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Characterization of bioactive components from petiole of two varieties of colocasia seen in Kerala

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ABSTRACT

Colocasia is a genus of flowering plant belongs to the family Araceae native to South-Eastern Asia and the Indian subcontinent. Some species are widely cultivated and naturalized in other tropical and subtropical regions. Different varieties of Colocasia are seen in Kerala of which Colocasia aquatilis is labeled as an aggressive weed that grows in dense clusters along lake shores and riverbanks, displacing native shoreline vegetation. Qualitative analyses of two samples are done using a crude extract from both the varieties. Antimicrobial activity of wild variety is evaluated in both grams positive and gram negative bacteria using pure extract. Even though both comprise the same genus Colocasia, significant differences were seen in the result of these varieties. In our work, we have used two common varieties of Colocasia one is edible variety Colocasia gigantea and another one a weed Colocasia aquatilis.

Keywords: *Colocasia, C. gigantea, C. aquatilis, Extraction, Distillation, GC-MS, Qualitative Analysis, Antimicrobial Activity.*

1. INTRODUCTION

Colocasia is a genus of flowering plant in the family Araceae native to south-eastern Asia and the Indian ubcontinent. Some species are widely cultivated and naturalized in other tropical and subtropical region include tarul, elephant-ear, taro, cocoyam, dasheen, chembu, champadhupa, shavigegadde, and eddoe. Elephant-ear and cocoyam are also used for some other large-leaved genera in the Araceae, notably Xanthosoma and Caladium. The generic name is derived from the ancient Greek word kolokasion. It thrives in hot, humid conditions and is found growing in moist forests and wet areas in riparian habitats, riverbanks, along streams, marshes and canals or cultivated near farmhouses, in water fields or as under-planting in coconut groves. It prefers deep, well-drained, friable loams soil particularly alluvial loams, with a high water table. The plant has white, adventitious and fibrous root and a massive, fleshy, starchy modified subterranean stem (corm) at the base. At the apex of the corm is a whorl of petioles bearing large leaves with blades pointing obliquely downwards. Petioles are robust, uniformly light or dark green. Corms consist of the skin, cortex and core; the skin is rough, fibrous and brown coloured or covered with concentric rings of leaf scars and scales. It has purple, white, yellow or pinkish coloured flesh depending on the variety of corm.

2. MATERIALS AND METHODS

The two varieties of Colocasia petiole are collected. The two varieties that we choose are one weed variety the *Colocasia aquatilis* and an edible one the *Colocasia gigantea*. The samples are washed with distilled water and it is chopped into small pieces. The chopped samples are then dried by using Hot Air Oven maintained as 50°C for one day. The dried sample is grinded into powder form by using Mixer Grinder.

The extraction of the sample material for determining the components present is done by using Soxhlet Apparatus using Petroleum Ether as a solvent. The extracted sample is then subjected to Simple Distillation for removing the extraction solvent from the extract obtained. The components present in the two varieties are determined by Gas Chromatography-Mass Spectrometry (GCMS) Analysis.

Phytochemical analysis of two varieties is done for the detection of flavonoids, diterpenes, and phenols. Qualitative Analysis is done for the two varieties for the identification of the presence of components like carbohydrates, proteins, aminoacids, glycosides, starch, resins, alcohols, saponins, taninsetc using the crude extract.

Antibacterial Activity of the weed variety *Colocasia aquatilis* is checked against two categories of Bacteria which are *Staphylococcus aureus*, a Gram positive and *Escherichia coli*, a Gram negative bacteria using the Petroleum ether extract obtained after simple distillation.

2.1 Extraction

The two varieties of Colocasia petiole is extracted by using Soxhlet Apparatus. For the extraction procedure, 22gm each of two samples and 400 ml of Petroleum Ether was used as the extraction solvent. The temperature we set for the extraction process is 50°C because petroleum Ether has a boiling point range of 45-60°C. The process of extraction for one sample completed by 8 cycles in 3 hours.

2.2 Simple Distillation

Simple Distillation is done to remove the Petroleum Ether from the sample extract for getting the clear extract.

2.3 Antibacterial Activity

Antibacterial activity of the *Colocasiaaquatilis* extract is determined using 2 strains of bacteria, one gram-positive *Staphylococcus aureus* and other gram negative bacteria *Escherichia coli*. The 3 petriplates and discs are autoclaved using a pressure cooker for 30 minutes. The nutrient medium is prepared by taking 1.75g nutrient agar in 67.5 ml distilled water and kept at microwave oven for boiling. At an ear bearable temperature, it is poured into the autoclaved petriplates and allowed to solidify. The bacterial culture is prepared by taking a loopful of samples and dissolved in 1ml distilled water and mixed well by using vortex mixer. Taking 200µl of this bacterial sample and pour it into the petriplate and swab it using an L rod. Then insert the paper disc and add 2-3 drops of the *Colocasia aquatilis* extract over it and then kept at the incubator for 24 hours incubation for effective bacterial growth.

