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Determination of Bioactive Compounds from *Desmidorchis indica* Stem Extract using Spectrometry Techniques and Evaluation of its Biological Properties

Aarthi P. and Velavan S.*

PG and Research Department of Biochemistry, Marudupandiyar College (Affiliated to Bharathidasan University), Thanjavur, Tamil Nadu, India

**Corresponding Author*

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Abstract: Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyze *Desmidorchis indica* hydro-alcoholic extract to identify bioactive components in the stem and their medicinal uses. Mass spectra of the extract showed compounds that matched the National Institute of Standards and Technology library database (NIST). GC-MS analysis revealed 25 phytochemical compounds from retention duration-dependent peaks. The hydro-alcoholic plant stem extract included 1,2-benzenedicarboxylic acid, diethyl ester, hexadecanoic acid, and 3,7,11,15-tetramethyl-2-hexadecen-1-ol. The major compounds present in the extract include Carbamic acid, N-[10, 11-dihydro-5-(2-methylamino-1-oxoethyl)-3-5H-, Piperidine, 1-nitro, 9,12,15-octadecatrienoic acid, 9-octadecenoic acid, Hydrazinecarbo-thioamide, 2-cyclopentylidene, and 2-Propenamide. The DPPH radical scavenging assay determined the antioxidant capability of *Desmidorchis indica* extract. This study found that *Desmidorchis indica*'s pharmacological effects rely on its bioactive compounds, whereas the plant itself may be used to extract novel medicines with therapeutic promise.

Keywords: *Desmidorchis indica,* Stem extract, Gas Chromatography-Mass Spectrometry, DPPH

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Introduction

In terms of bioactive components, which play a significant part in the upkeep of human health, plants rank towards the top of the food chain (Sermakkani and Thangapandian, 2012). Herbal remedies are efficient, cheap, and safe (Sridhar *et al*., 2016). Traditional medicine, alternative medicine, dietary supplements, nutraceuticals, pharmaceuticals, and chemical entities for synthetic medications all draw heavily upon medicinal plants as a source of their active ingredients (Ncube *et al*., 2008). There is a significant need in the pharmaceutical industry

for new therapeutic agents, and natural medicines extracted from medicinal plants provide this need. Currently, there is a high need for plant-based medicines (Dhivya and Manimegalai, 2013).

The biological compounds that may be discovered in plants are referred to as secondary metabolites, and it is these chemicals that are responsible for the plants' medicinal capabilities. Secondary metabolites may be found in a wide variety of plant tissues, such as those found in the leaves, fruits, buds, stem, flowers, bark, and roots of a plant (Hussein and El-Anssary, 2019). When screening medicinal plants using a variety of techniques, such as chromatography and spectrometry, one may get pharmacological and chemical information that is helpful in finding plants with physiological activity (Juszczak *et al*., 2019). Due to the fact that it is a highly compatible approach, gas chromatography mass spectrometry (also known as GC-MS) is considered the gold standard for identifying and quantifying chemicals. It is possible to identify the unidentified organic compounds in a complex mixture by interpreting the spectra and comparing them to those of recognized chemicals. This process is called spectral interpretation (Thomas *et al*., 2013). The present study identified bioactive components present in hydro-alcoholic extracts of the *Desmidorchis indica* stem by f GC-MS analysis.

Materials and Methods

Collection of plant materials:

Desmidorchis indica plant was collected in March 2020 from Kathattipatti (Palaiyapatti North), Sengipatti Village, Thanjavur District. Dr. S. John Britto (Director, Rabiant Herbarium and Centre for Molecular Systematic, St. Joshphe's College, Trichy, Tamil Nadu, India) verified the plant. Rapinat Herbarium St. Joseph's College, Trichy, Tamil Nadu, India, has a voucher specimen (RSV01).

Preparation of extracts:

In the first step, the *Desmidorchis indica* stem was thoroughly cleaned of dust. After being air-dried at ambient temperature, the stem was ground into a rough powder. Powder was obtained after 24 h of hydro-alcoholic extraction. After pressing down to remove all of the alcohol, a semisolid extract resulted. A desiccator was employed to keep the extract usable until it was needed. Both polar and non-polar phyto-components of the plants were present in the extract.

GC MS analysis:

The Shimadzu 2010 plus, which includes an AOC-20i auto sampler and a gas chromatograph that is connected to a mass spectrometer, was used for the GC MS analysis. 51.25 minutes is the whole length of the GC. Each component's percentage share was determined by dividing the average peak area by the sum of all areas. Mass spectra and chromatograms were processed using Turbo Mass Ver 5.2.0 software (Srinivasan *et al*., 2013).

Identification of components:

The National Institute of Standards and Technology's (NIST) database of more than 62,000 patterns was used for GCMS interpretation. NIST's collection of spectra was searched for a match between the unknown component's and the known components' spectra. The components of the test materials were identified, together with their molecular weights and chemical structures (Dukes, 2013).

