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Extraction and Identification of Phytochemicals in N-hexane and Methanol Extracts of *Cocos Nucifera L.* Leaves

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Abstract The *Cocos nucifera L.* (Coconut palm) of Arecaceae family is found mainly in coastal areas. The extraction and analysis of various parts of plants reveal its application in natural medicine. In the present investigation, the preliminary phyto-chemical screening of *Cocos nucifera L.* leaves of Veraval region was performed using n-hexane and methanol solvents, which showed the presence of secondary metabolites like, phenols, alkaloids, tannins, terpenoids, phenols, etc. GC-MS (Gas Chromatography–Mass Spectrometry) analysis was done and Mass spectra of these compounds were matched with NIST (National Institute of Standards and Technology) library and were identified by comparing the peak area and retention time with literature and the interpretation was done. This shows presence of 11 bioactive compounds in n-hexane extract including mainly Squalene, Hexadecanoic acid, Moretenol, alpha-Tocopherol, Hexadecamethyl heptasiloxane, Eicosamethyl cyclodecasiloxane and others while the methanolic extract showed 9 bioconstituents like Squalene, Neophytadiene, Trans Phytol, A'-Neogammacer-22(29)-en-3-one and others. These bioconstituents show various biological activities like Antitumor, Antioxidant, Hypocholesterolmic, Immunostimulant, Antimycobacterial, Antiprotozoal, Anti-inflammatory, Antidepressant, Anti-inflammatory, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Neuroprotective activities and others. Thus further studies and isolation of these compounds may benefit in pharmaceutical and medicinal fields.

Keywords Cocos Nucifera L., GC-MS, Phytochemicals, Bioactive Compounds

1. Introduction

Natural materials wide applications have been found since long for its therapeutic purposes in India. Ancient ayurvedic preparations included the use of various portions of medicinal plants, which is still relevant today and increasing popularity as people become more health-conscious. Natural medicines are preferred by millions of people in the current phase due to their effectiveness, low toxicity, and overall health benefits [1]. Natural products are also biodegradable, making them environmentally beneficial. Plants have bioactive elements in their roots, leaves, fruits, inflorescence, stems, flowers, and other parts of their bodies that have a variety of physiological functions [2], [3]. Alkaloids, tannins, saponins, terpenoids, phytols, quinones, steroids, glycosides, fatty acids, and other phytochemicals found in plants have become increasingly important in the medical sector [4]–[7]. Plant extracts are widely used as antiviral, antiallergic, antifungal, antimicrobial, antibacterial, antioxidant, anti-inflammatory, antidiabetic,

antihypertensive, and others [8].

Cocos nucifera L. (The coconut palm) that belongs to the Arecaceae family, is planted in coastal locations. After the Philippines and Indonesia, India is the world's third-largest producer. Coconut is called as the "tree of life" since all of its parts have uses ranging from industrial to household to therapeutic. According to Indian mythology, it is known as a kalpvruksh or kalpataru (wish-granting tree) and offers livelihood to people in a variety of ways. The phytochemicals found in hexane and methanolic extracts of *Cocos nucifera L.* leaves, as well as their medicinal applications, are discussed in this study. Various chemical components present in plant extracts were identified using qualitative analysis tests and GC-MS (gas chromatography combined with mass spectrometry) analysis.

2. Materials & Method

Sample collection

Coconut leaves were collected from Veraval, Gir Somnath district, Saurashtra, Gujarat during the month of December, 2021. The coconut variety selected for the investigation is Dwarf variety. The leaves were cleaned thoroughly with water and allowed to shade dry for a week and then it was powdered using grinder.

Extraction of leaves

10g of the powdered leaves were allowed to soak in solvents like methanol and n-hexane for 24 hours. The extracts were then filtered using Whatman No 41 filter paper. Before filtering, the filter paper was moistened with methanol and hexane. Further, the GC-MS analysis and qualitative analysis for the phytochemicals screening were performed for both extracts.