2.4 GC-MS Analysis

The phytochemical investigation of petroleum ether extract was performed on a GC-MS equipment Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II. Experimental conditions of GC-MS system were as follows: VF5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. The flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 40°C raised to 250°C at 5°C/min and the injection volume was 1 µl. Samples dissolved in chloroform were run fully at a range of 50-650 m/z and the results were compared by using Nyquist library search programme.

2.5 Phytochemical Analysis

Phytochemicals are chemicals produced by plants through primary or secondary metabolism. We did the phytochemical analysis for the detection of flavonoids, diterpenes, phenols, carbohydrates, proteins, aminoacids, resins, alcohol, starch, saponins, glycosides, tannins.

3. RESULTS AND DISCUSSIONS

3.1 Phytochemical Analysis

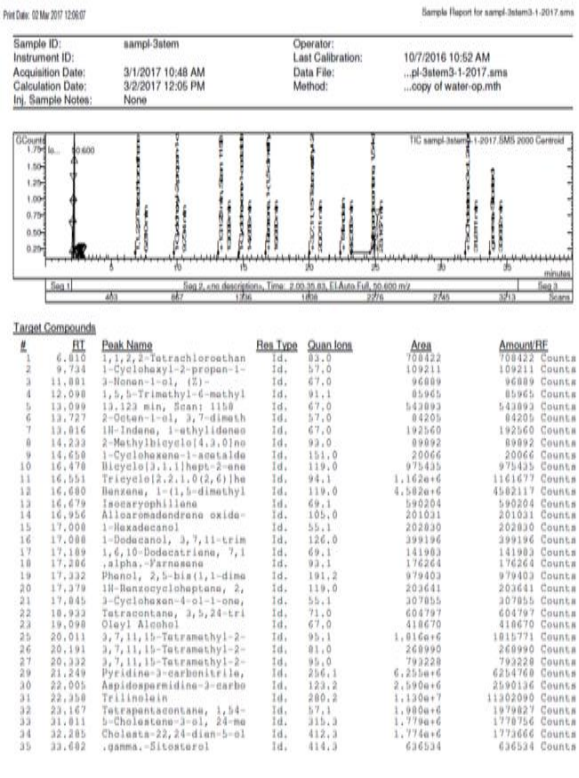
Qualitative Analysis	**C.gigantea**	**C. aquatilis**
Carbohydrates	Positive	Positive
Protein	Positive	Positive
Aminoacid	Positive	Positive
Starch	Negative	Negative
Saponins	Negative	Negative
Alcohol	positive	Positive
Resins	Positive	Negative
Glycosides	Positive	Positive
Tanins	Positive	Positive
Flavanoids	Negative	Positive
Diterpenes	Positive	positive
Phenols	Positive	Positive

3.2 Anti-Bacterial Assay

We obtained positive results for Colocasiaaquatilis extract in both gram positive (MRSA) and gram negative bacteria (E.coli).

3.3 GCMS Analysis

3.3.1 Colocasia Aquatilis



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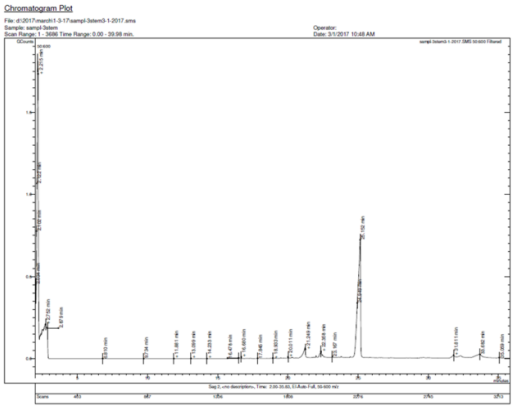
Calculation Date:	3/2/2017 12:05 PM	Method:	...copy of water-op.mth
Inj. Sample Notes:	None		

