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Brintha S
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Rajesh S
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Renuka R
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Santhanakrishnan VP
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Gnanam, R
a) Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
b) Present address: Imayam Institute of Agriculture and Technology (IIAT), Kannanur, Tamil Nadu, India

Correspondence
Rajesh S
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Phytochemical analysis and bioactivity prediction of compounds in methanolic extracts of *Curculigo orchioides* Gaertn.

Brintha S, Rajesh S, Renuka R, Santhanakrishnan VP and Gnanam R

Abstract
Curculigo orchioides Gaertn. a medicinal herb with diverse pharmacological activities has been considered as a wonder crop for people working in the herbal pharmaceuticals. Preliminary screening for the presence of phytochemicals in rhizome and leaf extracts of *C. orchioides* was carried out through qualitative tests. GC-MS analysis of methanolic extracts of leaves and rhizome revealed the presence of several compounds and were interpreted based on the reported compounds in NIST-Wiley library. PASS prediction analysis revealed the pharmacological activities of phytoconstituents in the extracts of *C. orchioides.*
In leaf extracts, the compound 2-Myristynoyl pantetheine covered the max peak area of 32.72%. Further in leaf extracts, 4-Acetyloxyimino-6,6-dimethyl-3-methylsulfanyl-4,5,6,7-tetrahydro benzo [c] thiophene -1 carboxylic acid methyl ester was also found to possess biological role in treatment of Alzheimer's disease, neurodegenerative disease and cognition disorders. In rhizome, the compound 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3']pyrrolo[4',5'-d] pyridazine covered maximum peak area (18.36%) which has predicted biological activity as an antiepileptic, for treating renal disease, heart failure and Alzheimer's disease.

Keywords: *Curculigo orchioides,* Phytochemicals, GC-MS analysis, PASS prediction

Introduction
Curculigo orchioides Gaertn. is a near threatened medicinal herb as per International Union for Conservation of Nature (IUCN) status (2012) belonging to the family Hypoxidaceae. The plant commonly referred to as Golden eye grass, Nilappanai, Kali Musli etc. has various medicinal properties like anticancer, anti-diabetic, anti-neurodegenerative and hepatoprotective activities etc., (Wang *et al.* (2012) [18], Chauhan and Dixit (2007) [4] and Gulati *et al.* (2015) [7]. It has been used time immemorial in traditional medicinal practices by people in China, India and few other Asian countries. It is also employed in Ayurveda and Unani herbal pharmaceuticals. Owing to its huge importance and exploitation, several people around the world have studied and experimented about it both *in vivo* and *in vitro* (Sahay *et al.* (2016) [12]. Nearly all of the results showed positive inference therapeutically.
Phytoconstituents are demanding because of its fewer side effects and most compatible to the human physiology (Sen *et al.* 2010) [13]. Presence of various important medicinal properties in *C. orchioides* necessitates understanding the herb's phytochemical composition by means of preliminary phytochemical screening and isolation of different compounds of medicinal importance using standard methods. Many compounds were isolated from *C. orchioides* using various solvent extracts. These compounds belong to different classes of secondary metabolites and can be tested for their *in vitro* or *in vivo* pharmacological activities in future, leading to a new and efficient drug development. Identification and structural elucidation of phytochemicals from plant species were successfully reported by Hites (1997) [9] and Gupta *et al.,* (2005) [8] through the use of analytical techniques like Thin Layer Chromatography, Nuclear Magnetic Resonance and Gas Chromatograph-Mass Spectrometry.
The present study was attempted to determine the bioactive compounds present in *Curculigo orchioides,* a near threatened herb using GC-MS technique for exploring its array of compounds that would possess pharmacological activities for subsequent use in therapeutics.

Materials and Methods
Plant materials for the phytochemical analysis were collected from the natural habitats surrounding areas of Nagercoil, Kanyakumari district, Tamil Nadu. The plant samples were authenticated as *Curculigo orchioides* Gaertn. by Botanical Survey of India (BSI), Coimbatore.