Phytochemical screening

Presence and identification of various secondary metabolites like phenols, alkaloids, quinones, saponins, tannins, steroids, terpenoids, flavonoids, amino acid were done using various tests as per the standard methods in literature. Qualitative analysis was performed in both n-hexane and methanol extracts for the identification as shown in Table 1.

GC-MS analysis

Gas Chromatography-Mass Spectrometry analysis was carried out on a ShimadzuQP2010 GC-MS instrument employed with BPX capillary column at 70 eV. Carrier gas helium at a constant flow of 1.4 ml/min was maintained. 1µl of the extract was injected at a temperature maintained to 250ºC and an ion source. The oven temperature was programmed to 250ºC. Total GC running time was 26 min. Peaks obtained in Mass Spectrometry were compared with Wiley and NIST (National Institute of Standard and Technology) compound library.

Table 1. Qualitative Phytochemical screening in n-hexane and methanol extracts of Cocos nucifera L.

Preliminary Phytochemical Screening		
Tests for phytoconstituents	**Results in n-hexane**	**Results in Methanol**
Alkaloids (Mayer test)	+	+
Alkaloids (Wagner test)	+	+
Saponins	-	+
Tannins	+	+
Steroids	-	+
Terpenoids	+	+
Flavonoids	-	+
Phenols	+	+
Quinone	-	+
Amino acid	-	-

Note: + present, - absent

3. Results

Secondary metabolites in plants are responsible for various bioactivities of plants. The first step is preliminary phytochemical screening (as shown in Table 1) to determine the presence of such chemical compounds. In the present investigation, qualitative analysis of leaves of *Cocos nucifera L.* shows the positive results for alkaloids analyzed by Wagner and Mayer tests for both the hexane and methanol extracts. Formation of green colour confirmed the presence of tannins in both the extracts. Furthermore, appearance of brown colour in both the extracts shows the presence of terpenoids. However, flavonoids, saponins, steroids and quinones were found to be absent in hexane extract. Green coloured solution with ferric chloride solution shows presence of phenols in both extracts. Tests for amino acids were found to be negative for both extracts.

GC-MS analysis of n-hexane extract of Dwarf variety showed presence of 11 phytoconstituents whereas 9 phytoconstituents were found in methanolic extract. Table 2 and Table 3 show the details of the components identified respectively for the extracts of hexane and methanol by comparing the mass spectra and retention time with the Wiley and NIST library data [9]–[11] showing its structure, molecular formula, molecular weight, name, area percentage and retention time. Figure 1 and Figure 2 show the GC-MS chromatogram of phytochemicals found in extracts of methanol and n-hexane leaves of *Cocos nucifera L.*

Table 2. Phytocomponents identified by GC-MS analysis of n-hexane leaf extract of Cocos nucifera L.

Sr. No.	R. T	Area%	Name of Compound	Molecular formula	Mol. weight
1.	13.544	0.39	Hexadecamethyl heptasiloxane	$C_{16}H_{48}O_6Si_7$	532
2.	14.155	1.97	Hexadecanoic acid	$C_{16}H_{32}O_2$	256
3.	14.598	0.53	Eicosamethyl cyclodecasiloxane	$C_{20}H_{60}O_{10}Si_{10}$	740
4.	15.161	1.58	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS)	$C_{20}H_{40}O$	296
5.	15.264	0.61	Cis-Octadec-9-enal	$C_{18}H_{34}O$	266
6.	15.566	0.39	Tetracosamethyl cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	888
7.	16.023	10.68	alpha-Tocopherol	$C_{29}H_{50}O_2$	430
8.	17.529	0.69	Hexadecamethyl heptasiloxane	$C_{16}H_{48}O_6S_{i7}$	532
9.	20.732	1.24	1H-Purin-6-amine, [(2-fluorophenyl)methyl]	$C_{12}H_{10}FN_5$	243
10.	21.468	55.84	Squalene	$C_{30}H_{50}$	410
11.	24.115	23.74	Moretenol	$C_{30}H_{50}O$	426

