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Ethno Med, 18(1): 69-80 (2024)
PRINT: ISSN 0973-5070 ONLINE: ISSN 2456-6772 **DOI: 10.31901/24566772.2024/18.01.668**

GC-MS Based Identification of Bioactive Phytocompounds in Methanolic Extract of *Peperomia dindygulensis* Miq. and Their Antimicrobial Activities against Pathogens

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KEYWORDS Antibacterial. Genus *Pepreromia*. Metabolomic Profiling. Pharmacological Actions. Phytochemicals

ABSTRACT The genus *Peperomia* belongs to the family Piperaceae and has extraordinary ethnomedicinal significance. Characterising *Peperomia dindygulensis* Miq's bioactive ingredients was the objective of the current study. The gas chromatography and mass spectroscopy analysis of the methanol extract from this plant led to the identification of 30 compounds that principally contain steroids, sesquiterpenes, alkaloids, fatty acids, and fatty acid esters. Of these some important compounds such as trans-13-Octadecenoic acid, cis-Methyl 11-eicosenoate, methyl 13-phenyl-tridecanoate, 6-hydroxy-5,14,14-trimethyl-15,19-dioxapentacyclo [11.7.0.01,16.02,10.05,9]icos-12-en-18-one, obacunone and 7-Deacetoxy-7-hydroxy gedunin with higher area percentages revealed a large number of biological activities against pathogens. Also, these phytoconstituents have been linked to anti-cancer, anti-inflammatory, anti-microbial, anti-angiogenic, and antioxidant properties. The preliminary anti-microbial assay was carried out with five different extracts against four bacteria (*Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas* and *Salmonella enterica*) and four fungal (*Candida albicans*, *Aspergillus flavus*, *Rhizopus oryzae* and *Aspergillus niger*) strains. Methanol extract of the whole plant showed the best activity against microbes while the remaining extracts showed moderate activity.

INTRODUCTION

The analysis and extraction of plant material are critical to the development, modernisation, and quality assurance of herbal medicines. Due to the existence of phytochemicals, which have specific physiological effects on humans, medicinal plants have been utilised as treatments for human diseases for ages (Narayanamoorthi et al. 2015). Modernising herbal therapy involves analysing and extracting plant materials in significant ways. Sources of novel pharmaceuticals can be found aplenty in natural crude drug extracts obtained from plant species (Sunita and Ganesh 2017). A significant fraction of medicines used in modern medicine are either synthesised from lead compounds with natural origins or directly extracted from plants (Anirban et al. 2021).

The genus *Peperomia* Ruiz and Pavon is a species-rich genus usually found in tropical areas, which also contain some significant medicinal plants. *P. dindygulensis*, an herb used in conventional medicine for the treatment of malignancies and asthma has been shown to include secolignans, tetrahydrofuran lignans, polyketides, and other alkaloids (Govindachari et al. 1998; Wu et al. 2006; Wang et al. 2012). Secolignans are potent new chemotherapy agents with a wide range of biological effects, comprising the ability to reverse multidrug resistance and have anti-cancer, anti-angiogenic, anti-inflammatory, anti-HIV, antiparasitic, antiviral, and antibacterial properties (Wu et al. 2006). Pep E has anti-proliferative activity against prostate cancer cell lines and causes its apoptosis (Li et al. 2019), and Pep B and E have toxicity to human umbilical vein endothelial cancer cells stop the growth of lung cancer cells that are aggressive (Lin et al. 2011).

Objectives

The current study is primarily concerned with two important objectives:

1. Identifying potentially bioactive compounds in the methanolic extract of *P. dindygulensis* using the GC-MS technique.
2. Evaluation of the antimicrobial potentialities of *P. dindygulensis* solvent extracts against various bacterial and fungal pathogens

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MATERIAL AND METHODS

Test Sample and Chemical Extraction

The whole plant *P. dindygulensis* was collected from Paderu, Visakhapatnam, Andhra Pradesh, India ($18^0$10'10.79" N $82^0$46'58.32" E) in January 2022 and the specimen is identified by Professor S.B. Padal, Department of Botany, Andhra University and herbarium specimen submitted in AUV herbarium with accession number 25504. The entire plant was collected, air dried and then used an electric blender to grind it into a fine powder. Powdered material underwent extraction through a continuous hot Soxhlet apparatus, employing five distinct solvents: methanol, acetone, chloroform, water, and n-hexane, as detailed in the work of Harborne (1984).

Preliminary Phytochemical Screening

The screening of phytochemicals including alkaloids, saponins, tannins, steroids, flavonoids, terpenoids, anthraquinones, glycosides, cardiac glycosides, and phenols was done on a total of five extracts from this plant using a standard procedure (Stein 1990; Balamurugan et al. 2019).

The extraction values were determined by employing the formula as follows:

Yield (percentage) = W1/W2 x 100

Where:

W1 = The weight of the extract after solvent evaporation.

W2 = The dry weight of the plant sample.