The powdered dried leaf and rhizome was subjected to solvent extraction and used for phytochemical screening.

Phytochemical analysis

Extraction of sample

Leaves and rhizomes of *C. orchioides* were used for phytochemical studies. The leaves were collected from plants maintained in the green house of Centre for Plant Molecular Biology and Biotechnology, TNAU, Coimbatore. The leaves were washed with tap water, rinsed with distilled water and blotted gently between the folds of filter paper. Later, they were made into smaller pieces and shade dried for seven days. Rhizomes were dug up from the soil, washed thoroughly with tap water in order to remove all the soil particles adhering to the rhizomes. Then they were washed with distilled water and blot dried. Following that, they were cut into small pieces to facilitate easy drying and kept for shade drying for 14 days. Shade dried leaves and rhizomes were finely powdered using a blender. The samples were transferred to conical flasks and directly extracted with four different solvents namely, water, methanol, chloroform and hexane (1:10 w/v of sample: solvent) and kept in orbitary shaker for 24 h at 125 rpm. The extract was allowed to settle and then filtered through a Whatmann filter paper No: 42 (125 mm) and the solvent layer was allowed to evaporate and the filtrate was used for further analysis.

Preliminary phytochemical screening

The leaf and rhizome extracts of *Curculigo orchioides* were screened for the presence of alkaloids, carbohydrates, saponins, flavonoids, phenols, tannins, glycosides, diterpenes, triterpenes etc as reported by Tiwari *et al.* (2011) [15], Iyengar (1995) [11], Siddiqui and Ali (1997) [14] and Hossain *et al.* (2013) [10].

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The rhizomes and leaves of *C. orchioides* were shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to stoppered flask, and treated with methanol for infusion. After 24 h, the extract was filtered and evaporated to dryness using a vacuum distillation unit. The final residue obtained was then subjected to GC-MS analysis.

GC-MS analysis of these extracts were performed using Thermo GC ultra Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with an Elite-I, fused RMS 5 silica capillary column composed of 100% Dimethyl poly siloxane). Detection was through an electron ionization system with ionizing energy of 70 eV. Helium (99.999%) was used as carrier gas at constant flow rate of 1 ml /min and the sample injection volume of 1µl was employed with the sample split ratio of 10:1. Temperature for injector and the ion source was set at 250 °C and 260 °C, respectively. The oven temperature was programmed from 110 °C with an increase of 5°C/min, up to 260 °C, ending with a 3 min isothermal at 260 °C. Mass spectra were documented with a scan interval of 0.5 seconds and fragments from 50 to 650 Da. Percentage amount of each component was calculated by comparing its average peak area to the total area. Turbo mass software was used for handling mass spectra and chromatograms.

Identification of components

Identification of components and the interpretation of mass spectrum of GC-MS were conducted using the National Institute of Standards and Technology (NIST) database with retention values of more than 90000 compounds (https://www.nist.gov/srd/nist-standard-reference-database-1a-v14). The unknown components were compared with the known components based on spectra stored in the NIST and Wiley library. Based on the analysis, the name, molecular weight and the components of the test materials were ascertained.

Prediction of biological activity of substances

In order to investigate the biological activity of the compounds, the predictions of biological activity spectra on the basis of their structural formula were obtained using PASS (Prediction of activity spectra for biologically active substances). A set of pharmacological effects, specific toxicities and mechanisms of action that might be exhibited by the compounds were predicted in PASS online database (Filimonov *et al.,* 2014) [6].

Results and Discussion

Among different solvents used for the extraction of metabolites and preliminary analysis of the phytochemicals, methanolic extract of rhizome revealed the presence of alkaloids, carbohydrates, steroids, saponins, phenols and flavonoids which is similar to the reports of Agrahari *et al.* (2010) [1]. The rhizome extract also showed the presence of tannins and flavones as reported by Asif (2012) [2]. The chloroform extract of the rhizome of *C. orchioides* was analysed and has been detected with the presence of alkaloids and triterpenes. The water extract of the rhizome of *C. orchioides* was analysed, and detected with the presence of flavones, triterpenes, saponins and diterpenes. The hexane extract of the rhizome showed the presence of steroids alone.