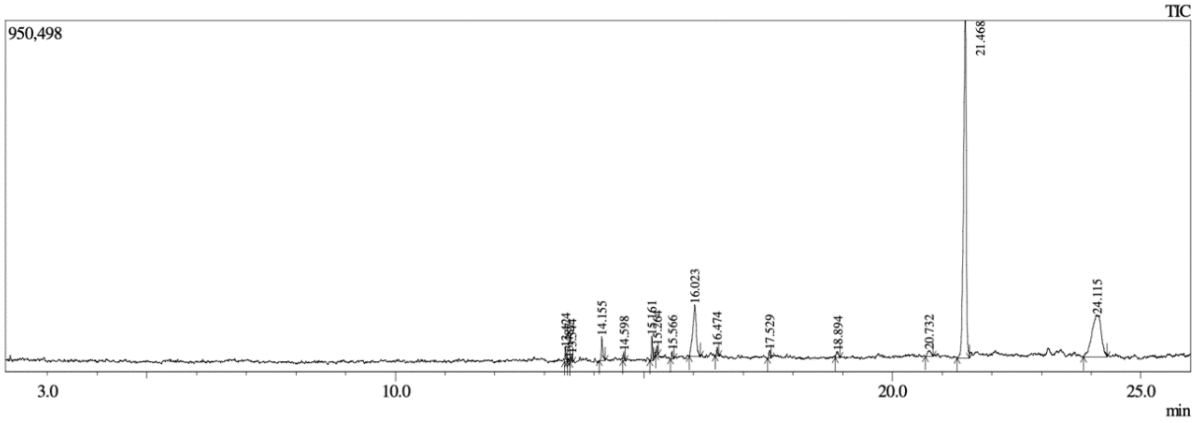

Figure 1. GC-MS Chromatogram of Phytochemicals in n-hexane leaf extract of Cocos nucifera L.

Table 3. Phytocomponents identified by GC-MS analysis of methanol leaf extract of Cocos nucifera L.

Sr. No.	R. T	Area%	Name of Compound	Molecular formula	Mol. weight
1.	9.045	9.06	Ethanone, 1-(2-hydroxy-5-methylphenyl)	$C_9 H_{10} O_2$	150
2.	13.425	22.73	Neophytadiene	$C_{20} H_{38}$	278
3.	13.495	3.49	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]	$C_{20} H_{40}$	280
4.	13.590	4.59	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]	$C_{20} H_{40} O$	296
5.	13.715	7.75	Neophytadiene	$C_{20} H_{38}$	278
6.	14.150	7.20	Hexadecanoic acid	$C_{16} H_{32} O_2$	256
7.	15.160	3.87	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]	$C_{20} H_{40} O$	296
8.	21.420	29.45	Squalene	$C_{30} H_{50}$	410
9.	24.035	6.08	A'-Neogammacer-22(29)-en-3-one (Hopenone b)	$C_{30} H_{48} O$	424