In vitro antioxidant activity:

DPPH radical-scavenging activity was determined by the method of Shimada *et al.* (1992).

Results and Discussion

Identification of bioactive compounds in Desmidorchis indica stem by GC MS analysis:

Throughout history, people have recognized and relied on traditional medicine due to its effectiveness. Therefore, medicinal plants may be seen as a storehouse for a wide range of bioactive chemicals with pharmacological potential (Aye *et al*., 2019). Twenty-five chemicals were identified by GC-MS analysis of *Desmidorchis indica* stem (hydro-alcoholic) (Fig. 1). Active ingredients are

Table 1: Identification of active compounds in *Desmidorchis indica* stem using GCMS

Peak	R. Time	Area %	Height %	Molecular Formula	Molecular Weight	Name of the compounds
1	7.029	2.67	2.10	$C_9H_{10}FeO_4$	238	Tricarbonyl[2-5.eta.-{(e)-2-methyl-2,4-pentadien-1-ol}] iron
2	9.998	5.52	5.14	$C_{12}H_{14}O_4$	222	1,2-benzenedicarboxylic acid, diethyl ester
3	13.054	3.20	3.24	$C_7H_{16}O$	116	3-Heptanol
4	13.258	3.65	1.07	$C_{17}H_{19}N_3O$	281	1-(benzotriazoyl-1-yl)-3-(1-cyclohexenyl) propargyl ethyl ether
5	13.918	12.23	9.91	$C_{16}H_{32}O_2$	256	Hexadecanoic acid
6	15.208	2.33	2.40	$C_{11}H_{20}O_2$	184	2-[3'-(1"-hydroxy-1"-methylethyl)-2',2'-dimethylcyclobutyl] ethanal
7	15.453	6.89	7.94	$C_{20}H_{40}O$	296	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
8	15.592	3.08	2.60	$C_{20}H_{23}N_3O_3$	353	Carbamic acid, N-[10,11-dihydro-5-(2-methylamino-1-oxoethyl)-3-5H-
9	15.844	8.95	5.21	$C_8H_{15}N$	125	3-Azabicyclo[3.2.2]nonane
10	15.925	2.52	4.68	$C_{11}H_{10}N_2O_5S$	282	7-ethyl-3-methyl-2-nitro-4-oxo-4,7-dihydrothieno[2,3-b]pyridine-5-carbo
11	15.973	5.68	6.45	$C_6H_{11}N$	97	Hexanenitrile
12	16.067	2.50	5.01	$C_5H_{10}N_2O_2$	130	Piperidine, 1-nitro-
13	16.133	2.86	4.88	$C_{27}H_{52}O_4Si_2$	496	9,12,15-octadecatrienoic acid
14	16.175	2.21	4.32	$C_{18}H_{34}O_2$	282	9-Octadecenoic acid
15	16.243	7.79	4.96	$C_{14}H_{22}N_2O_2$	250	Quinoxalin-2-one, decahydro-3-(3,3-dimethyl-2-oxobutenylideno)-
16	16.409	2.38	3.96	$C_{23}H_{27}NO_3$	365	9-(4-hydroxyphenyl)-3,3,6,6-tetramethyl-3,4,6,7,8a,9-hexahydro
17	16.483	2.14	3.84	$C_{14}H_{22}N_2O_2S$	282	Ethyl (1-adamantylamino) carbothioylcarbamat
18	16.608	3.11	3.92	$C_{23}H_{42}OSi$	362	4-Dimethyl(phenyl)silyloxypentadecane
19	16.702	2.58	3.61	$C_{11}H_{17}N$	163	2,4-octadienenitrile, 5-propyl-, (e)
20	16.925	3.92	3.22	$C_9H_{19}NO$	157	2-butanone, 3,3-dimethyl-4-[(1-methylethyl)amino]-
21	17.283	2.72	2.34	$C_6H_{11}N_3S$	157	Hydrazinecarbothioamide, 2-cyclopentylidene
22	17.850	2.12	1.87	$C_4H_7NO_2$	101	2-Propenamide
23	18.100	3.29	2.22	$C_5H_9NO_2$	115	6-oxa-1-azabicyclo[3.1.0]hexane, 5-methoxy-siloxy)-
24	21.893	3.07	2.55	$C_{18}H_{37}NO_3Si_3$	399	Phenethylamine, N-methyl-.beta.,3,4-tris(trime
25	22.634	2.60	2.55	$C_5H_5Br_2ClO$	274	Cyclopropanecarbonyl chloride, 2,2-dibromo-1-methyl-