Analysis of the water extract of leaves of *C. orchioides* showed the presence of alkaloids and flavone, methanol extract of leaves showed the presence of carbohydrates, tannins, steroids, phenols, flavonoids and diterpenes, chloroform extract of leaves showed the presence of alkaloids and cardiac glycosides and hexane extract of leaves showed the presence of alkaloids, carbohydrates, steroids and glycosides (Table 1).

The results of GC-MS analysis revealed the presence of several bioactive constituents in the extracts of *C. orchioides.* The GC-MS chromatogram of methanolic extracts of the leaf and rhizome of *C. orchioides* is presented in Fig. 1 and Fig. 2 respectively.

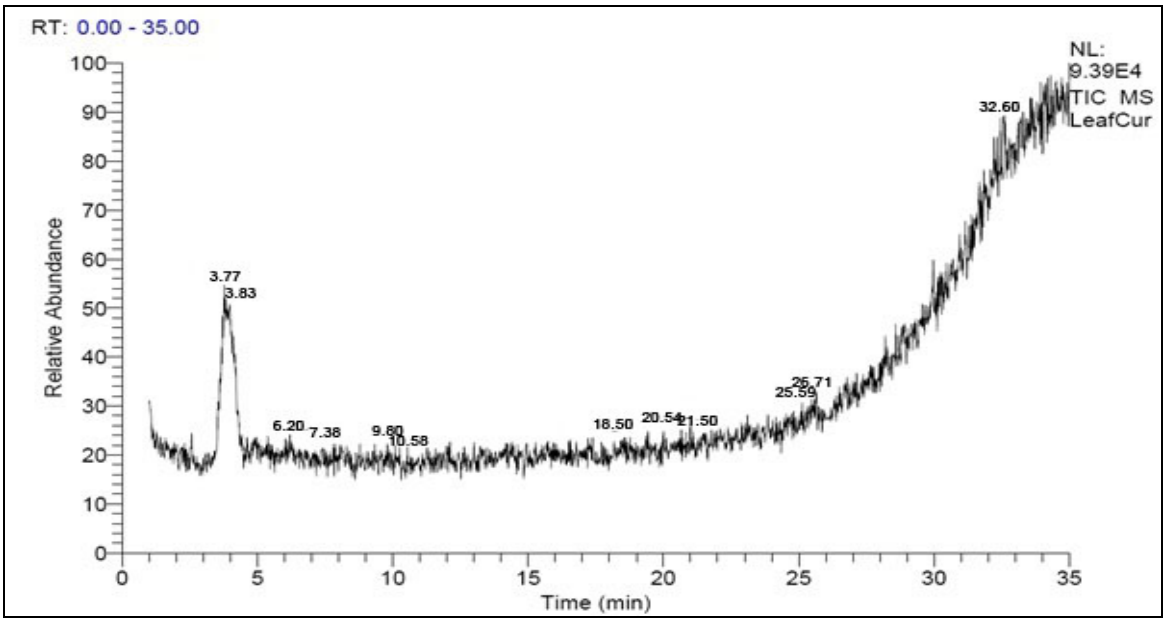

Fig 1: GC-MS Chromatogram of methanolic leaf extract of *C. orchioides*

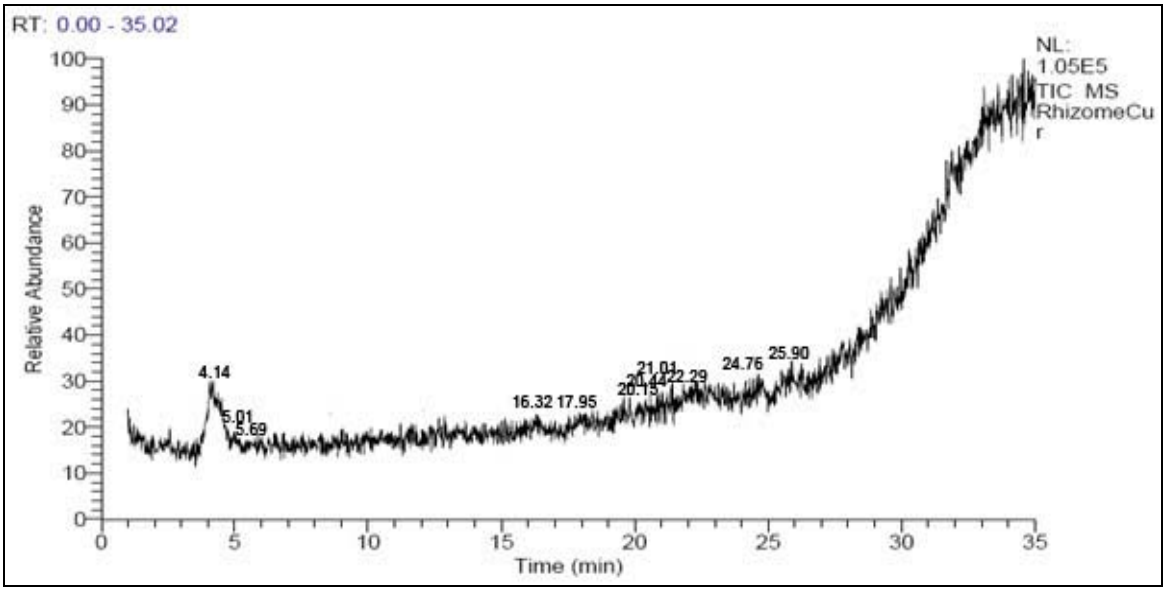

Fig 2: GC- MS Chromatogram of methanolic rhizome extract of *C. orchioides*

Table 1: Phytochemical screening of the solvent extracts of Leaf and rhizome extracts of *Curculigo orchioides*

S. No.	Phytochemicals	Qualitative test	Leaf extract of *C. orchioides*				Rhizome extract of *C. orchioides*			
