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Research Article

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Phytochemicals Screening and Antioxidant Activity of *C.nucifera* Flower Extracts

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Abstract

Coconut flower was collected. 200 ml chloroform was added, magnetic stirring (continuous stirring) was done for three days. 5 ml of DMSO and ethanol were added to dissolve the sample. 52.5mg of Hydrochloric acid (35% HCL 1.5ml) was mixed with 298.5ml of distilled water, which was added to the *Coconut flower* and kept in a magnetic stirrer (continuous stirring) for 3 days. After the extraction coconut sample was filtered, to the extract 5 to 10ml of ammonia was added altogether this was shaken in a separating funnel for 15 minutes. To this equal volume of chloroform was added and continue the shaking for another 15 minutes. The sample was further allowed to settle for 30 minutes in the separating funnel for complete separation of water and chloroform layer. Qualitative phytochemical screening of *C. nucifera* L. extract was performed by standard methods. *In vitro* antimicrobial activity was done in both chloroform extraction and acid base extraction by agar well diffusion method. Results observed after 24 hours incubation at 37°C showed significant inhibition. The chloroform extract of the flowers of *Cocos nucifera* L. was subjected for identification of bioactive compounds by GC-MS analysis. A total of nine major compounds were identified, majority of which were alkaloids. The extracts were subjected to frap assay for estimation of antioxidant. The result showed good antioxidant activity. The study for first time has reported antioxidant and antibacterial properties of chloroform and acid base extract of coconut flower extract. The study concludes at secondary metabolites alkaloids, tannins, flavonoids, terpenoids, cardiac glycosides present in the extract might have exhibited the antioxidant and antibacterial properties.

Keywords: C.Nucifera, Phytochemicals Screening, Antioxidant activity, GC-MS.

Introduction

Many source of modern drugs were isolated from the natural resource. 80% of the world's population used medicinal plants as the only available medicine especially in developing countries. (Bhagunt *et al.*, 2008). 4000 years of ago the use of Coconut for food and its application in Ayurvedic medicine were documented in India in Sanskrit (Dayrit 2007). 90% of the coconut production in India are seen in four south Indian states namely Kerala, Tamil Nadu, Karnataka, Andhra Pradesh (DebMandal and Mandal 2011). Coconuts are around 3000 million from the export earnings derived by India. Coconut is an ecofriendly crop which permits coexistence of multi-species plants. It enriches soil fertility in association with other crops. The source of various chemical compound is the versatile coconut tree (DebMandal and Mandal 2011). There are two groups of coconut, tall and dwarf. The tall varieties grow slow. It bear fruit 6 to 10 years after planting. Its copra, oil and fiber are of good quality. It hardy, and lives up to 120 years. As male flowers mature earlier than the female flowers. This type is highly cross-pollinated. Nuts mature within period of 12 months after pollination. The dwarf varieties are fast growing and bear early 4 to 5 years. Due to overlapping of male and female phases, the dwarf varieties are self-pollinated. The nuts are yellow, red, green, orange colored. These are less hardy and require favorable climatic conditions and soil type for better yield (DebMandal and Mandal, 2011).

C. nucifera L. belongs to the family Arecaceae, sub family Cocoideae (DebMandal and Mandal 2011). *C. nucifera* L. is called as Coconut tree, is a perennial monocot tree. The tree was first native in the Southest Asia and Melanesia. It was widely distributed in the tropics and sub- tropics in the world (Chan and Elevitch 2006). In the tropical regions, the Coconut *(C. nucifera* L.) is an important fruit tree. Fruit can be used in variety of foods and beverages. The endosperm tissue, is the edible part of the Coconut fruit (Yong *et al.*, 2009). The coconuts cotyledon is also known as Coconut Apple, Sprout and Pearl, It is a white or creamy, spongy structure, formed during the germination of zygotic embryo. They form the basis of nutrition for the developing plant. Cotyledons possesses parenchyama cells with few vascular tissues. Even though the coconut cotyledons are consumed by people at large, they have been explored only for their role in clonal propagation (Nguyen *et al*., 2015).

