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GC-MS and HPLC analysis of *Cocos nucifera* (L.) flowers

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Abstract
The phytochemical analysis of flowers of *Cocos nucifera* was investigated. GC-MS also helpful to find out component present in *Cocos nucifera* flower extract. The chemical components identification is studied from the flower extract on its retention time and their mass spectrum supports the data from Wiley library.The quantification of polyphenolic compounds are also identified with the help of HPLC analysis. In conclusion, *Cocos nucifera* flower extract contains various kinds of phytochemicals.

Keywords: GC-MS, flower, compounds, HPLC

1. Introduction
Cocos nucifera (L.) is a significant member of the family Arecaceae (Palm family) popularly known as Coconut, coco, coco-da-bahia, or Coconut-of-the-beach. The plant is basically from Southeast Asia (Malaysia, Indonesia and the Philippines) and the islands between the Indian and Pacific Oceans (Lima *et al*., 2015) [7]. Every part of it is advantageous to mankind for numerous functions including food, drinks, fibers, building materials and chemicals discovering their way into an immense range of modern day products. The coconut maintain a source of meat, milk, oil, fibers, vitamins and minerals provides enormous health assistance beyond its nutritional content.
Solangih and Iqbal (2011) [14] revealed that the constituents of the liquid albumen were identified as vitamin B, nicotinic acid (B3, 0.64 mg/ml), pantothenic acid (B5, 0.52 mg/ml), biotin (0.02 mg/ml), riboflavin (B2, 00.01 ng/ml), folic acid (0.003 mg/ml), with trace quantities of vitamins B1, B6, and C, pyridoxine, thiamine, folic acid, amino acids, L-arginine, plant hormones (auxin, 1,3-diphenylurea, cytokinin), enzymes (acid phosphatase, catalase, dehydrogenase, diastase, peroxidase, RNA polymerases), and growth-promoting factors (Solangih and Iqbal, 2011) [14].
The existence of 1-butyl-2-cyclohexenol (46.84%), benzaldehyde (4.42%) and Globulol (4.07%) revealed from *Goniothalamus umbrosus* of ethyl acetate extract analysis of GC-MS (Abdelwahab *et al*., 2009) [1]. By GC-MS method in four *Staphylea* species, it found four tocopherols, three sterols, amyrine, cycloartenol, actinidiolide and linolenic acid (Lacokova *et al*., 2007) [6]. Zhen *et al*. (2008) [15] reported the phytohormones from coconut water. They were determinate the various classes phytohormones, including indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), abscisic acid (ABA), gibberellic acid (GA), zeatin (Z), N6-benzyladenine (BA), α-naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) in young Coconut water (CW). The present work to study about the phytochemical constituents of *Cocos nucifera* flower extract and to find out the GC-MS and HPLC profiling.

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

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

2.3. GC-MS Analysis
GC MS analysis of these extracts was carried out by following the method of Hema *et al.* (2010) [5].Chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Elite-1, fused silica capillary column (30 m ´ 0.25 mm ID ´ 1 m df, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 2 ml was employed (Split ratio of 10:1) injector temperature 250°C; ion-source

temperature 280°C.

The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass.

2.4. Quantification of polyphenolic compounds by HPLC

High performance liquid chromatography (HPLC) LC-20AT prominence gradient system Shimadzu, Kyoto, Japan was used for the present study. Volume injection was 10µl in the gradient system. Mobile phase consists of water: methanol: acetic acid (25:74:1). The flow rate of the samples 0.8 ml/min as well as standard and sample were detected in the range of 280 nm. The stock solutions of standard and samples were maintained at 5.0mg/ml of methanol. Identification of the compounds was done by comparison of their retention time with Gallic acid.

3. Results

3.1. GC-MS Analysis of ethanol extract of *Cocos nucifera* L.

The GC-MS analysis revealed the presence of compounds from the ethanol extract of flower of *Cocos nucifera*. On comparison of the mass spectra of the constituents with the NIST library, the ç-Sitosterol, Stigmast, quercetin and fatty acid components also observed such as Hexadecanoic acid, 6-Octadecenoic acid (Fig. 1-3). The above mentioned isolated compounds from the methanol extract of *Cocos nucifera* flower seem to posses the reported biological activity (Fig 1 to1b).