			Water (H_2O)	Methanol (CH_3OH)	Chloroform ($CHCl_3$)	Hexane (C_6H_{14})	Water (H_2O)	Methanol (CH_3OH)	Chloroform ($CHCl_3$)	Hexane (C_6H_{14})
1	Alkaloids	Wagner' test	+	-	+	+	-	+	+	-
2	Carbohydrates	Benedict's test	-	+	-	+	-	+	-	-
3	Glycosides - Cardiac	Legal's test	-	-	+	+	-	-	+	-
4	Diterpenes	Copper acetate test	-	+	-	-	+	-	-	-
5	Flavonoids	Lead acetate test	-	+	-	-	-	+	-	-
6	Flavones	Siddiqui and Ali, 1997	+	-	-	-	+	+	-	-
7	Phenols	Ferric Chloride test	-	+	-	-	-	+	-	-
8	Saponins	Foam test	-	-	-	-	+	+	-	-
9	Steroids	Hossain, 2013	-	+	-	+	-	+	-	+
10	Tannins	Iyengar, 1995	-	+	-	-	-	+	-	-
11	Triterpenes	Salkowski's test	-	-	-	-	+	-	+	-

Qualitative analysis shows visually observable product and depicted as presence (+) or absence (-) of phytochemicals in the extracts.

The compounds in the samples were detected and analysed using NIST library based on their molecular weight, peak area and retention time. The phytoconstituents with highest peak area percentage are selected and tabulated for leaf (Table 2) and rhizome extracts (Table 3).

