activity of alkaloid fractions from berberis microphylla G. Forst and study of synergism with ampicillin and cephalothin. Molecules, 21(1), 76.
- Pervaiz, A., Khan, R., Anwar, F., Mushtaq, G., A Kamal, M., & Khan, H. (2016). Alkaloids: an emerging antibacterial modality against methicillin resistant Staphylococcus aureus. Current pharmaceutical design, 22(28), 4420-4429.
- Cushnie, T. T., Cushnie, B., & Lamb, A. J. (2014). Alkaloids: an overview of their antibacterial, antibiotic enhancing and antivirulence activities. International Journal of Antimicrobial Agents, 44(5), 377-386.
- Sparg, S., Light, M. E., & Van Staden, J. (2004). Biological activities and distribution of plant saponins. Journal of ethnopharmacology, 94(2-3), 219-243.
- 51. Johnson, A. M. (2013). Saponins as Agents Preventing infection Caused by Waterborne Pathogens.112 pages.
- 52. Oyekunle, M. A., Aiyelaagbe, O. O., & Fafunso, M. A. (2006). Evaluation of the antimicrobial activity of saponins extract of Sorghum bicolor L. Moench. African journal of Biotechnology, 5(23), pp. 2405-2407.
- Abu-Shanab, B., ADWAN, G. M., Abu-Safiya, D., Jarrar, N., & Adwan, K. (2005). Antibacterialactivities of some plant extracts utilized in popular medicine in Palestine. Turkish journalof biology,28(2-4), 99-102.
- Biswas, B., Rogers, K., McLaughlin, F., Daniels, D., & Yadav, A. (2013). Antimicrobialactivities of leaf extracts of guava (Psidium guajava L.) on two gram-negative and gram-positive bacteria. International journal of microbiology, 2013:746165. doi: 10.1155/2013/746165.
- Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. The Scientific World Journal, 2013:162750. doi: 10.1155/2013/162750.
- 56. Mbaveng, A. T., Ngameni, B., Kuete, V., Simo, I. K., Ambassa, P., Roy, R., ... & Meyer, J. M. (2008). Antimicrobial activity of the crude extracts and five flavonoids from the twigs of Dorstenia barteri (Moraceae). Journal of ethnopharmacology, 116(3), 483-489.
- 57. Cushnie, T. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. International journal of antimicrobial agents, 26(5), 343-356.
- 58. Wang, T. Y., Li, Q., & Bi, K. S. (2018). Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. asian journal of pharmaceutical sciences, 13(1), 12-23.
- Chen, X., Mukwaya, E., Wong, M. S., & Zhang, Y. (2014). A systematic review on biological activities of prenylated flavonoids. Pharmaceutical biology, 52(5), 655-660.
- Procházková, D., Boušová, I., & Wilhelmová, N. (2011). Antioxidant and prooxidant properties of flavonoids. Fitoterapia, 82(4), 513-523.
- 61. Prior, R. L., & Cao, G. (2000). Antioxidant phytochemicals in fruits and vegetables: diet and health implications. HortScience, 35(4), 588-592.
- 62. Rojas, J. J., Ochoa, V. J., Ocampo, S. A., & Muñoz, J. F. (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. BMC complementary and alternative medicine, 6(1), 2.

- Simoben, C., Ibezim, A., Ntie-Kang, F., N Nwodo, J., & L Lifongo, L. (2015). Exploring cancer therapeutics with natural products from African medicinal plants, part I: xanthones, quinones, steroids, coumarins, phenolics and other classes of compounds. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 15(9), 1092-1111.
- 64. Kaur, A., Nain, P., & Nain, J. (2012). Herbal plants used in treatment of rheumatoid arthritis: a review. Int J Pharm Pharm Sci, 4(Suppl 4), 44-57.
- Serrano, J., Puupponen-Pimiä, R., Dauer, A., Aura, A. M., & Saura-Calixto, F. (2009). Tannins: current knowledge of food sources, intake, bioavailability and biological effects. Molecular nutrition & food research, 53(S2), S310-S329.
- 66. Banso, A., & Adeyemo, S. O. (2007). Evaluation of antibacterial properties of tannins isolated from Dichrostachys cinerea. African Journal of Biotechnology, 6(15), pp. 1785-1787.