Pharmacological properties such as antioxidant, antitumor, anti-helminthic, anti-inflammatory, anti-arthritic, anti-diabetic and antimicrobial activities were reported to have various constituents of *C. nucifera* L. (Lima *et al*., 2015). *Coconut flower* nectar is full of vitamins and minerals which contains 17 amino acids, minerals and vitamin C; Has a broad spectrum of B vitamins (vitamin B1, B2, B3 and B6); and is high in potassium, magnesium, zinc and iron (Ebuna *et al*., 2002 ; Hussein *et al*., 2016). Low glycemic sap extruded from the Coconut blossoms is available as *Coconut flower* nectar syrup. The *Coconut flower* is available as Ayurveda formulations and value added products for human consumption (Soumya *et al*., 2014). Nutritionists said that coconuts contain vitamins, electrolytes, fiber and few minerals like potassium, phosphorus & manganese (Nneli and Woyike, 2008). Many inflammatory disorders like postnatal changes are healed by the flowers of *C. nucifera* which also has a traditional use. Food technology based studies are underway to use its powder as an alternate to wheat flour by considering its gluten free mature, nutritional value and natural sweetness. The flower of *C. nucifera* (L.) may also be a natural and delicious alternative to wheat and grain. Which is not explored yet. In India, the coconut flower infusions as tea are used for treating the disorders associated with menstrual cycle (Berlin Grace and Monisha 2022).

The knowledge on the bioactive phytochemicals and their pharm ecological effects in the extracts of Coconut flower are few. In this study, the traditional usage of Coconut flowers as medicinal agent provoked us to explore the bioactive

compounds and evaluate its antibacterial properties (Soumya *et al.*, 2014).

Materials and methods

Sample Collection:

Coconut flower (*Cocos nucifera*) was collected from local market.

Plant source and solvent extraction:

Chloroform extraction: Chloroform extraction was performed as follows. 100gm Coconut flower was taken, and mashed well. 200 ml chloroform was added. Magnetic Stirring (continuous stirring) was done for 3 days. If required 120 ml of chloroform was added during stirring. Weight of the extract before evaporation and after evaporation was found to be (51.34gm & 54.60gm respectively). 5 ml of DMSO and ethanol were added to dissolve the sample. Phytochemical test was also performed.

Acid base extraction: Acid base extraction was performed as per the method followed by (Youbin *et al.*, 2014)

100gm Coconut flower was taken, and mashed well. 52.5mg of Hydrochloric acid (35% HCL 1.5ml) is mixed with 298.5 ml of distilled water which is added to the coconut flower and keep in magnetic stirrer (continuous stirring) for 3 days. After the extraction coconut sample was filtered, to the extract 5 to 10 ml of ammonia was added altogether shake this in a separating funnel for 15 minutes to this add equal volume of chloroform continue the shaking for another 15 minutes further allow the sample to settle for 30 minutes in the separating funnel for complete separation of water and chloroform layer. Each layer was separated for further analysis.

Qualitative phytochemical test analysis of chloroform extraction and acid base extraction: Qualitative phytochemical analysis of chloroform extraction and acid base extraction was performed as per the method followed by Evans 2009.

Qualitative phytochemical screening of *C. nucifera* L. flower extract.

The different qualitative chemical tests were performed for establishing profile of the extract for its chemical composition. The following standard tests were performed on extracts to detect various phyto constituents present in them.

Alkaloids test:

Alkaloids - Wagner's reagent preparation:

- 1 ml of iodine
- 9 ml of distilled water
- 0.6 g in potassium iodide

Procedure: A clean sterile test tube was taken and 0.5 ml sample, 1 ml of distilled water, 2 ml of Wagner's reagent was added. It was observed for the reddish brown precipitate.

Tannins test:

Tannins reagent:

0.1gm ferric chloride

Procedure: A clean sterile test tube was taken and 5 ml of extract, 0.1gm ferric chloride was added. Tubes were observed for the development of brownish green or blue black color

Saponins test:

Procedure: A clean sterile test tube was taken and 0.3 ml distilled water, 1 ml well shaken extract, a few drops of oil was added.

Flavonoids test:

Flavonoids - NaOH reagent preparation:

- 0.8gm NAOH
- 10 ml distilled water

Procedure:

A clean sterile test tube was taken and 1 ml extract, 1 ml NaOH was added. It was observed for intension yellow color

Terpenoids Test:

Procedure: A clean sterile test tube was taken and 1 ml of extract, 0.4ml chloroform, 0.6 ml sulphuric acid was respectively added. It was observed for the development of reddish brown interface.