Table 2: Phytoconstituents in leaf extract detected by GC-MS

S. No.	Retention Time (min)	Compound name	Area (%)
1	3.83	2-Myristynoyl pantetheine	32.72
2	7.38	9-Hexadecenoic acid	0.20
3	9.80	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-	1.71
4	10.58	Cyclobarbital	0.36
5	18.50	Thieno[2,3-c]furan-3-carbonitrile,2-amino-4,6-dihydro-4,4,6,6-tetramethyl-	2.97
6	20.54	Testosterone, 17-O-(t-butyldimethylsilyl)-	1.06
7	21.50	2-Myristynoyl-glycinamide	0.60
8	25.59	N-[3,5-Dinitropyridin-2-yl]glutamic acid	2.51
9	26.71	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	2.26
10	32.60	4-Acetyloxyimino-6,6-dimethyl-3-methylsulfanyl-4,5,6,7-tetrahydro-benzo[c]thiophene-1-carboxylic acid methyl ester	4.09

Table 3: Phytoconstituents in rhizome extract detected by GC-MS

S. No	Retention Time (min)	Compound name	Area (%)
1	4.14	2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'-d] pyridazine	18.36
2	5.01	EPPS	0.53
3	5.69	Sarreroside	0.90
4	16.32	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	4.88
5	17.95	Ergosta-5,22-dien-3-ol, acetate, (3á,22E)-	3.33
6	20.15	Digitoxin	0.81
7	20.44	Ethyl iso-allocholate	0.64
8	21.01	Rhodopin	0.54
9	24.76	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,	4.13
10	25.90	Cyclobarbital	3.88

The large list of compounds identified by GC-MS analysis was compared with compounds in NIST library and more detailed analysis was done to selected phytochemicals from primary data. Some of their activity were identified and reported based on PASS prediction analysis.

It was found that a drug named Cyclobarbital is available both in leaves (0.36 %) and rhizome (3.88 %) of *C. orchioides* methanolic extracts, which is used to treat insomnia as reported by Breimer *et al.* (1976) [3]. Another compound Vitamin D3 is also present in both leaf (1.71 %) and rhizome (4.13 %) methanolic extracts, which is reported to be anti-osteoporotic whereas a cardiac glycoside called digitoxin was present in methanolic extracts of rhizome (0.81 %), which is reported to be involved in complex cell-signal transduction mechanisms, resulting in selective control of human tumor as reported by Varadharajan *et al.* (2012) [16]. Ethyl iso-allocholate, a steroid compound found in the methanolic rhizome extract (0.64 %) of present study corroborates with findings of Daffodil *et al.* (2012) [5] in ethanolic extracts of same species. The compound was reported to exhibit antimicrobial, antiasthma, anti-inflammatory, anticancer and diuretic activities. The activities of other selected compounds are tabulated for leaf (Table 4) and rhizome extracts (Table 5).

Table 4: Pharmacological activity of phytochemicals in leaf extracts of *C.orchioides* detected by GC – MS

S. No	Compound Name	Molecular formula	MW (kDa)	Pharmacological activity
1	4-Acetyloxyimino-6,6-dimethyl-3-methylsulfanyl-4,5,6,7-tetrahydro-benzo[c]thiophene-1-carboxylic acid methyl ester	$C_{15}H_{19}NO_4S_2$	341	Alzheimer's disease treatment, Cognition disorders treatment, Calcium regulator, Neurodegenerative diseases treatment
2	Thieno[2,3-c]furan-3-carbonitrile,2-amino-4,6-dihydro-4,4,6,6-tetramethyl-	$C_{11}H_{14}N_2OS$	222	Analgesic, Antianginal, Analgesic, non-opioid, Antihypertensive, Antiarthritic, Dementia treatment, Neurotransmitter uptake inhibitor
3	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	$C_{16}H_{14}N_2O_4$	298	Neurotransmitter uptake inhibitor, Chemosensitizer, Benzoate-CoA ligase inhibitor, Insecticide, Antineoplastic (melanoma)
4	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)- (Calcitriol)	$C_{27}H_{44}O_3$	416	Prostaglandin-E2 9-reductase inhibitor, Antieczematic, Antiosteoporotic etc.
5	Testosterone, 17-O-(t-butyldimethylsilyl)-	$C_{25}H_{42}O_2Si$	402	Testosterone 17beta-dehydrogenase (NADP+) inhibitor, Prostate disorders treatment, Antineoplastic etc.
6	Cyclobarbital	$C_{12}H_{16}N_2O_3$	236	Testosterone 17beta-dehydrogenase (NADP+) inhibitor, Anesthetic general, Anticonvulsant, Neurotransmitter antagonist, Skeletal muscle relaxant
7	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	CYP2J substrate, Antieczematic, Mucomembranous protector, Phosphatidylglycerophosphatase inhibitor

