WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 6, 1152-1158. Research Article ISSN 2277–7105

PHYTOCHEMICAL STUDIES AND GC-MS ANALYSIS OF THE RHIZOME OF *CURCUMA AROMATICA*

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Article Received on
04 April 2017,

Revised on 25 April 2017,
Accepted on 15 May 2017

DOI: 10.20959/wjpr20176-8580

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ABSTRACT

The present study was aimed to carry out the detailed phytochemical analysis of the rhizome of *Curcuma aromatica*. Qualitative phytochemical screening of the methanolic extract of the rhizome revealed the presence of many components such as alkaloids, carbohydrates, flavanoids, reducing sugars. GC-MS analysis was also carried out to detect the phyto constituents present in the methanolic extract of the rhizome of *Curcuma aromatica*.

KEYWORDS: *Curcuma aromatica*, Phytochemical screening, GC-MS.

INTRODUCTION

Medicinal plants are widely used by the traditional medicinal practitioners to cure different diseases due to their world-wide availability and fewer side effects. The herbal medicines occupy distinct position right from the primitive period to present day. Natural products have been a source of drugs for centuries.[1] India has a wealth of medicinal plants most of which have been traditionally used in Ayurveda, Unani systems of medicine and by tribal healers for generation. The medicinal value of this plant lies in the bioactive phytochemical constituents that produce definite physiological effect on human body. These natural compounds signify the base of modern drugs as we use today. Phyto components are the natural bioactive compounds found in the plants. These phyto components work with nutrients and fibers to from an integrated part of human defense system against various disease and stress condition. In the last few years gas-chromatography mass-spectrometry has become firmly established as a key technological platform for metabolite profiling in plant.[2] GCMS based metabolome analysis has profound applications in discovering the mode of

action of drugs or herbicides and helps unravel the effect of altered gene expression on metabolism and organism performance in biotechnological applications.

Curcuma aromatica Salisb (family Zingiberaceae), a traditional Chinese herb, is now widely cultivated and used as a traditional herbal drug in India, China and Southeast Asia. In Thailand, the rhizome and roots of *Cu. Aromatica* are frequently used in cosmetics and spas for skin nourishment and ceremonial dye.[3] Pharmacological study on *Cu. aromatica* volatile oil reveals various medical activities such as promotion of blood circulation to remove blood stasis and treatment of cancer[4] and has been investigated for possible medicinal benefits, including antioxidant[5] and anti-inflammation properties.[6]

MATERIAL AND METHOD

(i)Preparation of plant material

The rhizome samples of plant *C.aromatica* was collected from Kerala. The sample were dried at 60°C for 2 days in an oven. They were then macerated to powder form with a mixer grinder.

(ii) Preparation of sample

About 20 grams of the plant sample powdered were soaked in 100 ml methanol individually. It was left for 24 hours so that alkaloids, flavonoids and other constituents if present will get dissolved. The methanol extract was filtered using Whatman No.1 filter paper and the residue was removed. It was again filtered through sodium sulphate in order to remove the traces of moisture.

(iii)Phytochemical screening

The phyto-components of the aqueous and methanolic extracts of the rizhome of *C.aromatica* were qualitatively analyzed in detail as per the standard methods.[7-9]

(iv) Gas chromatography – Mass Spectrum analysis

The GC-MS analysis of the plant extract was made in a (QP 2010 Plus SHIMADZU) instrument under computer control at 70 eV. About 1µL of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 minutes. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, bigger was the signal obtained which was then processed by a

computer. The time from when the injection was made (Initial time) to when elution occurred is referred to as the Retention time (RT).While the instrument was run, the computer generated a graph from the signal called Chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the Gas chromatography column into the detector. The X-axis showed the RT and the Y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass .The M/Z (Mass / Charge) ratio obtained was calibrated from the graph obtained, which was called as the Mass spectrum graph which is the fingerprint of a molecule.

Before analyzing the extract using Gas Chromatography and Mass Spectroscopy, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1ml per minute. The electron gun of mass detector liberated electrons having energy of about 70eV.The column employed here for the separation of components was Elite 1(100% dimethyl poly siloxane).

(v) Identification of components

The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST08.LIB9, WILEY8.LIB10 library sources were used for matching the identified components from the plant material.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis of aqueous and methanolic extracts of rizhome of *Curcuma aromatica.* The results of qualitative phytochemical analysis of aqueous and methanolic rizhome extract of *Curcuma aromatica* given in Table 1. Results indicate the presence of many phyto-components in the extract.

Phytochemical constituents of Curcuma species were reported by various authors.[10] Tannins, phenolics, alkaloids and flavonoids have been suggested to be involved in antibacterial activities.[11] GC-MS of the methanolic extract of the rizhome of *Curcuma aromatica* is

presented in Table 2. The fragmentation patterns of the mass spectra were compared with those of the known compounds stored in the National Institute of Standards and Technology (NIST) research library. In the GC-MS analysis, many active components were detected. The identification of phytochemical compounds was based on peak area, molecular weight and molecular formula. The results of the GC-MS profile can be used as pharmacognostical tool for the identification of the compounds with different chemical structures. The presence of various bioactive compounds confirms the application of *Curcuma aromatica* for various ailments by traditional practitioners.

Table 1: Phytochemical screening of methanolic extract of *Curcuma aromatica* Rhizome.