Cardiac glycosides test:

Cardiac glycosides – ferric chloride reagent preparation:

- 1ml ferric chloride
- 3 ml distilled water

Procedure: A clean sterile test tube was taken and 1 ml extract, 1 ml distilled water, 500 µl ferric chloride, 500 µl glacial acetic acid was added. Then sulphuric acid was added drop by drop. The presence of the brown ring indicate the presence of cardiac glycosides.

Quantitative alkaloids estimation:

Caffeine standard -1mg/ml

Acidified methanol - 50% methanol (with water + 0.1 ml of glacial acetic acid) Acetic acid - 5% solution
Hydrogen peroxide - 0.1% Cupric chloride - 0.02% Ammonia solution – 15 N

Procedure:

3 ml acidified methanol, 2 ml H_2O_2 ,2 ml cupric chloride, 1 ml 15N ammonia was added in five different test tubes with samples ranges of (100µl,200µl,300µl,400µl,500µl) each. It was incubated at 30 minutes and read at 475 nm. (Vaidya *et al.*, 2020).
Formula:

Concentration of unknown = Absorbance of unknown × Concentration standard

Absorbance of standard

Estimation of Antioxidant by frap:

1% Potassium ferric cyanide:

A sterile beaker was taken and 0.25 grams of potassium ferric cyanide was added in 25 ml of distilled water.
1% Ferric chloride solution:

A sterile beaker was taken and 0.2 grams of ferric chloride was added in 25 ml of distilled water.
10% Trichloro acetic acid solution:

A sterile beaker was taken and 2.5 grams of trichloroacetic acid was added in 25 ml of distilled water.

Procedure:

100 µl of coconut sample was taken and mixed with 400 µl of same solvent in 1:4 dilution.
1.25 ml of phosphate buffer (0.2M) and 1.25 ml of potassium ferric cyanide (1%) was added to the coconut sample and it was thoroughly mixed.
The mixture was incubated in hot air oven at 50°C for 30 minutes.
The reaction mixture was cooled after incubation.
Then 1.25 ml of (10%) trichloroacetic acid was added and it was thoroughly mixed.
The reaction mixture was then transferred to centrifuge tube and centrifuge the same at 3000 rpm for 10 minutes.
After centrifugation, 1.25 ml of upper layer of solution was transferred from the centrifuge tube to a new test tube.
Then 1.25 ml of distilled water was added to the test tube and it was mixed well.
0.5 ml of 1% freshly prepared ferric chloride solution was added to the test tube and it was mixed well and color change was observed.
The absorbance was measured using visible spectrometer at 700 nm.
Ascorbic acid can be used as a standard.

Gas Chromatography- Mass Spectrometry (GC-MS) analysis:

The Thermo GC- Trace Ultra VER: 5.0 (Bremen, Germany) and Mass Spectroscopy (MS) DSQ II electron ionization mode with ionization energy of 70 eV was used in our GC- MS analysis for the chloroform extraction of *Cocos nucifera* L. flowers to identify the biologically important compounds. The column temperature was set to 80°-250° at 8°/ min rate. The temperatures of 280° and 290° were set for the GC injector and MS transfer respectively. The major carrier gas used was is helium and its flow rate was 1.0 ml/min. Then 1 µl volume of sample was used for the GC-MS analysis as per the method adopted by (Berlin Grace and Monisha 2022).

Result

Phytochemical analysis of chloroform extraction:

The phytochemical analysis of the chloroform extract was and acid base extraction performed by standard methods and the results are given in table 1.