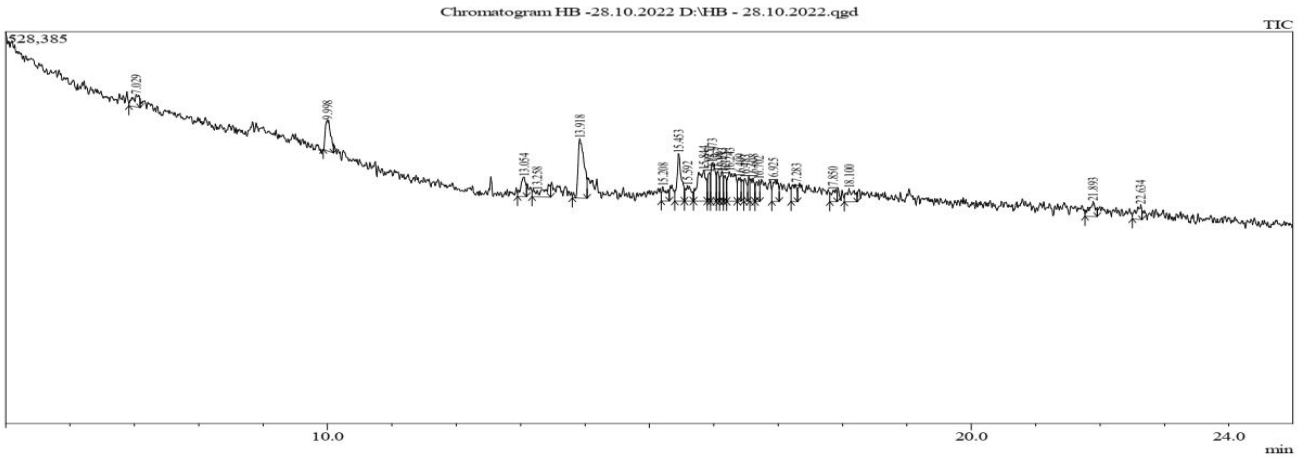

Fig. 1: Chromatogram of *Desmidorchis indica* stem.

Table 2: Biological activity of compounds identified in *Desmidorchis indica* stem using GCMS

R. Time	Name of the compounds	Biological activity**
9.998	1,2-benzenedicarboxylic acid, diethyl ester	Antimicrobial and Antifouling
13.918	Hexadecanoic acid	**Antioxidant**, Pesticide, Nematicide, Hypocholesterolemic, Anti androgenic, hemolytic, Flavor, Alpha reductase inhibitor, Antibacterial and antifungal
15.453	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Antimicrobial, anti-inflammatory, precursor for the manufacture of synthetic forms of vitamin E and vitamin K1 used in the fragrance industry and used in cosmetics, shampoos, toilet soaps, household cleaners, detergents cancer-preventive, antimicrobial, anti-diuretic.
15.592	Carbamic acid, N-[10,11-dihydro-5-(2-methylamino-1-oxoethyl)-3-5H-	Antimicrobial and insecticides
16.067	Piperidine, 1-nitro-	Antibacterial and antifungal
16.133	9,12,15-octadecatrienoic acid	Anti-inflammatory, Insectifuge Hypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic and Anticoronary
16.175	9-Octadecenoic acid	Anticancer, antimicrobial Anemiagenic, Insectifuge, Antiandrogenic, Dermatitigenic activity
17.283	Hydrazinecarbothioamide, 2-cyclopentylidene	Inhibitor of Ras protein and Carbonic anhydrase enzyme as potential anticancer agent
17.850	2-Propenamide	Antimicrobial and Antifungal

**Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database].

included in Table 1 along with their RT, molecular formula, MW, and concentration (%). Traditional healers' use the plant to treat a wide range of conditions which is supported by its chemical makeup, which contains a number of bioactive chemicals. To be sure, we might expect good findings from isolating particular phytochemical elements and testing their biological function (Table 2). Unknown compounds were identified by cross-referencing their peaks in the GC-MS spectra with those of known compounds in the NIST library (Ravindra and Mahendra, 2009) and creating a table containing the compound's name, molecular formula, and molecular weight (Peter *et al*., 2012).

Various biological activities were detected in the GC-MS study of a hydro-alcoholic extract from the stem of *Desmidorchis indica*. Plasticizer and antimicrobial/antifouling agent 1,2-benzenedicarboxylic acid, diisooctyl ester (Heinonen *et al*., 1998) Terpene compound 3,7,11,15-tetramethyl-2-hexadecen-1-ol showed antimicrobial, anticancer, anti-diuretic, and anti-inflammatory activity (Jegadeeswari *et al*., 2012; Upgade and Anusha, 2013); hexadecanoic acid and palmitic acid have antimicrobial activities, antioxidant, pesticide, nematicide, and alpha reductase (Mujeeb *et al*., 2014). Based on the results of a GC-MS study of a hydro-alcoholic extract of *Desmidorchis indica* stem, it has been hypothesized that the plant's biological properties include several components with antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Further investigation and isolation of the plant may reveal newer molecules that might be useful in the study of therapeutic and pharmacological activities.