Library Search Results

SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
784	806	STIGMAST-5-EN-3-OL, (3á,24S)-	44.22	C29H50O	414	0.29
783	805	ç-Sitosterol	44.22	C29H50O	414	0.29
777	816	Stigmast-5-en-3-ol, (3á)- (CAS)	33.87	C29H50O	414	0.29
747	769	Stigmast-5-en-3-ol, (3á)- (CAS)	33.87	C29H50O	414	0.29
741	862	Stigmast-5-en-3-ol, (3á,24S)- (CAS)	44.22	C29H50O	414	0.29
716	793	á-Sitosterol	33.87	C29H50O	414	0.29
706	746	Stigmastan-3-en-6-ol	4.82	C29H50O	414	0.29
695	745	I-22,23-Dihydrostigmasterol	3.30	C29H50O	414	0.29
673	758	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	1.30	C33H54O3	498	0.29
672	767	1-Heptatriacotanol	1.25	C37H76O	536	0.29

Fig 1: GC-MS analysis of ethanol extract of *Cocos nucifera* L.

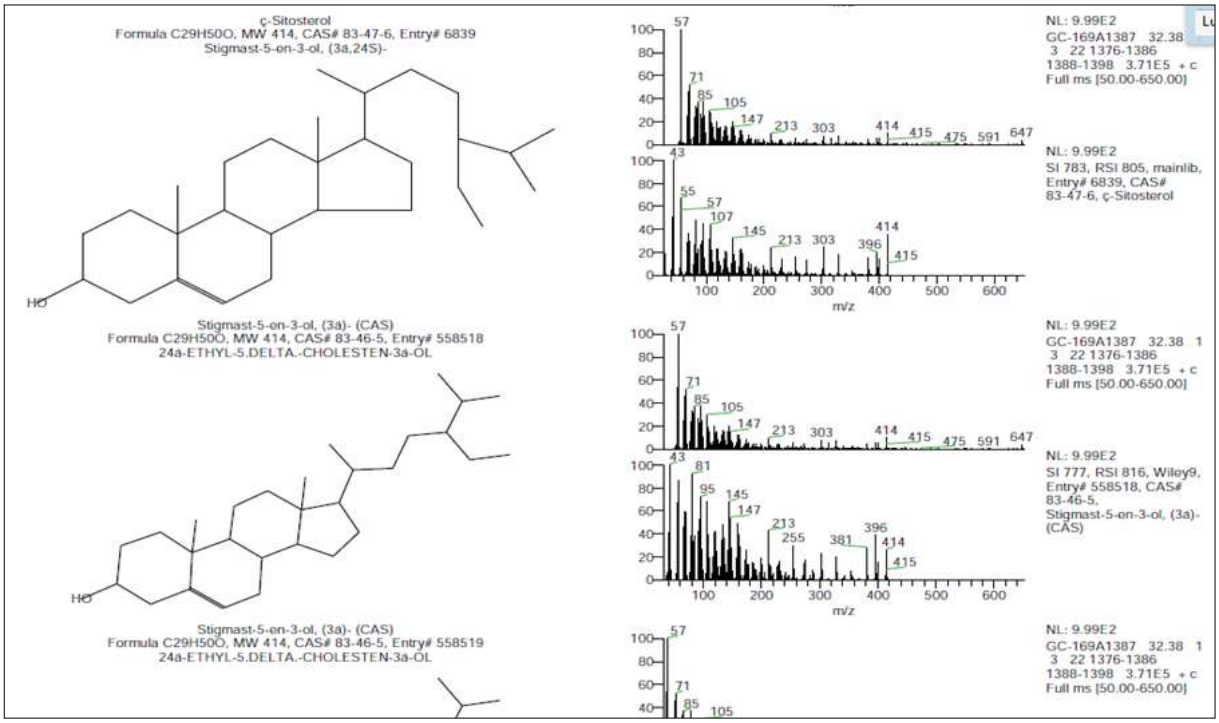

Fig 2: Spectrum analysis

Library Search Results

SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
747	759	QUERCETIN 7,3',4'-TRIMETHOXY	22.06	C18H16O7	344	0.04
739	803	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3á,5Z,7E)- (CAS)	16.46	C30H52O3Si	488	0.04
739	803	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3á,5Z,7E)-	16.46	C30H52O3Si	488	0.04
734	735	Lucenin 2	13.26	C27H30O16	610	0.04
725	738	DI-(9-OCTADECENOYL)-GLYCEROL	9.63	C39H72O5	620	0.04
721	743	9-Octadecenoic acid (Z)- (CAS)	8.13	C18H34O2	282	0.04
712	740	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)- (CAS)	5.90	C27H52O4Si2	496	0.04
712	740	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	5.90	C27H52O4Si2	496	0.04
710	734	Oxiraneoctanoic acid, 3-octyl-, cis- (CAS)	5.44	C18H34O3	298	0.04
701	746	1-Monolinoleoylglycerol trimethylsilyl ether	3.95	C27H54O4Si2	498	0.04

Fig 3: GC-MS analysis of ethanol extract of *Cocos nucifera* L.