GC-MS Analysis

The analysis of extracts was conducted utilizing the Agilent Technologies GC-MS instrument (GC-8890, GC/MS 5977 MSD). The injection mode employed was split, with a split flow rate of 18 mL/min and a purge flow rate of 3 mL/minute. Oven temperature was carefully regulated within the range of 75°C to a maximum of 360°C. Two distinct columns, namely the Polar Columns (DB-WAX) and HP-5 MS UI, were employed, with a helium gas (99.99%) serving as the carrier gas at a flow rate of 1.21 mL/minute. Column temperature settings were maintained between 60°C and 325°C. The total duration of the GC-MS runs encompassed 53.5 minutes. Electron ionization (EI) mode was employed to ionize the sample components, utilizing an energy level of 70 eV.

Determination of the Antibacterial Activity of Crude Bioactive Compounds

Using the agar well diffusion method, the antibacterial activity was evaluated (Balouiri et al. 2016). The test pathogens (*Pseudomonas* sp. MTCC 129, *Salmonella enterica* MTCC 98, *Streptococcus mutans* MTCC 497 ^{T} and *Staphylococcus aureus* MTCC 96) were inoculated in nutrient broth agar plates. The 5 mm diameter drilled wells on agar plates were supplied with 20 µL of plant extract. Following a 24-hour incubation period at 37°C, the plates were examined. The diameter of the inhibition zone was measured to estimate the antibacterial activity following incubation. The findings of each test were averaged after being run three times. The positive and negative controls were streptomycin 100µg/ml and DMSO, respectively.

Analysis of Antifungal Activity

Antifungal activity was assessed using the agar well diffusion method (Magaldi et al. 2004). The zone of inhibition was used to assess antifungal activity against the test fungus species (*Aspergillus flavus* MTCC 2798, *A. niger* MTCC 282, *Candida albicans* MTCC 183, and *Rhizopus oryzae* MTCC 2726). 5 mm diameter wells were drilled on a potato dextrose agar (PDA) using a sterilised cork-borer. After that, 20 ìL of the sample solutions were added to the wells. After 48-hour incubation at 26°C, the inhibition zone's diameter was determined. The reference antifungal drug used was fluconazole 30 g/ml, while DMSO was used as the negative control. Triplicates of each experiment were run.

RESULTS AND DISCUSSION

A total of five different extracts from *P. dindygulensis* were prepared in methanol, acetone, chloroform, aqueous, and n-hexane. Each extract's proportion of soluble chemicals was calculated, and the findings are shown in Table 1. This plant showed the highest percentage of

Table 1: Percentages of the soluble extract of various solvent extracts

The solvent used for extraction	*Weight of the powered material*	*The volume of the solvent*	*Weight of the soluble extract*	*Percentage of the extract*
Methanol	25gm	250ml	3.16	12.65
Acetone	25gm	250ml	4.52	18.1
Chloroform	25gm	250ml	3.18	12.72
Aqueous	25gm	250ml	4.14	16.5
N-Hexane	25gm	250ml	3.11	12.4

Source: Authors

soluble compounds in acetone extract (18.1%) followed by distilled water (16.5%). The lowest yield was reported in hexane (12.4) followed by methanol (12.65%).

Preliminary Qualitative Phytochemical Analysis

The current investigation found that the different *P. dindygulensis* extracts contained a variety of compounds, including terpenes, flavonoids, alkaloids, cardiac glycosides, phenols, glycosides, steroids, saponins, and flavonoids (Table 2). However, all five extracts included phenols, tannins, and terpenoids. Except for n-hexane, practically all extracts included alkaloids and flavonoids. A variety of secondary metabolites, including alkaloids, phenols, tannins, anthraquinones, terpenoids, anthocyanins, steroids, coumarins, and flavonoids, were detected in both methanol and acetone extracts. These secondary metabolites were present in a medium variety in the aqueous extract. The least amount of secondary metabolites is present in n-hexane and chloroform extracts when compared to all other solvent extracts. Both the methanol and the aqueous extracts contained primary metabolites such as carbohydrates, proteins, and fatty acids. The methanol extract exhibited higher levels of steroids, phenols, coumarins, and alkaloids.

Antimicrobial Activity

About 4 strains of bacteria, two from each gram-negative (*S. enterica* and *Pseudomonas* sp.) and gram-positive (*S. mutans* and *S. aureus*) and 4 fungal species, 1 from a non-filamentous human pathogen (*Candida albicans*), 2 filamentous plant pathogens (*Aspergillus flavus*

Table 2: Preliminary qualitative phytochemical analysis of various extracts of *P. dindygulensis*

Plant constituents	*Aq*	*Me*	*Ac*	*Ch*	*Nh*
Carbohydrates	++	++	+	-	-
Proteins	+	+	-	-	-
Fixed oils and fats	-	+	-	-	-
Gums and mucilage	-	+	+	-	+
Alkaloids	-	++	+	+	-
Anthraquinones	-	+	+	+	+
Phenols	++	++	+	+	+
Tannins	+	++	++	+	+
Flavonoids	+	+	+	+	-
Terpenoids	+	+	+	+	+
Glycosides	-	-	+	-	++
Cardiac glycosides	+	+	-	-	-
Saponins	-	-	-	-	+
Steroids	+	++	+	-	+
Anthocyanins	+	+	+	-	-
Coumarins	+	++	++	-	-