Table 1 Results of phytochemicals screening

S.no	Phytochemicals	Chloroform extraction	Acid basextraction
1	Alkaloids	+	+
2	Tannins	-	+
3	Saponins	-	-
4	Flavonoids	+	-
5	Terpenoids	+	+
6	Cardiac glycosides	+	+

The Results of phytochemicals screening

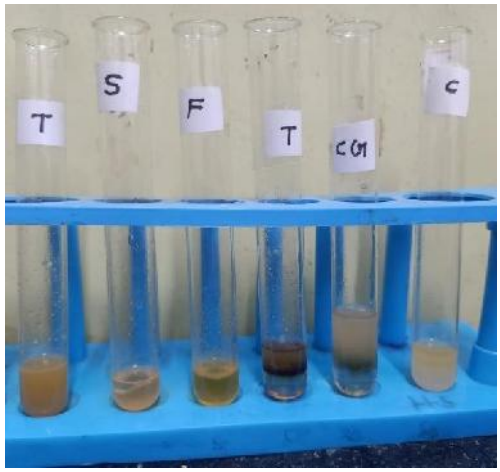


Fig. 1

The phytochemical screening showed development of yellow color confirming the presence of flavonoids and brown color ring confirming the presence of terpenoids, cardiac glycosides in the chloroform extraction. Fig. 1; Table (1)

Phytochemical analysis of acid base extraction:

The phytochemical analysis of the acid base extract was performed by standard methods and the results are given in table1.

The results of phytochemical screening

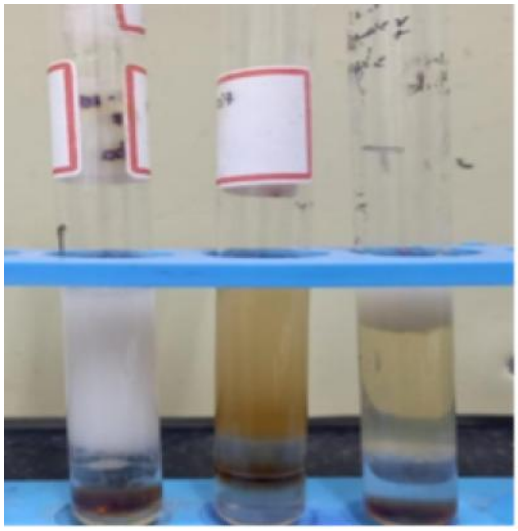


Fig. 2 a (t) b (tp) c (cg) **Fig. 3**

The phytochemical screening showed development of brown color ring confirming the presence of tannins (a), terpenoids (b), cardiac glycosides (c), in the acid base extraction fig 2&3, : Table(1)

Alkaloids test:

The alkaloids test done by the chloroform extract and acid base extract was performed by standard methods and the results are given fig 4.

The results of phytochemical screening

Fig. 4

The phytochemical screening showed development of brown color confirming the presence of alkaloids in the chloroform extraction and acid base extraction fig 6, : Table (1)

Quantitative of alkaloids estimation:

The quantitative analysis of alkaloids estimation done by the chloroform extract and acid base extract was performed by standard methods and the results are given table 2 & 3. Graph 1& 2. Fig 5.

The total alkaloids content of coconut flower was high in acid base extraction, with the OD value of 1.656 and concentration value of 269.268mg. The value of chloroform was low with OD value of 1.084 and concentration value 1.76.260mg.

The results of alkaloids estimation

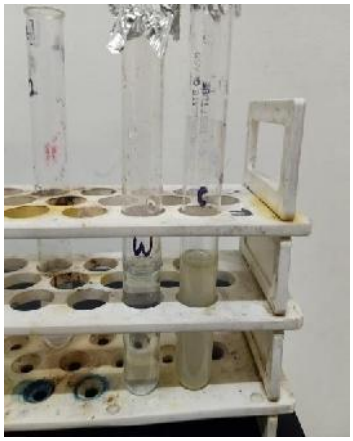


Fig. 5

Table 2: standard curve of alkaloids estimation

S.no	Concentration (µl/ml)	Optical density
S_1	20µl	0.208
S_2	40µl	0.296
S_3	60µl	0.373
S_4	80µl	0.452
S_5	100µl	0.514