S.No	Phyto constituents	Aqueous	Methanol
1.	Alkaloids	+	+
2.	Steroids	+	+
3	Tannins	+	-
4	Phenols	+	+
5	Flavonoids	+	-
6	Glycosides	+	-
7	Saponins	+	-
8	Terpenes	+	+
9	Reducing Sugar	+	-
10	Carbohydrates	+	+

+ = indicates the presence of constituents, - = indicates the absence of constituents.

Table 2: GCMS Analysis of Methanolic Extracts Of Curuma aromatica Rhizome.

NO	R. TIME	AREA	AREA %	NAME	MOLECULAR FORMULAS	MOLECULAR WEIGHT g/mole
1	3.429	138454	0.41	Benzene Methyl (CAS) Toluene	$C_6H_5CH_3$	92
2	3.594	241895	0.71	Butanoic Acid 2-methyl –methyl ester	$C_6H_{12}O_2$	116
3	4.348	393462	1.15	Pentanoic acid, methyl ester	$C_6H_{12}0_2$	116
4	6.799	93840	0.27	Camphene	$C_{10}H_{16}$	136
5	9.602	36267	0.11	1-Octanol (CAS) Octilin	$C_8H_{18}O$	130
6	9.765	380989	1.12	Undecane (CAS) n-Undecane	$C_{11}H_{22}$	156
7	10.912	542605	1.59	Bicyclo [2,2,1] heptan-2-one, 1,7,7- trimethyl	$C_{10}H_{17}NO$	167
8	13.925	353168	1.03	Cyclohexasiloxane, Dodecamethyl	$C_{12}H_36O_6Si_6$	444
9	14.116	42262	0.12	2 – METHOXY-4-VINYLPHENOL	$C_9H_{10}O_2$	150
10	15.858	90880	0.27	BETA-ELEMENE	$C_{15}H_{24}$	204
11	16.069	293654	0.86	`TRANS-ALPHA-BERGAMOTENE	$C_{15}H_{24}$```	204
12	16.963	76732	0.22	Germacrene B (CAS) 1,5,Cyclodecadien	$C_{15}H_{24}$	204
13	17.404	365645	1.07	Trans – beta – Famesene s$(E) – beta - farmesene	$C_{15}H_{24}$	204
14	17.575	141910	0.42	Benzoic acid 2 (1-oxopropyl) – methyl ester	$C_7H_6O_2$	122
15	18.419	11299999	33.07	Benzene 1 (1,5-dimethyl – 4 – hexenyl) – 4 - methyl	$C_{15}H_{22}$	202
16	19.078	441232	1.29	Curzerene	$C_{15}H_{20}O$	216
17	19.408	1919744	5.62	CEDR – 8 – ENE $$ DIEPI – ALPHA - CEDRENE	$C_{15}H_{24}$	204
18	20.719	149123	0.44	SESQUISABINENE HYDRATE	$C_{15}H_{26}O$	222
19	21.043	286597	0.84	Germacrene B (CAS) 1,5, Cyclodecadiene	$C_{15}H_{24}$	204
20	21.789	3289770	9.63	1,2, Benzene di carboxylic acid diethyl ester	$C8H_6O_4$	166
21	22.183	1135188	3.33	9,17, HYDROXY ANDROSTA – 1,4 – DIEN – 3 – ON	$C_{19}H_{26}O_3$	302
22	23.199	205491	0.60	CYCLO OCTASILOXANE HEXADECANE	$C_{16}H_{34}$	226
23	23.593	131458	0.38	3 – Cyclohexen 1 – ol 2 – (1,5- dimethyl – 4 – hexenyl)	$C_6H_{10}O$	98
24	24.329	1093482	3.20	Germacrone	$C_{15}H_{22}O$	218
25	24.513	136706	0.40	2, Heptanone 6 – methyl – 6 – 3 – methyl – 3	$C_7H_{14}O$	114
26	25.183	7261440	21.25	Pheno, 2 – methyl 5 – (1,2,2- trimethyl cyclopentan)	C_8H_{16}	112
27	25.680	1038396	3.04	1,3 Diphenyl 1,3,5,5 – Tetra methyl 1 Disilazane	$C_{16}H_{23}NSi_2$	285

28	27.195	238525	0.70	1,2 BENZANE DICARBOXYLIC ACID	$C_8H_6O_4$	166
29	27.391	605062	1.77	BORAZINE 2,4,6 – TRIPHENYL – 1,3,5 – TRIPHENYL	$C_{21}H_{24}B_3N_3$	350
30	28.644	183346	0.54	1,2, Benzene dicarboxylic acid, dibutyl ester	$C_{16}H_{22}O_4$	370
31	29.365	209987	0.61	DISILANE 1,1,2,2 – TETRAMETHYL 1,2 – DI IODO	$C_4H_{12}I_2Si_2$	322
32	31.465	228739	0.67	PENTAMETHYL PHENYL DISILANE	$C_{16}H_{26}Si_2$	274
33	32.565	425912	1.25	4- P – Chlorophenyl – 2 – dimethylamino – 5 – nitrosoth	$C_9H_9N_3O_2$	191
34	33.915	246365	0.72	PENTAMETHYL PHENYL DISILANE	$C_{20}H_{26}Si_2$	140
35	46.059	450648	1.32	1- 2- (FLUOROPHENYL) -4 - FLUOROP	$C_{22}H_{16}F_2N_2$	346

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