Section A-Research paper

Phytochemical, HRLCMS and antitubercular studies on *Hibiscus calyphyllus* flowers

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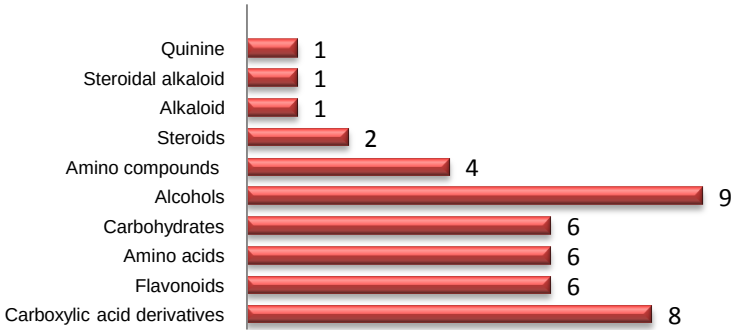
ABSTRACT

The phytochemical constituents present in *H. calyphyllus* flowers were studied by preliminary phytochemical tests and HRLCMS analysis. The HRLCMS analysis revealed the presence of fifty two versatile compounds including eight phenolic compounds, eight carboxylic acid derivatives, six flavonoids, six amino acids, six carbohydrates, nine alcohols, four amino compounds, two steroids, one alkaloid, one steroidal alkaloid and a quinine. The crude extracts of *H. calyphyllus* possess moderate antitubercular activity.



Key words: *Hibiscus calyphyllus*, Phytochemical analysis, HR-LCMS studies, Antitubercular activity

1. Introduction

Hibiscus calyphyllus is a perennial plant and it is commonly known as lemon-yellow rosemallow.[1] The leaves, roots and stems of *H. calyphyllus* are used as a food, medicine and

construction purposes respectively.[2-5] It is cultivated as an ornamental plant in various places and this plant parts are also used to produce oils.[6] Furthermore, natural products play a vital role in the drug discovery process. Simultaneously, the process has some technical barriers to screening, isolation, characterization and optimization. Several technological and scientific developments - including improved analytical tools are aid to overcome the above barriers.[7] In this connection, we have planned to analyze the phytochemical constituents present in *H. calyphyllus* flowers using HRLC-MS studies. In our continuous work on the plant *H. calyphyllus*,[8] the main objectives of the present investigation is to assess the phytochemical profile of *H. calyphyllus* flowers and to analyze antitubercular activity of the crude extracts.

2. Materials and Methods

All the organic solvents used were of analytical grade. High Resolution Liquid Chromatograph Mass Spectrometer (HRLCMS) is recorded on 1290 Infinity UHPLC System,1260 infinity Nano HPLC with Chipcube, 6550 iFunnel Q-TOFs, Agilent Technologies, USA.

3. Experimental

3.1. Plant Material

H. calyphyllus flowers were collected from Pottal Pudur (8°47'29.4"N 77°23'40.3"E), Tenkasi district of Tamil Nadu, India in month of January 2021 and were authenticated by Dr. K. Petchimuthu, Taxonomist, Sri KGS Arts College, Srivaikundam.

3.2. Extraction and isolation

About 500 g of the flowers of *H. calyphyllus* were collected and were immersed with 1 L ethanol for one week. The extract was filtered and the solvent was removed *in vacuo* afforded the 32 g brown pasty mass of the crude extract. It was stored in refrigerator for further analysis. A portion of the extract (25 g) was mixed with 20 mL of hot ethanol and 50 g of silica gel for column chromatography 60-120 mesh to get slurry. The slurry was dried and packed in soxhlet extractor and were consecutively extracted with different solvents namely n-hexane, chloroform, ethyl acetate and acetonitrile. Each of extract obtained from the different solvents were concentrated in rotary evaporator and then stored in refrigerator for further analysis.