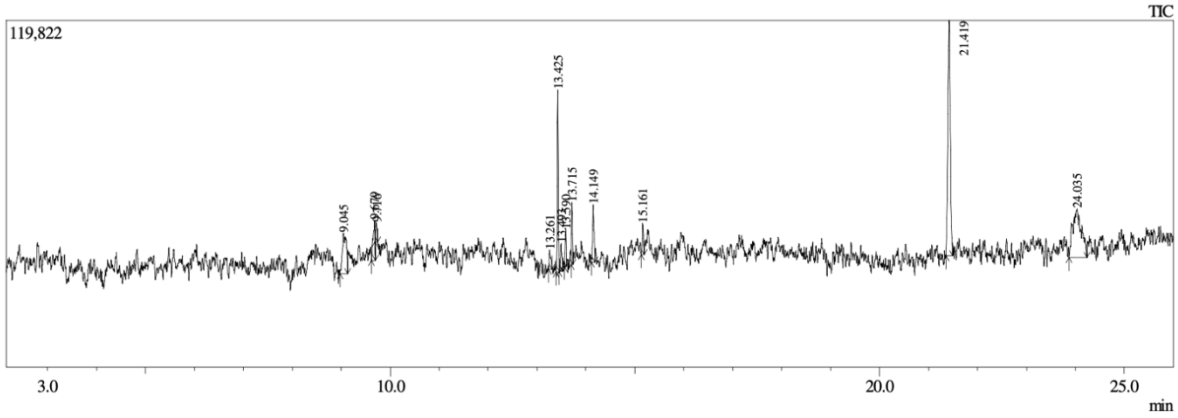

Figure 2. GC-MS Chromatogram of Phytochemicals in methanol leaf extract of Cocos nucifera L.

4. Discussion

As shown in Table 4 and Table 5, the triterpenoids like Squalene identified in n-hexane (55.84% peak area, R.T. 21.468) as well methanolic (29.45% peak area, R.T. 21.420) extracts are found to have Antitumor, Antibacterial, Antioxidant, Pesticide, Cancer preventive, Lipoxygenase inhibitor, Hypocholestremic, Immunostimulant activities. Hexadecanoic acid (1.97% peak area, R.T. 14.155) in n-hexane and (7.20% peak area, R.T. 14.150) in methanol extract, is a saturated fatty acid that exhibits Antioxidant, Anti-cancer, Hypocholesterolmic, 5-alphareductase, Nematicide, Lubricant, inhibitors activity, Pesticide, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor activities. Vitamin E (alpha tocopherol) (16.023% peak area, R.T. 10.68) in n-hexane extract is an immunity booster and acts as an Antioxidant. Moretenol (23.74% peak area, R.T. 24.155), a triterpenoid found in n-hexane extract possesses Antimycobacterial, Antiprotozoal, Anti-inflammatory activities. Neophytadiene, a sesquiterpenoids is found to show Antimicrobial, Antivenom, Antidepresseur Anti-inflammatory and Neuroprotective activities in methanol extract with 22.73% peak area at 13.425 R.T. Ethanone, 1-(2-hydroxy-5-methylphenyl) belonging to Alkyl Phenyl Ketone class acts as 17-beta-hydroxysteroid dehydrogenase-Inhibitor, Testosterone-Hydroxylase-Induc cer and Aryl-Hydrocarbon-Hydroxylase-Inhibitor having peak area of 9.06% and 9.045 R.T. A'-Neogammacer-22(29)-en-3-one (Hopenone b) in methanolic extract have been reported as Hypocholesterolemic and antioxidant at 6.08 R.T and 24.0359 % peak area. Trans Phytol (2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]] identified in both n-hexane (1.58% peak area, R.T. 15.161) as well as methanol (3.87% peak area, R.T. 15.160) extract is a Neuroprotective, Antimicrobial, Anti-inflammatory, Anticancer, Anti-diabetic, Antioxidant, Anti-diuretic properties, Antidepressant, Anticonvulsant. Therefore, n-hexane and methanol extracts of leaves of *Cocos nucifera L.* are found to possess various biological activities and recommended for further studies.

Table 4. Reported Biological activity and class of compounds for phytochemicals identified in hexane leaf extract of Cocos nucifera L.