Graph 1: standard curve of alkaloids estimation

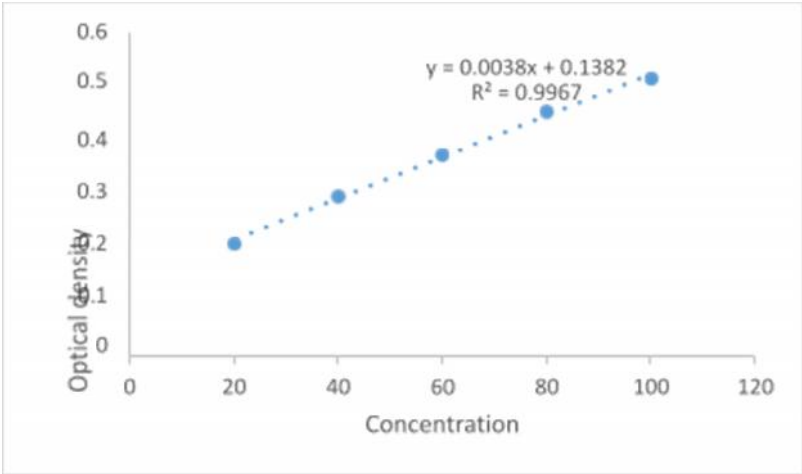
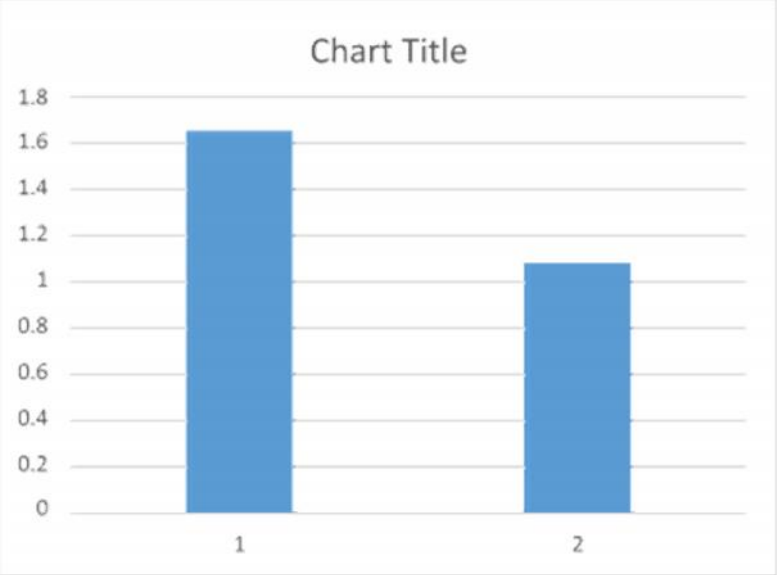


Table 3: Estimation of alkaloids Acid base extraction and Chloroform extraction

Components	Sample volume	Optical density	Concentration volume
Acid base extraction	200µl	1.656	269.268mg
Chloroform extraction	200µl	1.084	176.260mg

Graph 2: Estimation of alkaloids 1. Acid base extraction and 2. Chloroform extraction



Antioxidant frap:

The antioxidant frap was done with the chloroform extract and acid base extract was performed by standard methods and the results are given table 4&5. Graph 3& 4. Fig 6.

The results of antioxidant activity

Color intensity was observed as shown in the fig 8. Which indicates the presence of antioxidant present in the sample.

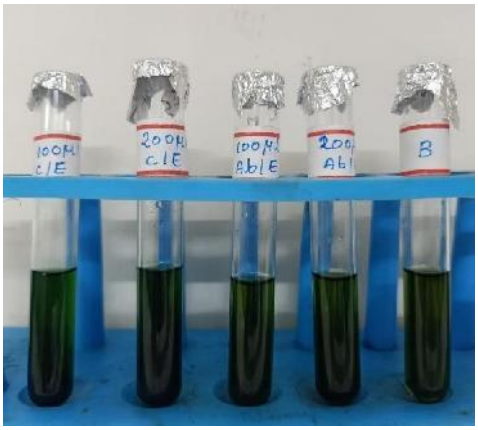


Fig.6

Table 4: Standard curve of antioxidant activity

S.no	Concentration (µg/ml)	Optical density
1	100	0.19
2	200	0.32
3	300	0.58
4	400	0.75
5	500	1.02