3.3. Antitubercular activity

MABA assay to determine the MIC (minimum inhibitory concentration) of the plant extracts against *Mycobacterium tuberculosis* H37Rv was tested. Serial two-fold dilutions of each drug were prepared directly in a sterile 96-well microtiter plate using 100 µl 7H9-S. A growth control containing no antibiotic and a sterile control were also prepared on each plate. Sterile water was added to all perimetre wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags and incubated at 37^{o}C in normal atmosphere. After 7 days incubation, 30 µl of alamar blue solution was added to each well, and the plate was re-incubated overnight. A change in color from blue (oxidized state) to pink (reduced) indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in color.[9-10]

4. Results and Discussion

4.1. Preliminary Phytochemical Analysis

The preliminary phytochemical analysis of different extracts of *H. calyphyllus* flower exposed the presence of phenols, acids, flavonoids, alkaloids, steroids, tannins, saponins, glycosides, and terpenoids and the results are presented in **Table 1.**The crude ethanolic extract contains steriods, reducing sugar, phenolic compound, saponin, xanthoproteins, tannins, flavonoids and aromatic acids.

Table 1: Primary phytochemical analysis of *Hibiscus calyphyllus* flower

S. No	Phyto constituents	Results for different extracts				
		***n*-Heaxane**	**$CHCl_3$**	**EtOAc**	**CH_3CN**	**EtOH**
1	Steriods	--	--	++	++	++
2	Triterpenoids	--	++	--	++	--
3	Reducing sugar	++	--	++	--	++
4	Alkaloids	--	--	++	++	--
5	Phenolic compound	++	--	--	--	++

6	Saponin	++	++	++	--	++
7	Xanthoproteins	++	--	++	--	++
8	Tannins	--	++	++	--	++
9	Flavonoids	--	--	--	++	++
10	Aromatic acids	--	++	++	--	++

4.2. HRLCMS Studies

The HRLCMS analysis revealed the presence of fifty two versatile compounds including eight phenolic compounds, eight carboxylic acid derivatives, six flavonoids, six amino acids, six carbohydrates, nine alcohols, four amino compounds, two steroids, one alkaloid, one steroidal alkaloid and a quinine. This state that the extract contains 15% phenolic compounds, 15% carboxylic acid derivatives, 11% flavonoids, 12% amino acids, 12% carbohydrates, 17% alcohols, 8% amino compounds, 4% steroids, 2% alkaloid, 2% steroidal alkaloid and 2% of quinine and it is represented in **Table 2.** The HRLCMS chromatogram of ethanolic extract of *H. calyphyllus* flowers are shown in **Fig.1** and the percentage of compounds present in the ethanolic extract is shown in **Fig.2.**