Aq: aqueous extract; Me: Methanolic extract; Ac: acetone extract; Ch: chloroform extract; Nh: n-hexane extract; (-) = negative (absent), (+) = Positive (slightly present), (++) = Positive (Abundantly present).
Source: The authors give data from their analysis in the form of a table

and *A. niger*) and 1 opportunistic human pathogen (*Rhizopus oryzae*) were exposed to crude extracts of Methanol (Me), Acetone (Ac), Chloroform (Ch), Aqueous (Ch), and n-hexane (Nh) (10mg, 5mg, and 2.5mg of each). Tables 3 and 4 were showing the results of the zone of inhibition tests against bacteria and fungi, respectively. *Streptococcus mutans* were most effectively inhibited by the methanol extract at concentrations of 10 mg, 5 mg, and 2.5 mg in zones measuring 20, 18, and 16 mm, respectively. Acetone and chloroform extracts displayed strong inhibitory effects against the same microbe (*S. mutans*) in comparison to methanol. Except for *Pseudomonas*, three organisms were not inhibited by aqueous extracts. Only the gram-positive bacteria *S. mutans* and *S. aureus* were susceptible to the inhibitory effects of n-hexane extracts. Only methanol and chloroform extracts displayed the lowest inhibitory activity at their maximum concentrations when used against *Salmonella enterica*, which exhibited the highest resistance to all plant extracts. Gram-negative bacteria *Salmonella* was highly resistant, *Pseudomonas* was moderately tolerant to *P. dindygulensis* plant crude extracts, whereas gram-positive bacteria *S. mutans* and *S. aureus* were more vulnerable.

Only *C. albicans* was affected by all five extracts, whereas methanol and water extract demonstrated inhibitory efficacy against all four fungal strains. At its maximum dose (10 mg), acetone extract had the greatest growth-inhibiting activity against *C. albicans* (12.3mm). While *C. albicans* is more vulnerable than the other three fungi, *Rhizopus oryzae* is the most resistant of the four. Research has demonstrated that alkaloids and polyphenols exhibit pronounced antimicrobial properties against a diverse spectrum of pathogenic bacteria and fungi (Othman et al. 2019). The methanol extracts exhibit strong antibiotic activity against bacteria, which suggests that active components are present in large amounts and it also exhibited higher concentrations of phthalic acid, di (2-propyl pentyl) ester, which has antimicrobial effects (Osuntokun 2019). This could be the cause of *P. dindygulensis*' remarkable antimicrobial potential.

GC-MS Spectral Data Analysis

Methanol extract demonstrated the highest level of microbial growth inhibition among the different solvent extracts studied, so GC-MS was used to analyse the comprehensive phytochemical profiling. The aforementioned experimental method led to the identification of multiple peaks in the GC-MS spectral data of the methanol extract of *P. dindygulensis*, which suggested the existence of 30 different compounds. Figure 1 displayed the chromatogram of the GC-MS. By evaluating the compounds' mass fragmentation patterns and retention indices in the Spectral Library and Database based on the licensed NIST 2017 Library and were operated using Open Lab CDS version 2.5 software, each of these compounds was identified and described. The compounds reported in GC-MS spectral data are included in Table 5 along with their peak area percentages, chemical class, molecular formula, molecular weight, structural features, and biological activities in order of retention times.

The biological activities of important compounds were investigated by a survey of the literature. The researchers observed that most of the compounds possess a variety of advantageous pharmacological and therapeutic effects. Phthalic acid, di (2-propyl pentyl) ester (37.68), 6-hydroxy-5,14,14-trimethyl-15, 19-dioxapentacyclo [11.7.0.01,16.02,10.05,9] icos-12-en-18-one (16.88), 7-deacetoxy-7-hydroxy gedunin (10.24), cis-methyl 11-eicosenoate (5.14) and methyl 13-phenyl-tridecanoate (2.93) were the most prevalent significant chemicals in respective of their area percentages. The insignificant substances included salvigenin (0.95), cis-13-eicosenoic acid, methyl ester (1.96), obacunone (1.93), ethyl iso-allocholate (1.05), cholesta-22, 24-dien-5-ol, 4, 4-dimethyl (0.97) and cis-13-eicosenoic acid, methyl ester (1.96). Less than 0.9 percent of the compounds were leftovers.

P. dindygulensis contains numerous bioactive compounds. Wu et al. (2006) isolated thirteen secolignans, including Peperomin A, B, C, and E, and Lin et al. (2011) isolated two more secolignans, Peperomin G and H, all of which have anticancer properties. Wang et al isolated tetrahydrofuran lignans in 2012. The entire phytochemistry of *P. dindygulensis* is summarised by Duan et al. (2019) and 87 identified chemicals are discussed, including lignans (43), flavone glycosides (15), and polyketides (17), steroids (4), fatty acids (2) and aromatic compounds (6). In this study, about 30 phytocompounds were

Table 3: Antibacterial activity of different extracts (Aq, Me, Ac, Ch &Nh) at the dosages of 10mg, 5mg, and 2.5mg