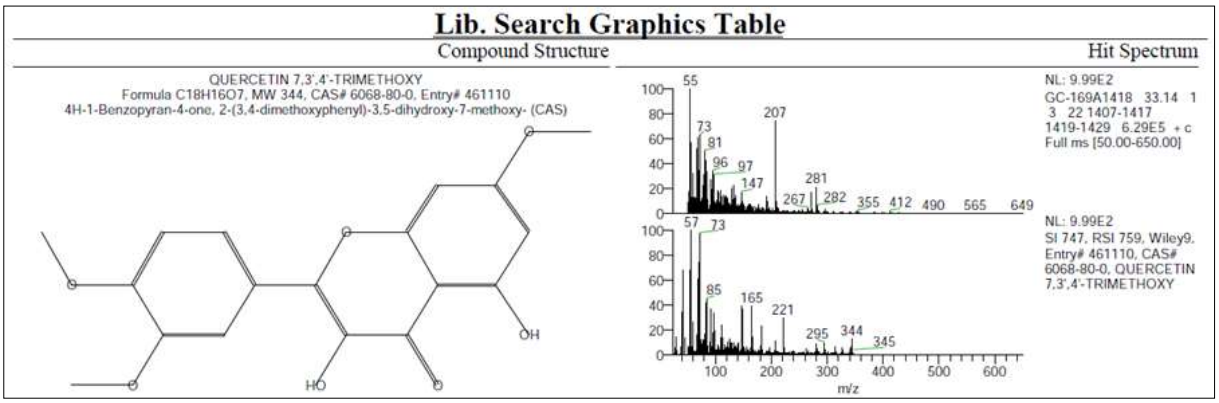

Fig 4: Mass spectrum analysis

Library Search Results

SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
441	488	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- (CAS)	10.88	C30H50	410	14.31
429	618	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- (CAS)	7.24	C30H50	410	14.31
429	618	2,6,10,14,18,22-TETRACOSAHEXAENE, 2,6,10,15,19,23-HEXAMETHYL-	10.88	C30H50	410	14.31
424	617	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- (CAS)	7.24	C30H50	410	14.31
420	472	Squalene	7.24	C30H50	410	14.31
417	667	1-Bromo-3,7-dimethyl-2,6-octadiene	4.82	C10H17Br	216	14.31
417	667	Geranyl bromide	4.82	C10H17Br	216	14.31
411	457	Supraene	10.88	C30H50	410	14.31
410	604	3,7,11-Tridecatrienenitrile, 4,8,12-trimethyl-	3.69	C16H25N	231	14.31
409	603	3,7,11-Tridecatrienenitrile, 4,8,12-trimethyl- (CAS)	3.69	C16H25N	231	14.31

Fig 5: GC-MS analysis of ethanol extract of *Cocos nucifera* L.

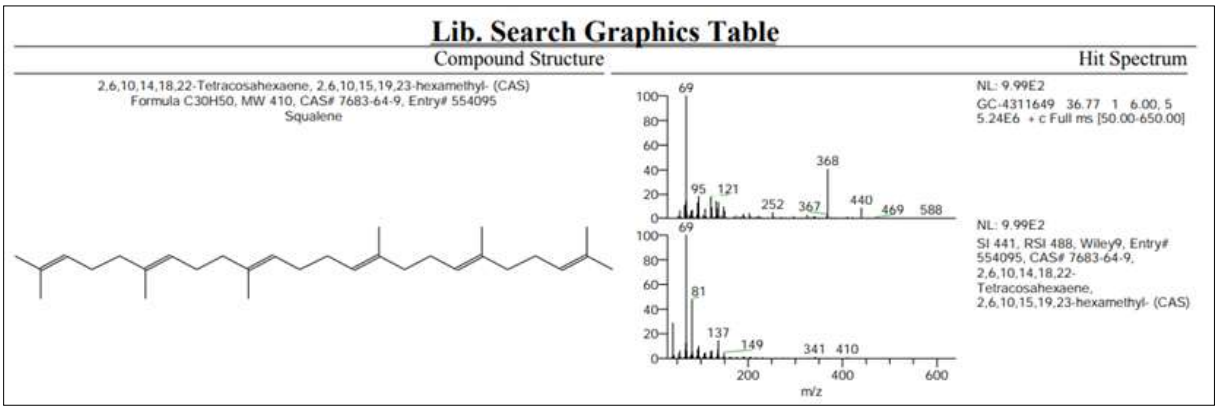

Fig 6: Mass spectrum analysis

3.2. HPLC analysis of ethanol extract of *Cocos nucifera* L.

In this study, ethanol extract was subjected to HPLC analysis for determination of phytoconstituents Peaks of chromatograms were confirmed by comparing its retention time with those of reference standards. Gallic acid was detected at 3.691minutes and furthermore unidentified compounds were detected. The result was depicted in Table 4and Fig 4.