Graph 3: Standard curve of antioxidant activity

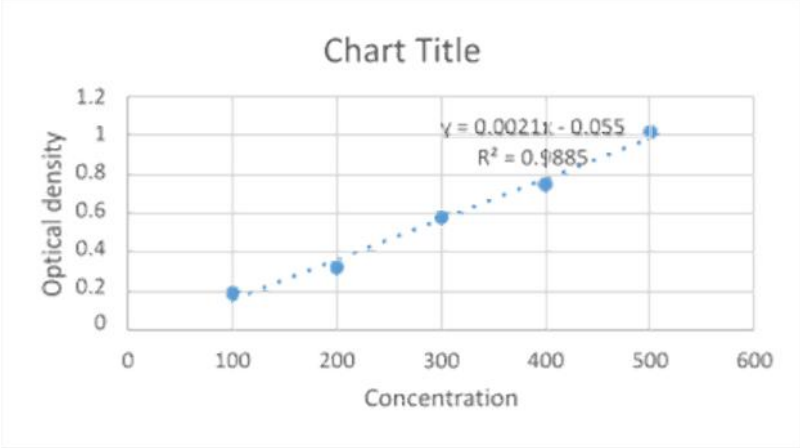


Table 5: Antioxidant activity of Acid base extraction and Chloroform extraction

S.no	Concentration (µl/ml)	Acid base extraction	Chloroform extraction
1	100µl	2.813	2.020
2	200µl	2.837	1.997

Graph 4: Antioxidant activity of Acid base extraction and Chloroform extraction

5.1 Gas Chromatography/ Mass Spectrometry (GC-MS) analysis:

Library Search Report

Data Path : D:\GC-MS-Year-2023\230123_GCMS Screening\
Data File : 1097058.D
Acq On : 23 Jan 2023 17:04
Operator : ADMIN
Sample : 1097058
Misc
ALS Vial : 4 Sample Multiplier: 1

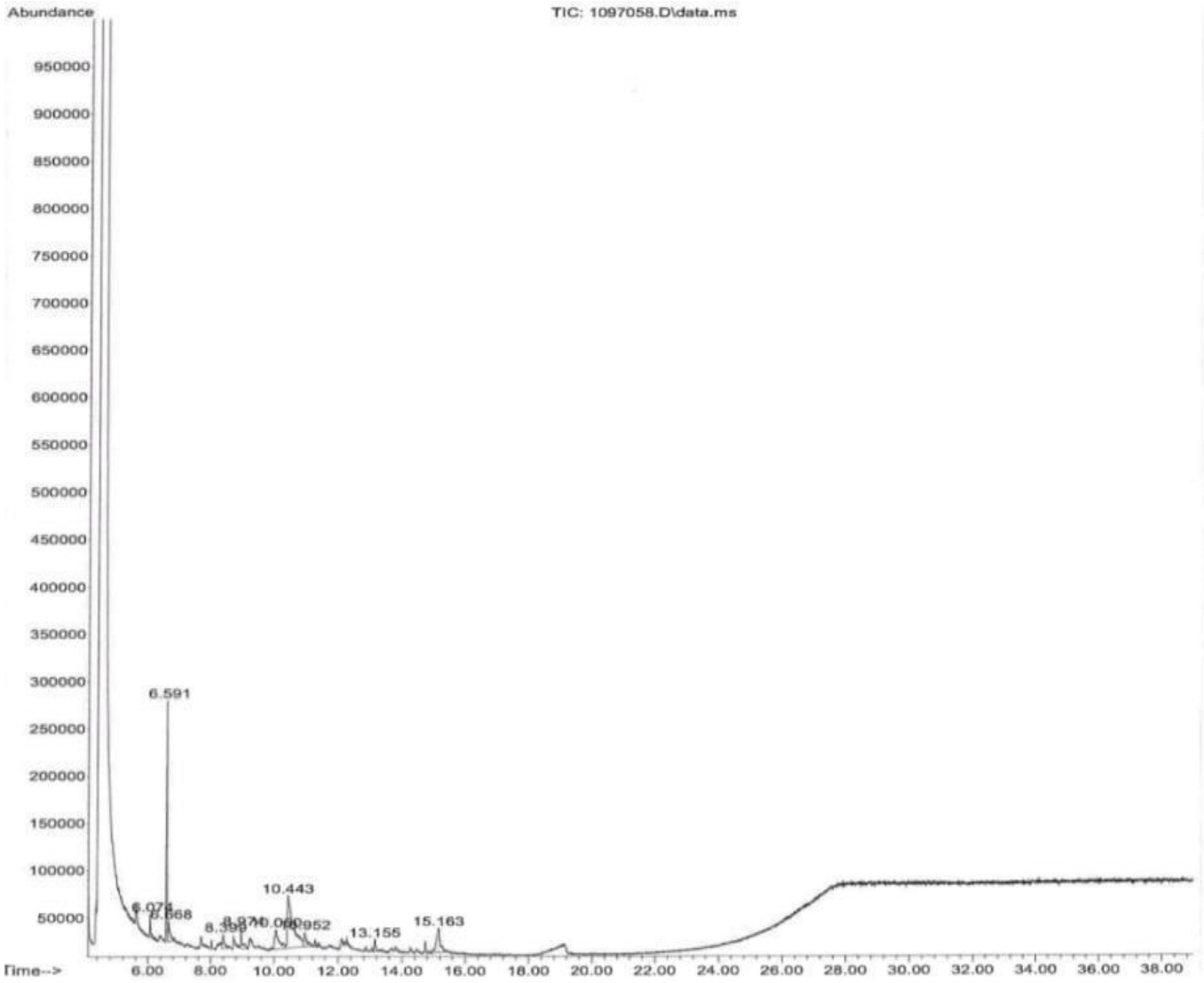
Search Libraries: C:\Database\NIST11.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator 1097058.E