- 67. Ashok, P. K., & Upadhyaya, K. (2012). Tannins are astringent. Journal of Pharmacognosy and Phytochemistry, 1(3), 45-50.
- Lila, M. A. (2007). From beans to berries and beyond. Annals of the New York academy of Sciences, 1114(1), 372-380.
- 69. Bartnik, M., & Facey, P. C. (2017). Glycosides. In Pharmacognosy (pp. 101-161).
- 70. Chikezie P.C., Ibegbulem C.O., Mbagwu F.N. (2015) Medicinal Potentials and Toxicity Concerns of Bioactive Principles. Med Aromat Plants, 4(3), 15 pages.
- Soulef, K., Abdelouahab, Y., & Dalal, B. (2014). Effect of glycosides extract of the medicinal plant Glycyrrhiza glabra L. from the region of Mlilli (southeast of Algeria) on the growth of some human pathogenic bacteria. Journal of Scientific & Innovative Research, 3(1), 28-34.
- 72. Joshi, B., Sah, G. P., Basnet, B. B., Bhatt, M. R., Sharma, D., Subedi, K., ...& Malla, R.(2011).Phytochemical extraction and antimicrobial properties of different medicinal plants: Ocimum sanctum (Tulsi), Eugenia caryophyllata (Clove), Achyranthes bidentata (Datiwan) and Azadirachta indica (Neem). Journal of Microbiology and Antimicrobials, 3(1), 1-7.
- Chakraborty, M., & Mitra, A. (2008). The antioxidant and antimicrobial properties of the methanolic extract from Cocos nucifera mesocarp. Food Chemistry, 107(3), 994-999.
- 74. Preetha, P. P., Devi, V. G., & Rajamohan, T. (2012). Hypoglycemic and antioxidant potential of coconut water in experimental diabetes. Food & function, 3(7), 753-757.
- 75. Silva, R. R., e Silva, D. O., Fontes, H. R., Alviano, C. S., Fernandes, P. D., & Alviano, D. S. (2013). Anti inflammatory, antioxidant, and antimicrobial activities of Cocos nucifera var. typica. BMC complementary and alternative medicine, 13(1), 107.
- 76. DebMandal, M., & Mandal, S. (2011). Coconut (Cocos nucifera L.: Arecaceae): in health promotion and disease prevention. Asian Pacific Journal of Tropical Medicine, 4(3), 241-247.
- Padumadasa, C., Dharmadana, D., Abeysekera, A., & Thammitiyagodage, M. (2016). In vitro antioxidant, antiinflammatory and anticancer activities of ethyl acetate soluble proanthocyanidins of the inflorescence of Cocos nucifera L. BMC complementary and alternative medicine, 16(1), 345.
- Valadez-Carmona, L., Cortez-García, R. M., Plazola-Jacinto, C. P., Necoechea-Mondragón, H., & Ortiz-Moreno, A. (2016). Effect of microwave drying and oven drying on the water activity, color, phenolic compounds content and antioxidant activity of coconut husk (Cocos nucifera L.). Journal of food science and technology, 53(9), 3495-3501.
- 79. Sulaiman, M., Tijani, H. I., Abubakar, B. M., Haruna, S., Hindatu, Y., Mohammed, J. N., & Idris, A. (2013). An overview of natural plant antioxidants: analysis and evaluation. Advances in Biochemistry, 1(4), 64-72.
- Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy reviews, 4(8), 118.
- Forman, H. J., Davies, K. J., & Ursini, F. (2014). How do nutritional antioxidants really work: nucleophilic tone and para-hormesis versus free radical scavenging in vivo. Free Radical Biology and Medicine, 66, 24-35.
- 82. Bennett, L. L., Rojas, S., & Seefeldt, T. (2012). Role of antioxidants in the prevention of cancer. Journal of Experimental & Clinical Medicine, 4(4), 215-222.
- Škrovánková, S., Mišurcová, L., & Machů, L. (2012). Antioxidant activity and protecting health effects of common medicinal plants. In Advances in food and nutrition research, 67, 75-139. Academic Press.