TIC sampl-3stem3-1-2017.SMS 2000 Centroid

Target Compounds

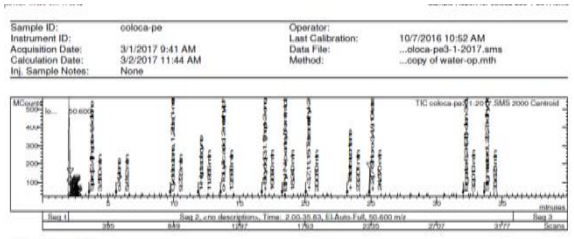
#	RT	Peak Name	Res Type	Quan Ions	Area	Amount/RF
1	6.610	1,1,2,2-Tetrachloroethan	Id.	83.0	708422	708422 Counts
2	9.731	1-Cyclohexyl-2-propen-1-	Id.	57.0	109211	109211 Counts
3	11.881	3-Nonen-1-ol, (Z)-	Id.	67.0	96889	96889 Counts
4	12.098	1,5,5-Trimethyl-6-methyl	Id.	91.1	85965	85965 Counts
5	13.099	13.123 min, Scan: 1150	Id.	67.0	543893	543893 Counts
6	13.727	2-Octen-1-ol, 3,7-dimeth	Id.	57.0	84205	84205 Counts
7	13.816	1H-Indene, 1-ethylidene-	Id.	67.0	192560	192560 Counts
8	14.233	2-Methylbicyclo[4.3.0]no	Id.	93.0	89892	89892 Counts
9	14.658	1-Cyclohexene-1-acetalde	Id.	151.0	20066	20066 Counts
10	16.478	Bicyclo[3.1.1]hept-2-ene	Id.	119.0	975435	975435 Counts
11	16.551	Tricyclo[2.2.1.0(2,6)]he	Id.	94.1	1.162e+6	1161677 Counts
12	16.680	Benzene, 1-(1,5-dimethyl	Id.	119.0	4.582e+6	4582117 Counts
13	16.679	Isocaryophillene	Id.	69.1	590204	590204 Counts
14	16.956	Allocaromadendrene oxide-	Id.	105.0	201031	201031 Counts
15	17.008	1-Hexadecanol	Id.	55.1	202830	202830 Counts
16	17.088	1-Dodecanol, 3,7,11-trim	Id.	126.0	399196	399196 Counts
17	17.189	1,6,10-Dodecatriene, 7,1	Id.	69.1	141983	141983 Counts
18	17.286	.alpha.-Farnesene	Id.	93.1	176264	176264 Counts
19	17.332	Phenol, 2,5-bis(1,1-dime	Id.	191.2	979403	979403 Counts
20	17.379	1H-Benzocycloheptene, 2,	Id.	119.0	203641	203641 Counts
21	17.845	3-Cyclohexen-4-ol-1-one,	Id.	55.1	307855	307855 Counts
22	18.932	Tetracontane, 3,5,24-tri	Id.	71.0	604797	604797 Counts
23	19.098	Oleyl Alcohol	Id.	67.0	418670	418670 Counts
25	20.011	3,7,11,15-Tetramethyl-2-	Id.	95.1	1.016e+6	1015771 Counts
26	20.191	3,7,11,15-Tetramethyl-2-	Id.	81.0	260990	260990 Counts
27	20.332	3,7,11,15-Tetramethyl-2-	Id.	95.0	793228	793228 Counts
29	21.249	Pyridine-3-carbonitrile,	Id.	256.1	6.255e+6	6254768 Counts
30	22.005	Aspidospermidine-3-carbo	Id.	123.2	2.590e+6	2590136 Counts
31	22.358	Trilinolein	Id.	280.2	1.130e+7	11302096 Counts
32	23.167	Tetrapentacontane, 1,54-	Id.	57.1	1.980e+6	1979827 Counts
33	31.811	5-Cholestene-3-ol, 24-me	Id.	315.3	1.779e+6	1778756 Counts
34	32.281	Cholesta-22,24-dien-5-ol	Id.	412.3	1.774e+6	1773666 Counts
35	33.682	.gamma.-Sitosterol	Id.	414.3	636534	636534 Counts



Chromatogram Plot

Fig 1: GC-MS analysis of *Colocasia Aquatilis*

3.3.2 Colocasia Gigantea



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Calculation Date:	3/2/2017 11:44 AM	Method:	...copy of water-op.mth
Inj. Sample Notes:	None		