	Extract		*Salmonella*		*Pseudomonas*		*Streptococcus mutans*			*Staphylococcus aureus*		
	10 mg	*5 mg*	*2.5 mg*	*10 mg*	*5 mg*	*2.5 mg*	*10 mg*	*5 mg*	*2.5 mg*	*10 mg*	*5 mg*	*2.5 mg*
Aq	-	-	-	10.6±0.57	9±1	7.6±0.57	-	-	-	-	-	-
Me	10±1	-	-	12.3±0.57	11±1	11.3±0.57	20.3±0.57	18±1	16±1	11.3±0.57	8.6±0.57	7.3±0.57
Ac	-	-	-	7.6±0.57	6.6±0.57	6.3±0.57	17±0	16±1	14±1	15.6± 1	11.6±0.57	10.3±0.57
Ch	7±0	-	-	8.6±0.57	8±0	7.3±0.57	16±0	14.6±0.57	14±1	10.6±0.57	6.6±0.57	-
Nh	-	-	-	-	-	-	10.5±0.7	8.6±0.57	6.3±0.57	7.6±0.57	7.6±0.57	-

*The values given are the representation of Mean ± S.D (n=3)
Aq: aqueous extract; Me: Methanolic extract; Ac: acetone extract; Ch: chloroform extract; Nh: n-hexane extract; "-" no zone inhibition. A diameter of the zone of inhibition less than 6 mm was considered inactive.
Source: The authors give data from their analysis in the form of a table

Table 4: Antifungal activity of different extracts (Me, Ac, Ch, W & Nh) at the dosages of 10mg, 5mg, and 2.5mg

	Extract		*Salmonella*		*Pseudomonas*		*Streptococcus mutans*			*Staphylococcus aureus*		
	10 mg	*5 mg*	*2.5 mg*	*10 mg*	*5 mg*	*2.5 mg*	*10 mg*	*5 mg*	*2.5 mg*	*10 mg*	*5 mg*	*2.5 mg*
Aq	8.6±0.57	7±1	6.3±0.57	12±0.57	11.3±0.57	11.3±0.57	11±1	10.3±0.57	7.6±0.57	6.3±0.57	-	-
Me	6.6± 057	6.3±0.57	-	9±1	6.6±0.57	6.6±0.57	13±0	9.6±0.57	8.6±0.57	6.6±0.57	6.3±0.57	-
Ac	12.3±0.57	7.3±0.57	7±0	-	-	-	-	-	-	-	-	-
Ch	8.3±0.57	7±1	6.3±0.57	-	-	-	-	-	-	-	-	-
Nh	10.6±0.57	7.6±0.57	7±0	-	-	-	-	-	-	-	-	-

*The values given are the representation of Mean ± S.D (n=3)
Aq: aqueous extract; Me: Methanolic extract; Ac: acetone extract; Ch: chloroform extract; Nh: n-hexane extract; "-" no zone inhibition. A diameter of the zone of inhibition less than 6 mm was considered inactive.
Source: The authors give data from their analysis in the form of a table

Table 5: Bioactive compound identified *P. dindygulensis* through GC-MS

S.No.	Compound name	RT	Mol. weight	Mol. formula	Area %	Activities
1.	Caryophyllene (β- caryophyllene)	13.90	204.26	$C_{15}H_{24}$	0.7	Antineoplastic, Sedative, Analgesic, and antibacterialanti-oxidant, anti-inflammatory, and painkilling (Narayanamoorthi et al. 2015; Hartsel et al.2016)
2.	Humulene(α caryophyllene)	14.72	204.35	$C_{15}H_{24}$	0.08	Anti-inflammatory and insect-repellant aromatic compound (Hartsel et al.2016; Russo et al. 2017)
3.	Alloaromadendrene	14.90	204.35	$C_{15}H_{24}$	0.11	Antioxidant, life span enhancer (Chan et al.2014)
4.	γ-muurolene	15.25	204.35	$C_{15}H_{24}$	0.11	Antifungal, antimicrobial, and anticancer activities (Duan et al. 2019; Ugbogu et al. 2022)
5.	1 HCyclopenta[1,3]cyclopropa[1,2] benzene,octahydro-7-methy-3-methylene syn; β-cubebene	15.37	204.35	$C_{15}H_{24}$	0.11	Analgesic, Anti-Bacterial, Anti-Inflammatory, anti-tumor, and Fungicide (Narayanamoorthi et al. 2015)
6.	β- Acorenol	15.80	222.37	$C_{15}H_{26}O$	0.15	Odorant in essential oils (Barbara et al. 2009)
7.	1-Naphthalenol, 1,2,3,4,4a,7,8, 8a-octa hydro-1,6-dimethyl-4-(1-methyl ethyl)-[oxyoctalineformate]	16.14	222.37	$C_{15}H_{24}O_2$	0.09	Anti-tumor, Analgesic, Anti-bacterial, and Anti-infla-(Narayanamoorthi et al. 2015) mmatory
8.	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl ethyl)-, (1S-cis)-	16.35	204.35 -	$C_{15}H_{24}$	0.17	
9.	Caryophyllene oxide	17.76	220.35	$C_{15}H_{24}O$	0.20	Food preservative, used in cosmetics and have anti-fungal activities (Yang et al.1999; Russo et al.2017)
10.	Apiol	18.63	222.23	$C_{12}H_{14}O_4$	0.18	Used for the treatment of menstrual disorders and as an abortifacient (Narayanamoorthi et al. 2015)
11.	(1R,7S, E)-7-Isopropyl-4,10-dimethylene cyclodec-5-enol	20.04	220.35	$C_{15}H_{24}O$	0.65	-
12.	Hexadecanoic acid, methyl ester	25.89	270.45	$C_{17}H_{34}O_2$	0.69	Anti-bacterial (Shaaban et al. 2021), Antiandrogenic, hemolytic, nematicide, insecticide, lubricant, and hypo-cholesterolemic (Sunita and Ganesh 2017)
13.	n-Hexadecanoic acidsyn; palmitic acid	26.79	256.42	$C_{16}H_{32}O_2$	0.21	Anti-inflammatory (Aparna et al. 2012), cytotoxic (Noori et al. 2013), Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor (Narayanamoorthi et al. 2015)
14.	Methyl 9-cis,11-trans-octadecadienoate	30.03	279.4	$C_{18}H_{31}O_2$	0.66	-
15.	cis-13-Octadecenoic acid, methyl ester	30.30	296.5	$C_{19}H_{36}O_2$	0.82	No activity reported
16.	trans-13-Octadecenoic acid, methyl ester	30.56	296.5	$C_{19}H_{36}O_2$	13.2	Anti-leukotriene—D4, irritant, anti-androgenic, cancer-preventive, hypocholesterolemic, 5-alpha reductase inhibitor, anemia-genic, insectifuge (Krishnamoorthy and Subramaniam 2014)
17.	Heptadecanoic acid, 16-methyl-, methyl ester	30.76	298.5	$C_{19}H_{38}O_2$	0.66	Anti-cancer properties (Kandasamy et al. 2012)
18.	cis-13-Eicosenoic acid, methyl ester	34.34	324.5	$C_{21}H_{40}O_2$	1.96	-