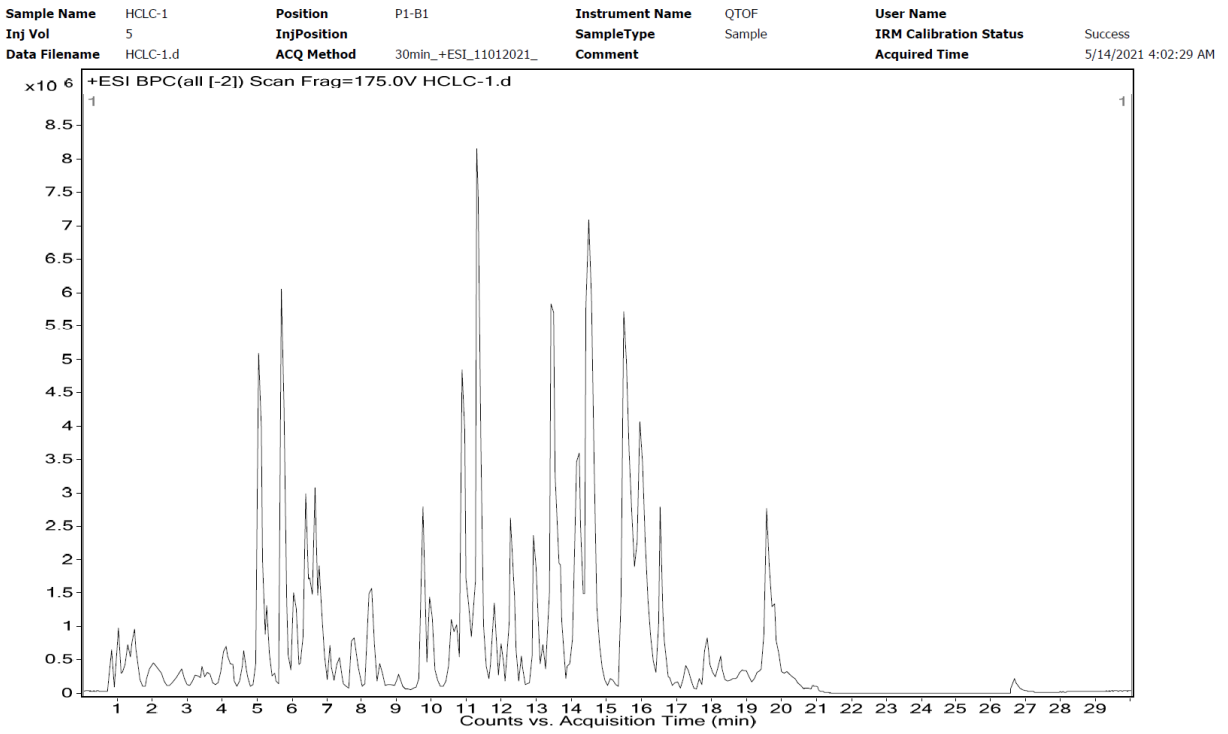


Fig.1. HRLCMS chromatogram of ethanolic extract of *H. calyphyllus* flowers

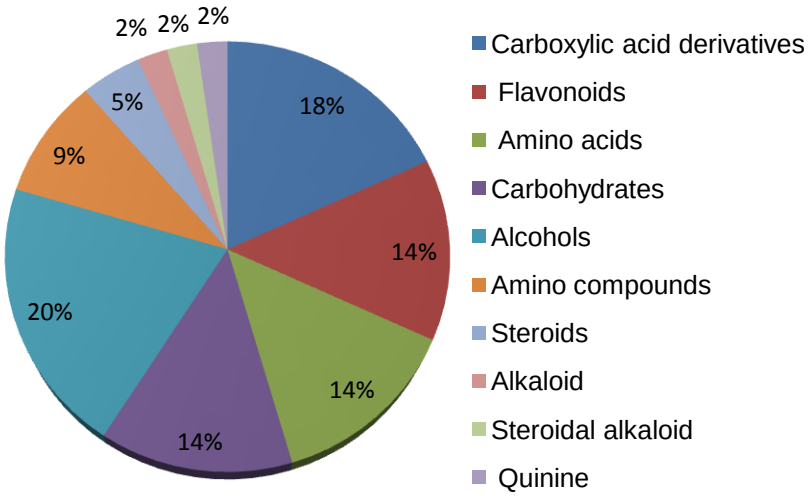


Fig.2. Percentage of compounds present in the ethanolic extract of *H. calyphyllus* flowers