#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	6.073	2.96	C:\Database\NIST11.L			
			2-Imino-3-methyl-6-nitrobenzothiazoline	68799	099700-95-5	37
			Carbazole, 2,4,7-trimethyl-	68726	078787-89-0	35
			Terephthalaldehydic acid, methyl ester, p-(O-methyloxime)	55193	033499-35-3	33
2	6.590	20.84	C:\Database\NIST11.L			
			7H-Dibenzo[b,g]carbazole, 7-methyl	128504	003557-49-1	64
			Cyclotetrasiloxane, octamethyl-	141482	000556-67-2	53
			5H-Naphtho[2,3-c]carbazole, 5-methyl-	128505	100025-44-3	50
3	6.667	4.67	C:\Database\NIST11.L			
			3,4-Difluoroanisole	20797	115144-40-6	64
			N-(3-Chlorophenyl)-2-hydroxyimino-acetamide	60193	1000143-61-6	37
			Propane, 2-chloro-	1009	000075-29-6	27
4	8.398	3.31	C:\Database\NIST11.L			
			7H-Dibenzo[c,g]carbazole	116628	000194-59-2	35
			6-Chloro-2,3-dimethyl-4-phenylquinoline	116526	022609-11-6	25
			Phenylpropylamine, N-acetyl-3,4,5-trimethoxy-	116311	112369-97-8	22
5	8.974	3.67	C:\Database\NIST11.L			
			Cyclopentasiloxane, decamethyl-	196316	000541-02-6	53
			3,4-Dihydroxybenzyl alcohol,tris(trimethylsilyl)-	187625	068595-79-9	46
			Cyclopentasiloxane, decamethyl-	196318	000541-02-6	38
6	10.063	10.21	C:\Database\NIST11.L			
			4-Methyl-6-trimethylsilyloxy-2-trimethylsilylsulfanyl-pyrimidine	132185	1000378-21-9	43
			Oxazole, 2-(1-naphthalenyl)-5-phenyl-	120279	000846-63-9	40
			Silane, dimethyl-2-propenyl(tetradecyloxy)-	154848	077774-33-5	33
7	10.443	35.96	C:\Database\NIST11.L			
			5-Hydroxymethylfurfural	11111	000067-47-0	76
			4-Mercaptophenol	11128	000637-89-8	53
			Benzenemethanol, 3-fluoro-	11296	000456-47-3	52
8	10.954	4.89	C:\Database\NIST11.L			
			6-Acetyl-.beta.-d-mannose	79686	1000130-09-9	12
			2-Piperidinone, 5-(carbamoyloxymethyl)-1-methyl-	50670	1000150-03-9	12
			5-Hydroxymethylfurfural	11110	000067-47-0	11
9	13.154	1.88	C:\Database\NIST11.L			
			1,5-Hexadiene-3-carboxylic acid, 1	48374	1000155-60-6	27

The Gas chromatography/Mass spectrometry (GC-MS) analysis of the chloroform extract was performed by the results are given in table 7.