In vitro antioxidant activity:

Antioxidant chemicals are substances that inhibit or slow the oxidation of other molecules. They prevent further damage from free radical chain reactions by giving an electron without becoming

Table 3: DPPH radical scavenging activity of *Desmidorchis indica* stem extract and compared with ascorbic acid

Concentrations (µg/ml)	% of inhibitions	
	Desmidorchis indica	**Std. (Ascorbic acid)**
20	19.14±0.09	21.27±0.16
40	29.78±0.18	37.44±0.38
60	48.08±0.28	55.74±0.54
80	70.63±0.41	73.19±0.65
100	85.95±0.67	90.63±0.83
IC_{50} (µg/ml)	59.18	53.51

Values expressed as Mean ± SD for triplicate

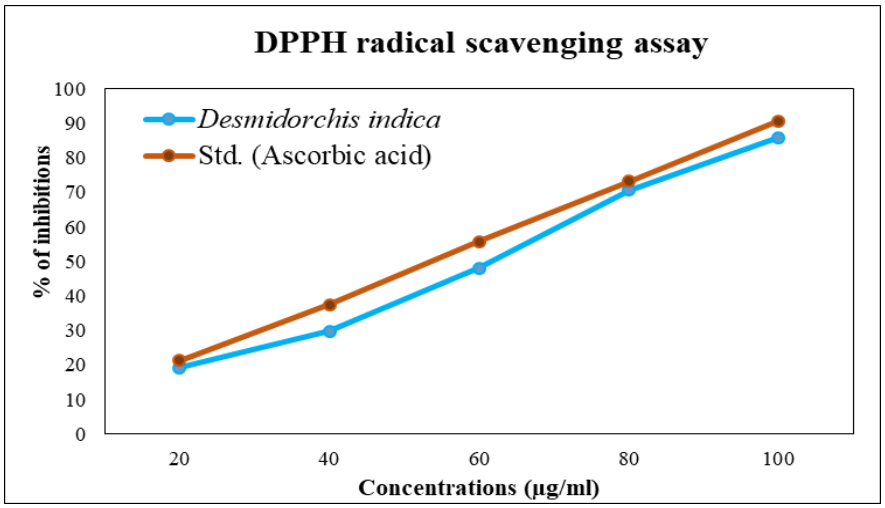

Fig. 2: DPPH radical scavenging activity of *Desmidorchis indica* stem extract and compared with ascorbic acid.

free radicals themselves, making them an essential component of modern biology. Antioxidants may be found in abundance in medicinal plants (Rice-Evans, 2004). The plasma antioxidant capacity is increased by natural antioxidants, and the risk of illnesses including cancer, heart disease, and stroke is decreased. The bioactive chemicals in the sample react with the stable free radical, a, a-diphenyl-picrylhydrazyl, in the DPPH technique (Table 3, Fig. 2). It undergoes a colour shift as it transforms into a,a-diphenyl- picrylhydrazine. The progressive shift in hue is a result of the presence of bioactive substances such phenolic compounds, flavonoids, terpenoids, and derivatives in the plant crude sample, which serve as free radical scavengers (Daffodil *et al*., 2012; Nishanthini *et al*., 2012).

Desmidorchis indica stem compared to ascorbic acid for its ability to scavenge DPPH radicals. The lowest DPPH-inhibiting activities were found for *Desmidorchis indica* (19.14%) and ascorbic acid (21.27%) in the 20 µg/ml concentration range, while the highest were found for the 100 µg/ml concentration range (85.95%) and 90.63 % (90.67 %), respectively. IC_{50} values for *Desmidorchis indica* and ascorbic acid were 59.18 µg/ml and 53.51 µg/ml, respectively. Potential DPPH activity and close to the norm may be found in the plant.

In this experiment, we evaluated the antioxidant power of ascorbic acid of varying doses to that of *Desmidorchis indica* stem. The stem of *Desmidorchis indica* showed the highest antioxidant activity, as measured by the inhibition concentration (IC_{50}). These results,

which confirm those from past research into other plants, imply that phenolic compounds contained in plants play an important role in shielding against the oxidative damage produced by free radicals (Velavan *et al*., 2007; Bera *et al*., 2015).

Conclusion

According to the GC-MS study, there are 25 phytochemical ingredients present in *Desmidorchis indica* stem, all of which contribute to the antioxidant activity. As a result, the existence of phytochemicals is the factor that is accountable for their curative benefits. Additional research is necessary in order to create new medications by making use of some of the bioactive substances that may be discovered in the stem of the *Desmidorchis indica* plant.

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