Table 5: Contd...

S.No.	Compound name	RT	Mol. weight	Mol. formula	Area %	Activities
19.	cis-Methyl 11-eicosenoate	34.54	324.54	$C_{21}H_{40}O_2$	5.14	Triggers the immune system and stimulates the production of inflammatory metabolites (Alqarni et al. 2019)
20.	Methyl 13-phenyl-tridecanoate	34.70	304.5	$C_{20}H_{32}O_2$	2.93	Anti-oxidant and anti-inflammatory (Zilani et al. 2021)
21.	Hexadecanoic acid, 1-(hydroxymethyl) -1,2-ethanediyl ester	37.72	568.9 g/mol	$C_{35}H_{68}O_5$	0.35	Antioxidant, anti-microbial (Kadhim et al. 2017)
22.	Phthalic acid, di(2-propyl pentyl) ester	38.44	390.55	$C_{24}H_{38}O_4$	37.68	Anti-microbial activity (Osuntokun 2019)
23.	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	40.56	365.5	$C_{21}H_{40}O_4$	0.22	Antimicrobial,Anticancer, Diureticand Anti-inflammatory (Hussein et al. 2016)
24.	4H-1-Benzopyran-4-one, 5-hydroxy -6,7-dimethoxy-2-(4-methoxyphenyl) -Syn; salvigeninnomodulatory,	45.68	328.31	$C_{18}H_{16}O_6$	0.95	neuroprotective effect. antitumor cytotoxic, immu and inhibits H2O2-induced cell apoptosis (Shia et al. 2020)
25.	Ethyl iso-allocholate	47.79	436.63	$C_{26}H_{44}O_5$	1.05	Anti-microbial, Diuretic, Anti-inflammatory, Antiasthma (Tyagi et al. 2017; Malathi et al. 2016)
26.	Cholesta-22,24-dien-5-ol, 4,4-dimethyl syn;14-Demethyllanosterol	48.19	412.7	$C_{29}H_{48}O$	0.97	Antibacterial, trypanocidal activity (Tyagi et al. 2017)
27.	6-hydroxy-5,14,14-trimethyl-15,19 -dioxapentacyclo[11.7.0.01,16.02, 10.05,9]icos-12-en-18-one	48.82	346.21	$C_{21}H_{30}O_4$	16.88	Not reported
28.	Obacunone	48.90	454.5	$C_{26}H_{30}O_7$	1.93	Anti-cancer activity (Shia et al. 2020; Kim et al. 2014)
29.	D-Homo-24-nor-17-oxachola-1,20, 22-triene-3,7,16-dione, 14,15:21, 23-diepoxSyn; 7-Deacetoxy-7-hydroxygedunin	50.18	440.5	$C_{26}H_{32}O_6$	10.24	Anti-bacterial (Okhale et al. 2013), Diuretic, Anti-inflammatory, Antiasthma (Tyagi et al. 2017)
30.	7a-Acetoxy-3-oxo-1,2-14b, 15b-diepoxy-meliacolide	50.66	498.22 -	$C_{28}H_{34}O_8$	0.56	

RT: retention time
Area %: relative percentage obtained from the peak area
Source: The authors give data from their analysis in the form of a table. Bio activities were collected through a literature survey (reference cited in table)

reported through GC-MS analysis. Of these, only hexadecanoic acid was previously reported by Chen et al. (2007), but the remaining compounds were discovered for the first time from this plant. The compounds identified by GC-MS analysis were categorised into terpenes, fatty acids, flavones, phenols, steroids, and other substances. Fatty acids (11 compounds) were the most representative compounds in terms of diversity, followed by sesquiterpenes (10 compounds), steroids (6), and phenols, flavones, and alkyl aryl esters, which had the fewest single compounds. However, alkyl aryl esters (Phthalic acid, di (2-propyl pentyl) ester) alone led in terms of area percentage, accounting for 37.68 percent, followed by steroids, limonoids, and fatty acids (26.84%). Tables 6 and 7 show several groups of components' relative abundance and diversity.