The GC-MS chromatogram of the chloroform extraction of the *Cocos nucifera* L.



Discussion

The phytochemical derived from medicinal plants have gained significant and considerable recognition as natural non- toxic therapeutic agents in recent past few years. All the valuable phytochemicals proven to have bioactive potency was identified based on the qualitative analysis in the coconut flower extract. Although many studies are available on the application of different plants parts the presence study conducted to know the presence of various phytochemical in the cotyledon of the coconut and evaluated their properties. The results of phytochemical analysis comprehensively validated the presence of therapeutically important phytochemicals. The qualitative analysis was performed in chloroform extract and acid base extract of *Cocos nucifer* L. flower extract. The qualitative phytochemical analysis of chloroform extract showed the presence alkaloids, flavonoids, terpenoids, cardiac glycosides, and acid base extract with the presence of alkaloids, tannins, terpenoids, cardiac glycosides. Alkaloids, terpenoids, cardiac glycosides were extracted in both the solvents irrespective of the solvent and extraction method. Chloroform extract was negative for tannins, saponins and acid base extract was negative for saponins, flavonoids as shown in table 1. Both the extracts showed absence of saponins similar to Kanchana *et al*., 2012 .Yields of different phytochemicals from plants extracts are found to vary depending on the method of extraction and the solvent used Udayaprakash *et al*., 2020.

Earlier studies has identified similar bioactive phytochemicals alkaloids, tannins, terpenoids, cardiac glycosides having valuable therapeutic index in acid base extracts of the flowers of *Cocos nucifera* L. by qualitative phytochemical analysis tests Berlin VM and Monisha M 2022; Kannaian *et al.*, 2020 . The result of the phytochemicals analysis show the presence of therapeutically important valuable secondary metabolites in the coconut flower extract.

Furthermore in the study, the chloroform extract of the flowers of *Cocos nucifera* L. was subjected for identification of bioactive compounds by GC-MS analysis. A total of nine major compounds were identified by their retention time. The national institute of standard and technology library was used for analysis of compounds and the result of GC-MS analysis revealed the presence of a list of compounds which includes few biologically active alkaloids. Alkaloids are wide group of naturally occurring compound with the nitrogen atom in their structure of alkaloids are used for treatment for neurological disorder, cancer and various infection disease.

Different radicals generated during the metabolism of biomolecules in the biological system have the potency to hinder normal activities and lead to various disease (Phaniendra et al., 2015). Hence a balance between oxidants and antioxidants in necessary. The extracts were subjected to frap assay for estimation of antioxidant. The result showed good antioxidant activity. The phytoconstituents such as flavonoids, tannins, protein and poly phenols are known to be responsible for antioxidant activity Hu *et al*., 2016.

The extract were subjected to *in vitro* screening of anti- microbial properties by agar well diffusion method. It was observed that the *C. nucifer* flower extract exhibited higher antimicrobial activity against all the tested bacteria (table 6). The highest zone of inhibition against Salmonella sp. formed in the chloroform extract and acid base extract with zone of inhibition of 22mm & 19mm respectively. The lowest zone of inhibition was against Staphylococcus aureus formed in the acid base extract. Similar results were obtained in in coconut oil extracts by Jeba pritha and Karpagam S 2018 ; and in coconut kernel by Dabesor *et al*., 2017. The significant finding of the study incudes that this is the first studied report on phytochemistry using acid base extraction and chloroform extract of coconut flower and to analyze its antimicrobial activity.

Conclusion

The phytochemical screening of chloroform extract and acid base extract of *Cocos nucifera* L.

flower extract have the demonstrated the presence of alkaloids, tannins, flavonoids, terpenoids, and cardiac glycosides which have many medicinal property. FRAP assay results showed good antioxidant activity. The phytochemical analysis of chloroform extract of Cocos nucifra flower extract reported the presence of bioactive alkaloids which might have contributed to the significant antimicrobial activity. The result of GC-MS analysis revealed the presence of few biologically active alkaloids. Due to the presence of various secondary metabolites coconut flowers are able to exhibit inhibitory effect against the growth of few pathogens The study concludes the coconut flower to be a potent nutrient source with antibacterial activity.

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