- Gutteridge, J. M. C., & Halliwell, B. (2010). Antioxidants: Molecules, medicines and myths. Biochemical and Biophysical Research Communications, 393 (4), 561–564.
- Krishnaiah, D., Sarbatly, R., & Nithyanandam, R. (2011). A review of the antioxidant potentialmedicinal plant species. Food and bioproducts processing, 89(3), 217-233.
- Nimse, S. B., & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. Rsc Advances, 5(35), 27986-28006.
- Fernandez-Garcia, E. (2014). Skin protection against UV light by dietary antioxidants. Food & function, 5(9), 1994-2003.
- Akpuaka, Ekwenchi, M., Dashak, D., & Dildar, A. (2013). Biological Activities of Characterized Isolates of n-Hexane Extract of Azadirachta indica A.Juss (Neem) Leaves. New York Science Journal, 6(6), 119-124.

- 89. Khan, K., Firdous, S., Ahmad, A., Fayyaz, N., Nadir, M., Rasheed, M., & Faizi, S. (2016). GC-MS profile of antimicrobial and antioxidant fractions from Cordia rothii roots. Pharmaceutical biology, 54(11), 2597-2605.
- Gergel, E., Konovalova, O. & V. H. (2013). GC-MS Analysis of Bioactive Components of Shepherdia argentea (Pursh.) Nutt. from Ukrainian Flora. The Pharma Innovation- Journal,2(6), pp: 7-12.
- Rahimian, H., Rasekh, F., Sharifimehr, S. & Tajick, M. A. (2014). Some of phytotoxic and antimicrobial compounds extracted from culture filtrates of Fusarium proliferatum FP85. Journal of Biodiversity and Environmental Sciences, 4(5), pp:245-251
- 92. Duraipandiyan, V., Gajendran, H., Girija, S., Kuppusamy, P. S., & Rajagopal, R. (2014). Chromatographic Characterization and GC-MS Evaluation of the Bioactive Constituents with Antimicrobial Potential from the Pigmented Ink of Loligo duvauceli. International Scholarly Research Notices. 2014:820745. doi: 10.1155/2014/820745.
- 93. Abdul Kaffoor, H., Arumugasamy, K., Jemimma, H. L. & Nantha Kumar, R. (2017). GC-MS Analysis Of Root And Aerial Parts Ethanolic Extract of Phyllanthus vasukii Parthipan et al., Sp. Nov. (Phyllanthaceae). International Journal of Ayurvedicand Herbal Medicine, 7(4):2672-2684.
- Varsha, K. K., Devendra, L., Shilpa, G., Priya, S., Pandey, A., & Nampoothiri, K. M. (2015). 2, 4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated Lactococcus sp. International journal of food microbiology, 211, 44-50.
- Padmavathi, A. R., Bakkiyaraj, D., Thajuddin, N., & Pandian, S. K. (2015). Effect of 2, 4-di-tert-butylphenol on growth and biofilm formation by an opportunistic fungus Candida albicans. Biofouling, 31(7), 565-574.
- 96. Belghit, S., Driche, E. H., Bijani, C., Zitouni, A., Sabaou, N., Badji, B., & Mathieu, F. (2016). Activity of 2, 4-Ditert-butylphenol produced by a strain of Streptomyces Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis, 6(2), 71-79.
- 97. Ahsan, T., Chen, J., Irfan, M., Wu, Y. & Zhao, X. (2017). Extraction and Identification of bioactive compounds (eicosane and dibutyl phthalate) produced by Streptomyces strain KX852460 for the biological control of Rhizoctonia solani AG-3 strain KX852461 to control target spot disease in tobacco leaf. AMB Express, 7(1):54. doi: 10.1186/s13568-017-0351-z.
- Agarwal M. & Tyagi T. (2016). Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of Pistia stratiotes L. and Eichhornia crassipes (Mart.) solms. Journal of Pharmacognosy and Phytochemistry 2017 Vol. 6(1), 195-26.
- Ponmathi S.A, Evanjaline, M. & Muthukumarasamy, S. Veerabahu, M. (2017). Determination of Bioactive Components of Barleria Courtallica Nees (Acanthaceae) By Gas Chromatography–Mass Spectrometry Analysis. Asian Journal of Pharmaceutical and Clinical Research, 10(6), 273.