In the present study, different phytocompounds obtained with the help of GC-MS analysis were also reported with anti-cancer, antimicrobial, anti-inflammatory, sedative, anti-asthma, analgesic, antioxidant, and painkiller properties. The compounds β-caryophyllene,β-cubebene, oxyoctalineformate, methyl 9-cis,11-trans-octadecadienoate, trans-13-octadecenoic acid, methyl ester, heptadecanoic acid, 16-methyl-, me-

Table 7: The relative abundance of different phytochemical groups

S. No.	*Grouped components*	*Relative area percentages*
1	Terpenes	2.37
2	Phenyl propenes	0.18
3	Fatty acids	26.84
4	Alkyl aryl ester	37.68
5	Flavones	0.95
6	Steroids	19.78
7	Limonoids	12.78

Source: Authors

Table 8: Pie chart on % of compounds showing different biological activities

S. No.	*Compounds name*	*Pharmacological action*
1	β- caryophyllene, 1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-[oxyoctaline formate], caryophyllene oxide, hexadecanoic acid, methyl ester, hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, phthalic acid, di (2-propylpentyl) ester, 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, ethyl iso-allocholate, 14-demethyllanosterol, and 7-deacetoxy-7-hydroxygedunin	Antimicrobial activity
2	β- caryophyllene, alloaromadendrene, palmitic acid, methyl 13-phenyl-tridecanoate, hexadecanoic acid and 1-(hydroxymethyl)-1,2-ethanediyl ester	Antioxidant activity
3	β-cubebene, 1-naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethy l-4-(1-methylethyl)-[oxyoctaline formate], methyl 9-cis,1 1-trans-octadecadienoate, trans-13-octadecenoic acid, methyl ester, heptadecanoic acid, 16-methyl-, methyl ester, 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, salvigenin and obacunone	Anti-cancer
4	β- caryophyllene, α caryophyllene, β-cubebene, 1-naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-[oxyoctaline formate], palmitic acid, trans-13-octadecenoic acid, methyl ester, cis-methyl 11-eicosenoate, methyl 13-phenyl-tridecanoate, 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester, ethyl iso-allocholate and 7-deacetoxy-7-hydroxygedunin	Anti inflamatory
5	β- caryophyllene	Sedative
6	β- caryophyllene, β-cubebene and 1-naphthalenol, 1,2,3,4,4a,7,8, 8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-[oxyoctaline formate]	Analgesic
7	Hexadecanoic acid, methyl ester, n-hexadecanoic acid and trans-13-octadecenoic acid, methyl ester	Anti-androgenic
8	Hexadecanoic acid, methyl ester and n-hexadecanoic acid	Nematicide/ insectiside
9	Hexadecanoic acid, methyl ester, palmitic acid, and trans-13-Octadecenoic acid, methyl ester	Hypocholestromic

Source: A review of the literature (references cited in table 5)

thyl ester, phthalic acid, di (2-propyl pentyl) ester, 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester, salvigenin, and obacunone are previously reported with anticancer activities. The compounds with the highest area percentages are discussed here for their biological activities. Phthalic acid, di (2-propyl pentyl) ester having anti-microbial properties (Osuntokun 2019), trans-13-octadecenoic acid methyl ester is significant because it qualities that are anti-leukotriene D4, anti-inflammatory, antiandrogenic, cancer-preventive, irritating, hypocholesterolemic, 5-alpha reductase inhibitor (Krishnamoorthy and Subramaniam 2014), 7-deacetoxy-7-hydroxygedunin is another important molecule having anti-bacterial (Okhale et al. 2013), diuretic, anti-inflammatory, and antiasthma (Tyagi and Agarwal 2017) properties. Cis-methyl 11-eicosenoate triggers the immune system and stimulates the production of inflammatory metabolites (Alqarni et al. 2019). Of the 30 compounds found in methanol extract, nearly 11 have antimicrobial and antioxidant properties, ten have cancer-fighting and cytotoxic properties, four have antioxidant properties, three have analgesic and hemolytic properties and two have asthmatic properties (Table 8). No activity has been reported from cis-13-eicosenoic acid, methyl ester, 6-hydroxy-5, 14, 14-trimethyl-15,19-dioxapentacyclo [11.7.0.01,16.02,10.05,9] icos-12-en-18-one and cis-13-octadecenoic acid, methyl ester till date. The structures of the compounds detected in GC-MS analysis are given in Figure 2.