TIC coloca-pe3-1-2017.SMS 2000 Centroid

Target Compounds

#	RT	Peak Name	Res Type	Quan Ions	Area	Amount/RF
1	3.550	Spiro[2.4]hepta-4,6-dien	Id.	90.9	109736	109736 Counts
2	5.612	m-Xylene	Id.	90.9	61857	61857 Counts
3	9.723	Cyclobutane, 1,2-bis(1-m	Id.	67.0	156429	156429 Counts
4	11.855	1-Heptadecyne	Id.	57.0	18959	18959 Counts
6	13.568	o-Toluylic acid, 2-methy	Id.	118.9	20664	20664 Counts
7	15.376	Benzenepropanoic acid, p	Id.	70.8	12600	12600 Counts
8	15.906	Naphthalene, 1,2,3,4-tet	Id.	159.1	96260	96260 Counts
9	16.481	Bicyclo[3.1.1]hept-2-ene	Id.	119.0	1.017e+6	1016669 Counts
10	16.554	Tricyclo[2.2.1.0(2,6)]he	Id.	94.1	1.133e+6	1132540 Counts
11	16.690	Bicyclo[3.1.1]hept-2-ene	Id.	119.0	4.866e+6	4865617 Counts
12	16.806	Isocaryophillene	Id.	91.1	146276	146276 Counts
13	16.958	Caryophyllene	Id.	93.9	79617	79617 Counts
14	17.115	Nonadecane, 1-chloro-	Id.	57.0	61660	61660 Counts
15	17.205	1,6,10-Dodecatriene, 7,1	Id.	69.0	189976	189976 Counts
16	17.306	.alpha.-Farnesene	Id.	93.0	225567	225567 Counts
17	17.345	Phenol, 2,5-bis(1,1-dime	Id.	191.1	845074	845074 Counts
18	17.393	Isocaryophillene	Id.	93.0	237850	237850 Counts
19	17.446	1H-Benzocycloheptene, 2,	Id.	119.0	68561	68561 Counts
20	17.680	Longifolene-(V4)	Id.	93.1	133323	133323 Counts
21	17.764	Naphthalene, 1,2,3,5,6,7	Id.	161.0	85918	85918 Counts
22	17.809	Naphthalene, 1,2,3,4,4a,	Id.	161.0	137589	137589 Counts
23	18.123	Tricyclo[3.1.0.0(2,4)]he	Id.	107.0	395683	395683 Counts
24	18.240	Ethyl N-(o-anisyl)formim	Id.	178.9	77772	77772 Counts
26	18.968	Tritetracontane	Id.	57.0	268732	268732 Counts
28	19.345	Propionic acid, 3-(m-ami	Id.	120.0	85208	85208 Counts
30	20.050	3,7,11,15-Tetramethyl-2-	Id.	95.0	1.024e+6	1023590 Counts
31	20.233	3,7,11,15-Tetramethyl-2-	Id.	67.0	122809	122809 Counts
32	20.302	1,2-Benzenedicarboxylic	Id.	149.0	999894	999894 Counts
33	20.375	3,7,11,15-Tetramethyl-2-	Id.	67.0	387253	387253 Counts
34	20.594	Tritetracontane	Id.	57.0	400814	400814 Counts
35	21.037	Palmitic anhydride	Id.	256.1	803044	803044 Counts
38	22.019	Phytol	Id.	71.0	698572	698572 Counts
39	22.267	Cyclopropaneoctanoic aci	Id.	67.0	579357	579357 Counts
40	22.563	Aspidospermidin-17-ol, 1	Id.	57.0	610343	610343 Counts
41	23.201	Tritetracontane	Id.	57.0	911242	911242 Counts
42	23.939	Tritetracontane	Id.	57.0	355325	355325 Counts
44	24.870	2-17-Chloro-3,4,9,10-tet	Id.	391.0	8.708e+7	87080816 Counts
45	32.013	Ergosta-8,24(28)-dien-3-	Id.	397.3	6.387e+6	6386759 Counts
46	33.623	Stigmasterol, 22,23-dihy	Id.	397.4	1.382e+6	1381798 Counts

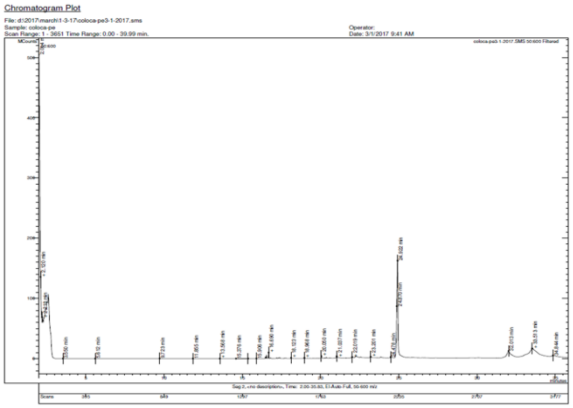

Fig 2: GC-MS analysis of *Colocasia Gigantea*

4. CONCLUSION

By analyzing the result it was concluded that the variety is having a wide range of active components which was not revealed earlier. GC-MS analysis was done for this variety of Colocasia for the first time. The research will pave the way to further analysis by using our work as a reference.

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