- 100. Jananie, R. K., Priya, V., & Vijayalakshmi, K. (2011). Determination of bioactive components of Cynodon dactylon by GC-MS analysis. NY Sci J, 4(4), 1-5.
- 101. Jose, M., Cyriac, M. B., Pai, V., Varghese, I., & Shantaram, M. (2014). Antimicrobial properties of Cocos nucifera (coconut) husk: An extrapolation to oral health. Journal of natural science, biology, and medicine, 5(2), 359.
- 102. Abushelaibi, A. A., Al Shamsi, M.S., & Afifi, H.S. (2012). Use of antimicrobial agents in food processing systems. Recent patents on food, nutrition & agriculture, 4(1), 2-7.
- 103. Rai, J., Randhawa, G. K., & Kaur, M. (2013). Recent advances in antibacterial drugs. International Journal of Applied and Basic Medical Research, 3(1), 3.
- 104. Reller, L. B., Weinstein, M., Jorgensen, J. H., & Ferraro, M. J. (2009). Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clinical infectious diseases, 49(11), 1749-1755.
- 105. Moshonas, M. G., & Shaw, P. E. (1990). Flavor evaluation of concentrated aqueous orange essences. Journal of agricultural and food chemistry, 38(12), 2181-2184.
- 106. Muller, P. M., & Lamparsky, D. (Eds.). (2012). Perfumes: art, science and technology. Springer Science & Business Media.
- 107. Zhang, J., Dou, J., Zhang, S., Liang, Q., & Meng, Q. (2010). Chemical composition and antioxidant properties of the essential oil and methanol extracts of rhizoma of Alpinia officinarum from China in vitro. African Journal of Biotechnology, 9(28). pp. 4414-4421.
- 108. Bokhari, T.H., Aslam, M.A., Hina, S., Rizvi, N.B., Rasool, N., Saif, M.J., Zubair, M., Hussain, A.I., Chatha, S.A. and Raiz, M., 2014. Mineral composition, phenolic profile, antioxidant and antimicrobial activities of Corchorus depressus roots extracts. Bulgarian chemical communications, 46(4), 788-794.
- 109. Zubair, M. F., Ajibade, S. O., Lawal, A. Z., Yusuf, S. A., Babalola, J. B., Mukadam, A. A., & Hamid, A. A. (2017). GC-MS analysis, Antioxidant and Antimicrobial Properties of Eclipta prostrata leaves. International Journal of Chemical and Biochemical Sciences, 11, 25-43.
- 110. Yoon, M.A., Jeong, T.S., Park, D.S., Xu, M.Z., Oh, H.W., Song, K.B., Lee, W.S. and Park, H.Y., (2006). Antioxidant effects of quinoline alkaloids and 2, 4-di-tert-butylphenol isolated from Scolopendra subspinipes. Biological and Pharmaceutical Bulletin, 29(4), 735-739.

- 111. Choi, S.J., Kim, J.K., Kim, H.K., Harris, K., Kim, C.J., Park, G.G., Park, C.S. and Shin, D.H., (2013). 2, 4-Di-tertbutylphenol from sweet potato protects against oxidative stress in PC12 cells and in mice. Journal of medicinal food, 16(11), 977-983.
- 112. Malek, S. N. A., Shin, S. K., Wahab, N. A., & Yaacob, H. (2009). Cytotoxic components of Pereskia bleo (Kunth) DC. (Cactaceae) leaves. Molecules, 14(5), 1713-1724.
- 113. Viszwapriya, D., Prithika, U., Deebika, S., Balamurugan, K., & Pandian, S. K. (2016). In vitro and in vivo antibiofilm potential of 2, 4-di-tert-butylphenol from seaweed surface associated bacterium Bacillus subtilis against group A streptococcus. Microbiological research, 191, 19-31.
- 114. Jia, Z., Patra, A., Kutty, V. K., & Venkatesan, T. (2019). Critical Review of Volatile Organic Compound Analysis in Breath and In Vitro Cell Culture for Detection of Lung Cancer. Metabolites, 9(3), 52.