CONCLUSION

In the present study, the medicinal herb of *P. dindygulensis* was chosen for the phytochemical characterisation and microbial analysis. The increased amount of phenolic compounds, alkaloids, steroids, flavonoids and tannins revealed by methanol and acetone extracts compared to water, chloroform, and n-hexane extracts explained why these two extracts had stronger antimicrobial activity among the five. A total of 30 peaks were identified by the GC-MS analysis of the methanol extract representing a greater variety of compounds. The majority of the chemicals found in this extract, including caryophyllene, cubebene, oxyoctaline formate, methyl 9-cis, 11-trans-octadecadienoate, trans-13-octadecenoic acid, methyl ester, phthalic acid, di (2-propyl pentyl) ester, 9-octadecenoic acid (Z), salvigenin and obacunone are proven to have antioxidant, antibacterial, anti-inflammatory, an-

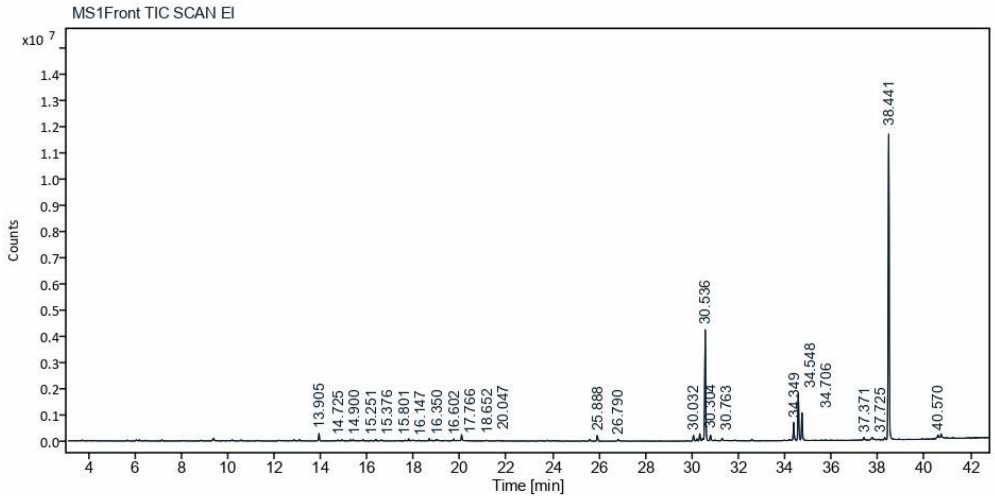

Fig. 1. GC-MS chromatogram of methanol extract of P. dindygulensis
Source: Authors

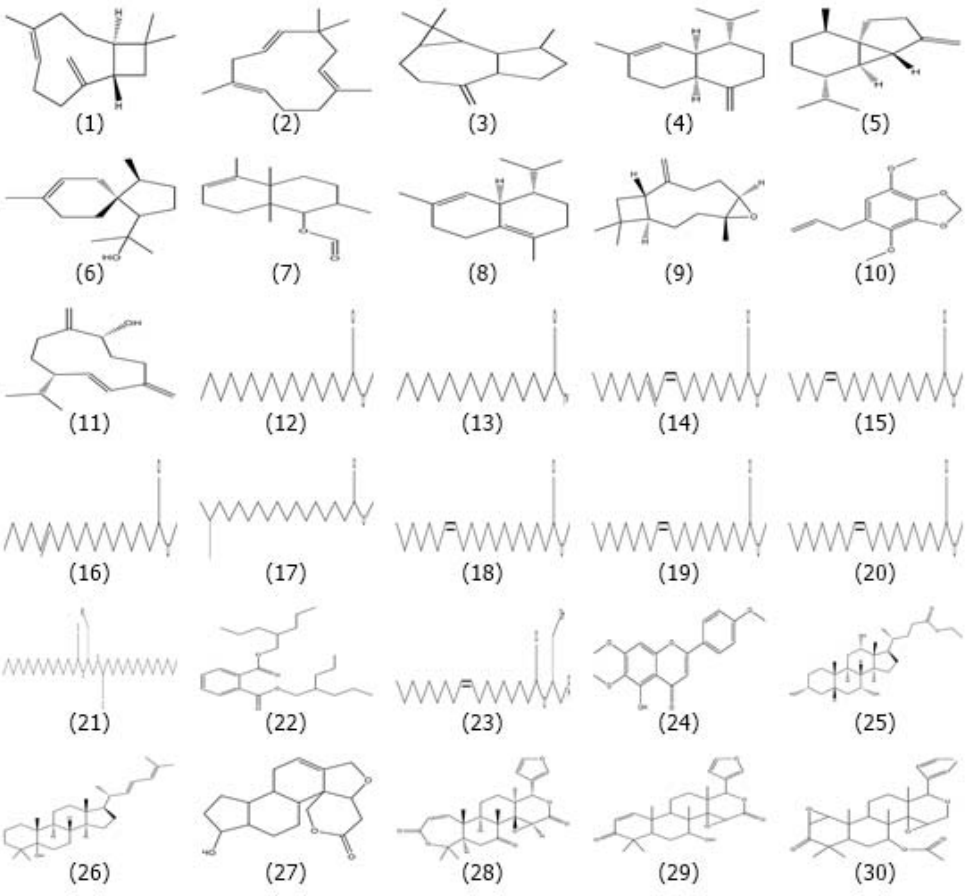

Fig. 2: Phytocompounds (Structures) from P. dindygulensis Compound numbering is given the same as in Table 5
Source: Authors

titumor and anti-cancer properties. Among the fungal strains examined, except *C. albicans*, all others showed simply moderate to frail antifungal action, and the antibacterial measure exhibited great adequacy. When antimicrobial potentialities of plant extracts were compared to those of standard extracts, methanol extract showed the highest (65%) resistance to *S. mutans*, whereas the other extracts demonstrated 25 to 50 percent. The bioactive compounds of *P. dindygulensis* methanolic extract showed effective antimicrobial activities as a substitute for the development of new antimicrobial agents as a medicine.