Table 4: Quantitative phytochemical analysis of *Cocos nucifera* l. flowers

S. No	RT	Area	% Area	Height
1	2.481	67611758	81.68	1042630
2	3.691	11120157	13.43	765519
3	4.087	3991210	4.82	215309
4	5.635	27893	0.03	3281
5	9.259	21487	0.03	736

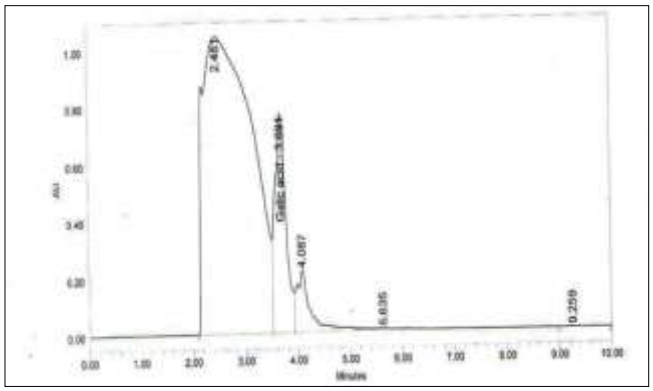

Fig 4: Percentage of phenolic activity of *Cocos nucifera* flowers

4. Discussion

In this study, next part of the investigation was GC-MS analysis of ethanol extract. According to highest phytochemicals constituents, ethanol extract was selected. On comparison of the mass spectra of the constituents with the NIST library, the ç-Sitosterol, Stigmast, Squalene quercetin and fatty acid components also observed such as Hexadecanoic acid, 6-Octadecenoic acid. The above mentioned isolated compounds from the methanol extract of *Cocos nucifera* fruits seem to posses the reported biological activity. In recently, Dhanya *et al.* (2018) [4] also observed the Squalene from Coconut shell extracts, which was responsible for the anticancer and antioxidant activity.

Gallic acid is a well-known antioxidant and antitumor agent (Daglia, 2012; Reedy *et al*., 2012) [2-11]. Its antimicrobial activity was also reported against Gram (+) bacteria including *S. aureus* (Saavedra *et al*., 2010) [13]. The authors have shown that the pure Gallic acid was more efficient than a commercial antimicrobial product, where it was used in association with streptomycin, demonstrating a synergic effect. In the present study, Gallic acid was detected with HPLC analysis. Davi *et al*. (2013) [3] were determined the Gallic acid from *Cocos nucifera.*

In this scenario, the screening of plant extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases, and about 20% of the plants or their extracts in the world have been submitted to biological or pharmacological tests (Rayne & Mazza, 2007) [10]. *Cocos nucifera* had number of volatile substance, which showed antimicrobial activity against bacteria and fungi (Luz *et al*., 2007) [8]. Manisha and Shyamapada (2011) [9] was reported inhibited the *Candida* species with *Cocos nucifera* containing volatile substance. In this present study, clinical isolates of bacterial genera and fungal were suppressed by the ethanol extract of *Cocos nucifera.* Oxidation is commonly known as chemical reaction and transfer electrons from the substance to oxidizing agent (Rishi and Sneha, 2012) [12].

The present study was carried out to explore antimicrobial, antioxidant, and anticancer potential of *Cocos nucifera*. Maximum antioxidant and antimicrobial activities were observed for ethanol extract which was correlated with flavonoids, phenols, tannins and carbohydrate, sterols and terpenoids contents. Bioactivity-guided screening of ethanol extracts revealed the presence of Squalene components, fatty acid and other volatile substance and all known to have useful bioactivities. This finding shows the importance of screening medicinal plants for antimicrobial, and antioxidant agents against resistant bacterial strains. The reported results should pave the way for more investigations.

5. Conclusion

The GC-MS study revealed that the presence of Sitosterol, Stigmast, quercetin and fatty acid components found in ethanol extract. Furthermore, HPLC analysis was carried out for determination of Gallic acid. These findings enhance the potential of *C. nucifera* flowers as an effective food supplement for improving the efficacy of different nutraceutical and pharmaceutical products for which further validation of the isolated flavonoids is warranted in the preclinical and clinical stages.

6. Acknowledgement
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7. References

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