Table 2: HRLCMS data of ethanolic extract of *H. calyphyllus* flowers

Compound Type	**Name of the Compound**	**RT**	**m/z**
Phenolic compounds	Narciclasine	4.063	307.0916
	Alfuzosin	4.53	407.2374
	Ankorine	5.124	336.2157
	Tamoxifen	5.187	354.226
	[4]-Gingerdiol 3,5-diacetate	5.295	352.2105
	Methyl 4,6-di-O-galloyl-β-D-glucopyranoside	6.813	481.0956
	Orthosporin	7.093	219.0645
	Semilepidinoside A	7.361	319.1279
Carboxylic acid derivatives	Saphenic acid methyl ester	5.929	265.0965
	D-Prephenyllactate	6.549	303.0486
	Citrinin	8.225	233.08
	23-Acetoxysoladulcidine	10.586	478.3356
	Cassine	11.138	280.2626
	10,20-Dihydroxyeicosanoic acid	16.539	349.272
	Octadecyl fumarate	17.298	351.288
	Dinoflagellate luciferin	17.902	593.2741
Flavonoids	Formylfusarochromanone	3.991	303.1329
	Glucodistylin	4.005	449.1065
	Tamoxifen	4.92	354.2264
	4’’-O-Acetylafzelin	5.192	495.075
	Dihydromyricetin	6.274	303.0489
	6’’-O-Malonylwistin	6.776	567.096
Amino acids	Arginyl-Proline	1.587	276.143
	N-(1-Deoxy-1-fructosyl)phenylalanine	2.115	310.1277
	D-Phe-Pro-Arg-CH_2Cl	5.569	468.2475
	L-N-(1H-Indol-3-ylacetyl)glutamic acid	7.819	309.0856
	Phytosphingosine	11.309	318.2997
	Pteroyltriglutamic acid	19.961	682.2182
Carbohydrates	3,5-Dihydroxyphenyl 1-O-(6-O-galloyl-beta-D-glucopyranoside)	4.62	423.0906
	Capsoside A	12.693	699.3548
	N-[(4-hydroxyphenyl)methyl]ethoxycarbothio amide 4'-(triacetylrhamnoside)	12.982	501.1903
	Glucosylceramide	14.051	664.4615
	Glucosylsphingosine	14.256	444.3303
	Acarbose	17.063	684.2126
Alcohols	Sphinganine	10.039	274.2732
	1-Tetradecanol	10.508	214.2526
	Ascorbyl stearate	10.805	460.3252
	Hydroxysintaxanthin 5,6-epoxide	11.014	480.3513
	Oleoyl Ethanolamide	11.499	308.2938
	Monoolein	11.687	356.3145
	Hypercalin B	13.257	518.3224
	Misoprostol	14.307	400.3046
	Kolanone	14.587	520.3387
Amino compounds	Dantrolene	5.193	319.0436

	Netilmicin	5.794	475.3236
	Citalopram aldehyde	7.895	295.1217
	3'-Sialyllactosamine	18.218	653.1804
Steroids	3-α,7-β,12-α-Trihydroxy-5alphacholan-24-oic Acid	16.519	426.3196
	Lanceotoxin A	19.605	621.305
Alkaloid	Dipyridamole	15.526	522.3537
Steroidal alkaloid	Solanocapsine	5.807	453.3419
Quinine	Tetracenomycin D1	13.914	337.0699

4.3. Antitubercular activity

The extracts were analyzed for the antimycobacterial activity; however, they showed no significant antitubercular activity and the results are presented in **Table 3.**

Table 3: Antitubercular activity of the crude extracts

S. No.	Compound Code	MIC (µg/mL)
1	HCE - 1	>25
2	HCPE - 2	>25
3	HCC - 3	>25
4	HCEA - 4	>25
5	Compound **I**	0.05
6	Isoniazid	0.1
7	Rifampicin	0.2
8	Ethambutol	1.56

*(HCE – 1, Ethanolic extract; HCPE – 2, n-Hexane extract, HCC – 3, Chloroform extract; HCEA – 4, Ethylacetate extract)

Conclusion

The phytochemical constituents present in *H. calyphyllus* flowers by preliminary phytochemical tests and HRLCMS analysis were discussed and this is the first record of the above studies in this plant. The photochemical investigation and HRLCMS analysis on *H. calyphyllus* ethanolic extract have exposed the presence of 15% phenolic compounds, 15% carboxylic acid derivatives, 11% flavonoids, 12% amino acids, 12% carbohydrates, 17% alcohols, 8% amino compounds, 4% steroids, 2% alkaloid, 2% steroidal alkaloid and 2% of quinine. However, the crude extracts

showed no significant antitubercular activity, the higher concentration in the chromatographic profile of ethanolic extract reveals that, the presence of significant bioactive compounds. From these results, it can be accomplished that ethanolic extract of *H. calyphyllus* flowers have great achievable as biomedicine for some diseases.

Acknowledgement

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