Table 5: Pharmacological activity of phytochemicals in rhizome extracts detected by GC – MS

S. No	Compound Name	Molecular formula	MW (kDa)	Pharmacological activity
1	2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3']pyrrolo[4',5'-d]pyridazine	$C_{20}H_{13}N_5O_2$	355	Renal disease treatment, Heart failure treatment, Antiepileptic, Alzheimer's disease treatment
2	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	$C_{28}H_{44}O_4$	444	Membrane integrity antagonist, Chymosin inhibitor, Antieczematic, Antiinflammatory
3	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (Calcitriol/ dihydroxy vitamin D3)	$C_{27}H_{44}O_3$	416	Vitamin, Antipsoriatic, Bone diseases treatment, Antieczematic, Autoimmune disorders treatment
4	Cyclobarbital	$C_{12}H_{16}N_2O_3$	236	Testosterone 17beta-dehydrogenase (NADP+) inhibitor, Anesthetic general, Anticonvulsant, Neurotransmitter antagonist, Skeletal muscle relaxant
5	Ergosta-5, 22-dien-3-ol, acetate, (3á,22E)- (Brassicasterol acetate)	$C_{30}H_{48}O_2$	440	Antihypercholesterolemic, Cholesterol antagonist, Caspase 3 stimulant, Prostaglandin-E2 9-reductase inhibitor, Chemopreventive, Antitoxic, Antieczematic, Bone diseases treatment, Antiparkinsonian, rigidity relieving, Acetylcholine neuromuscular blocking agent
6	Sarreroside	$C_{30}H_{42}O_{10}$	562	Respiratory analeptic, Antineoplastic (breast, leukemic, prostate, ovarian, colorectal and lung cancer), Chemopreventive, Antiprotozoal (Leishmania), Dementia treatment, Hepatoprotectant, Antieczematic
7	Digitoxin	$C_{41}H_{64}O_{13}$	764	Anesthetic general, Proliferative diseases treatment, Dementia treatment, Cardiotonic, Diuretic
8	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	Antieczematic, Signal peptidase I inhibitor, Alkenyl glycerol phosphor choline hydrolase inhibitor
9	Rhodopin	$C_{40}H_{58}O$	554	Mucomembranous protector, Lipid metabolism regulator, Antipsoriatic, Antineoplastic
10	EPPS	$C_9H_{20}N_2O_4S$	252	Amyloid beta precursor protein antagonist, Antiischemic (cerebral), Endoglycosylceramidase inhibitor, Amylo-alpha-1,6-glucosidase inhibitor, Acute neurologic disorders treatment, Ceramide glucosyltransferase inhibitor

In case of leaf extracts, the compound 2-Myristynoyl pantetheine covered the max peak area of 32.72%. A compound in Leaf extract, 4-Acetyloxyimino-6,6-dimethyl-3-methylsulfanyl-4,5,6,7-tetrahydro-benzo[c]thiophene-1-carboxylic acid methyl ester was also found to possess biological role in treatment of Alzheimer's disease, neurodegenerative disease and cognition disorders. In rhizome extracts, the compound 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'-d] pyridazine covered maximum peak area (18.36%) which has predicted biological activity as an antiepileptic, for treating renal disease, heart failure and Alzheimer's disease.

Conclusion

The analysis of *Curculigo orchioides*, an important medicinal herb suggests that the phytochemicals identified through GC-MS analysis are pharmacologically active compounds and the results from this experiment could provide insights into the bioactive compounds which can be potentially used to develop novel therapeutic agents for treating various ailments.

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