Sr. No.	Name of the Compounds	Class of Compounds	Reported Biological activity	Reference
1.	Hexadecamethyl heptasiloxane	Organosiloxane	Antibacterial, Antimicrobial	[12], [13]
2.	Hexadecanoic acid	Palmitic acid (Saturated fatty acid)	Antioxidant, Antiandrogenic, Nematicide, Hypocholesterolemic, Pesticide, Lubricant, Flavor, Hemolytic, 5-Alpha reductase inhibitor activities	[5], [14], [15]
3.	Eicosamethyl cyclodecasiloxane	Organohetero silanes	Antimicrobial, Antihelmintic, Antioxidant	[16]
4.	Trans Phytol [2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-]	Acyclic diterpenoids	Antimicrobial, Anti-inflammatory, Anticancer, Anti-diabetic, Antioxidant, Anti-diuretic properties, Antidepressant, Anticonvulsant, Neuroprotective	[17]–[22]
5.	Cis-Octadec-9-enal	Fatty aldehyde	Flavouring agent	[23], [24]
6.	Tetracosamethyl cyclododecasiloxane	Organohetero silanes	Hepatoprotective, Antispasmodic, Antirheumatic	[16]
7.	alpha-Tocopherol	Vitamins	Immunity Booster, Antioxidant	[25]–[27]
8.	Hexadecamethyl heptasiloxane	Organosiloxanes	Antimicrobial	[16]
9.	1H-Purin-6-amine, [(2-fluorophenyl)methyl]	Alkaloids or Purines	Antimicrobial, as a potent mechanism-based inhibitor of several enzymes like cholesterol acyltransferase, acyl coenzyme A, monoamine oxidase, Antifungal	[28], [29]
10.	Squalene	Triterpenoids	Antitumor, Antibacterial, Antioxidant, Pesticide, Cancer preventive, Lipoxygenaseinhibitor, Hypocholestremic, Immunostimulant	[30], [31]
11.	Moretenol	Triterpenoids	Antimycobacterial, Antiprotozoal, Anti-inflammatory	[32], [33]

Table 5. Reported Biological activity and class of compounds for phytochemicals identified in methanol leaf extract of Cocos nucifera L.

Sr. No.	Name of the Compounds	Class of Compounds	Reported Biological activity	Reference
1.	Ethanone, 1-(2-hydroxy-5-methylphenyl)	Alkyl Phenyl Ketone	17-beta-hydroxysteroid dehydrogenase-Inhibitor, Testosterone-Hydroxylase-Inducer, Aryl-Hydrocarbon-Hydroxylase-Inhibitor,	[16]
2.	Neophytadiene	Sesquiterpenoids	Antimicrobial, Antivenom. Antidepresseur Anti-inflammatory and Neuroprotective activities.	[34], [35]
3.	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]	Acyclic diterpenoids	Antimicrobial, Anti-inflammatory, Anticancer, Anti-diabetic, Antioxidant, Anti-diuretic properties, Antidepressant, Anticonvulsant, Neuroprotective	[17]–[22]
4.	Hexadecanoic acid	Palmitic acid (Saturated fatty acid)	Antioxidant, Lubricant, Anti-cancer, Hypocholesterolmic, 5-alphareductase, Pesticide, inhibitors activity, Antiandrogenic, Nematicide, pFlavor, Hemolytic, 5-Alpha reductase inhibitor activities.	[36]
5.	Squalene	Triterpenoids	Antitumor, Antibacterial, Antioxidant, Pesticide, Cancer preventive, Lipoxygenaseinhibitor, Hypocholestremic, Immunostimulant	[30], [31]
6.	A'-Neogammacer-22(29)-en-3-one (Hopenone b)	Hopanoids	Antioxidant, Hypocholesterolemic	[37], [38]

5. Conclusion

The preliminary phytochemical screening gives the basic information regarding the presence of compounds in the plants. In the present study, the hexane and methanolic extracts of *Cocos nucifera L.* revealed the positive tests for alkaloids, tannins, terpenoids, and phenols shown in Table 1. Further analysis using GC-MS of hexane and methanol extract of leaves of *Cocos nucifera L.* showed the presence of significant bioactive constituents like Squalene, Hexadecanoic acid, Moretenol, alpha-Tocopherol, Hexadecamethyl heptasiloxane, Eicosamethyl cyclodecasiloxane, Neophytadiene, Trans Phytol, 2-hexadecen-1-ol, 3,7,11,15-tetramethyl-[R-[R*,R*-(E)]], A'-Neogammacer-22(29)-en-3-one and others. These constituents exhibited important biological activities like Antitumor, Antioxidant, Immunostimulant, Antimycobacterial, Antiprotozoal, Anti-inflammatory, Antidepressant, Nematicide, Hypocholesterolemic, Pesticide, Neuroprotective, Lubricant activities and others. They should be further explored for various pharmaceutical and pharmacological applications.

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