- 115. Daudin, J.B., Monnet, D., Kavian, N., Espy, C., Wang, A., Chéreau, C., Goulvestre, C., Omri, S., Brézin, A., Weill, B. and Batteux, F., 2011. Protective effect of pristane on experimental autoimmune uveitis. Immunology letters, 141(1), pp.83-93.
- 116. Selvamangai, G., & Bhaskar, A. (2012). GC–MS analysis of phytocomponents in the methanolic extract of Eupatorium triplinerve. Asian Pacific Journal of Tropical Biomedicine, 2(3), S1329-S1332.
- 117. Brindha, P. & Sivasubramanian, R. (2013). In vitro Cytotoxic, Antioxidant & GC-MS Studies on Centratherum punctatum Cass. International Journal of Pharmacy and Pharmaceutical Sciences, 5(3), 364- 367.
- 118. Abdennebi, E., Belakhdar, G. & Benjouad, A. (2015). Determination of some bioactive chemical constituents from Thesium humile Vahl. J. Mater. Environ. Sci., 6(10), 2778-2783.
- 119. Shibula, K., & Velavan, S. (2015). Determination of phytocomponents in methanolic extract of Annona muricata leaf using GC-MS technique. Int J Pharmacognosy Phytochem Res, 7(6), 1251-1255.
- 120. Sanseera, D., Niwatananun, W., Liawruangrath, B., Liawruangrath, S., Baramee, A., & Pyne, S. G. (2012). Chemical composition and biological activities of the essential oil from leaves of Cleidion javanicum bl. Journal of Essential Oil Bearing Plants, 15(2), 186-194.
- 121. Logan, A. C. (2008). Linoleic and linolenic acids and acne vulgaris. The British journal of dermatology, 158(1), 201-202.
- 122. Masuda, T., Yamada, K., Maekawa, T., Takeda, Y., & Yamaguchi, H. (2006). Antioxidant mechanism studies on ferulic acid: identification of oxidative coupling products from methyl ferulate and linoleate. Journal of agricultural and food chemistry, 54(16), 6069-6074.
- 123. Shobier, A. H., Ghani, S. A. A., & Barakat, K. M. (2016). GC/MS spectroscopic approach and antifungal potential of bioactive extracts produced by marine macroalgae. The Egyptian Journal of Aquatic Research, 42(3), 289-299.
- 124. Brewer, M. S. (2011). Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. Comprehensive reviews in food science and food safety, 10(4), 221-247.
- 125. Li, A. N., Li, S., Zhang, Y. J., Xu, X. R., Chen, Y. M., & Li, H. B. (2014). Resources and biological activities of natural polyphenols. Nutrients, 6(12), 6020-6047.
- 126. García-Alonso, J., Ros, G., Vidal-Guevara, M. L., & Periago, M. J. (2006). Acute intake of phenolic-rich juice improves antioxidant status in healthy subjects. Nutrition research, 26(7), 330-339.
- 127. Koren, E., Kohen, R., & Ginsburg, I. (2010). Polyphenols enhance total oxidant-scavenging capacities of human blood by binding to red blood cells. Experimental Biology and Medicine, 235(6), 689-699.
- 128. Lee, M. T., Lin, W. C., Yu, B., & Lee, T. T. (2017). Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals- A review. Asian-Australasian journal of animal sciences, 30(3), 299.
- 129. Vadlapudi, V., & Kaladhar, D. S. V. G. K. (2012). Antimicrobial study of plant extracts of Datura metel L. against some important disease causing pathogens. Asian Pacific Journal of Tropical Disease, 2 (1), S94-S97.
- 130. Ahmad, A., Husain, A., Mujeeb, M., Khan, S.A., Najmi, A.K., Siddique, N.A., Damanhouri, Z.A. and Anwar, F., 2013. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pacific journal of tropical biomedicine, 3(5), 337-352.
- 131. Chan, K. W., Iqbal, S., Khong, N. M., Ooi, D. J., & Ismail, M. (2014). Antioxidant activity of phenolics-saponins rich fraction prepared from defatted kenaf seed meal. LWT-Food Science and Technology, 56(1), 181-186.