RECOMMENDATIONS

Methanolic extract of *P.dindygulensis* has been found to have potent antimicrobial capabilities under in-vitro conditions, whereas GC-MS analysis of this medicinal plant confirms this potential by identifying several secondary metabolites and bioactive compounds with effective antimicrobial properties. These discoveries have given the use of plant resources in traditional medicine a sound scientific foundation. It is strongly advised that more research should be done on this medicinal plant so that it can

withstand the clinical trials to develop plant-based natural medications.

ABBREVIATIONS

AUV: Andhra University Herbarium code

GC-MS: Gas Chromatography-Mass Spectrometry

MSD: Mass Selective Detector

HP-5 MS: (5%-phenyl)-methylpolysiloxane capillary column

UI: Ultra Inert

EI: Electron Ionisation

MTCC: Microbial Type Culture Collection and Gene Bank

DMSO: dimethyl sulfoxide

NIST: National Institute of Standards and Technology

CDS: Chromatography Data System

CONFLICT OF INTEREST

The authors declare that they hold no competing interests.

ACKNOWLEDGMENTS

The first author wishes to acknowledge the Indian Council of Medical Research (ICMR) for funding the research, as well as Dr. Ummidi Ravi Shankar of the Mic gene laboratory in Visakhapatnam for his assistance at various stages of this study. The authors would like to thank the Department of Botany at Andhra University for providing facilities and instruments for the research, as well as SAIF-IIT Madras for providing GC-MS services.

REFERENCES

Alqarni AMM, Dissanayake T, Nelson DJ et al. 2019. Metabolomic profiling of the immune stimulatory effect of eicosenoids on PMA-differentiated THP-1 cells. *Vaccines*, 7(4): 142.

Anirban C, Amrita P, Priya KG et al. 2021. GC-MS analysis and screening of anti-proliferative potential of methanolic extract of *Garcinia cowa* on different cancer cell lines. *Pharmacogn J*, 13(2): 347-361.

Aparna V, Dileep KV, Mandal PK et al. 2012. Anti-inflammatory property of n-Hexadecanoic acid: Structural evidence and kinetic assessment. *Chem Biol Drug Des*, 80(3): 434-439.

Balamurugan V, Fatima MAS, Sreenithi V 2019. A guide to phytochemical analysis. *Int. j. adv. res. innov. ideas educ.*, 5 (1) 236-245.

Balouiri M, Sadiki M, Ibnsouda SK 2016. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal*, 6(2): 71-79.

Barbara DZ, Ana CLA, Ana LPdeM et al. 2009. Screening of the odour-activity and bioactivity of the essential oils of leaves and flowers of *Hyptis passerine* Mart. from the Brazilian Cerrado. *J Braz Chem Soc*, 20(2): 322-332.

Chan WY, Wen HL, Fu LH et al. 2014. Essential oil alloaromadendrene from mixed-type *Cinnamomum osmophloeum* leaves prolongs the lifespan in *Caenorhabditis elegans*. *J Agric Food Chem*, 62(26): 6159-6165.

Chen L, Zhou Y, Dong JX 2007. Chemical constituents of *Peperomia dindygulensis*. *Chin. Tradit. Herb. Drugs*, 38(4):491-493

Doan TQH, Trong DV, Le N et al. 2019. Chemical composition and anti-inflammatory activity of the essential oil from the leaves of *Limnocitrus littoralis* (Miq.) Swingle from Vietnam. *Nat Prod Res*, 35(9): 1550-1554.

Duan Z, Wang Y, Xiao HX 2019. The *Peperomia dindygulensis*: A review of phytochemistry and pharmacology perspectives. *Asian J Tradit Med*, 14(4): 193-201.

Govindachari TR, Krishna KN, Partho PD 1998. Two secolignans from *Peperomia dindigulensis*. *Phytochemistry*, 49(7): 2129-2131.

Harborne JB 1984. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.* 2nd Edition. London: Chapman and Hall Enterprises.

Hartsel JA, Eades J, Hickory B et al. 2016. *Cannabis sativa* and Hemp. *Nutraceuticals*, 735-754.

Hussein JH, Mohammed YH, Imad HH 2016. Study of the chemical composition of *Foeniculum vulgare* using Fourier transforms infrared spectrophotometer and gas chromatography-mass spectrometry. *J Pharmacognosy Phytother*, 8(3): 60-89.

Kadhim MJ, Abeer FAR, Imad HH 2017. Determination of bioactive compounds of methanolic extract of *Vitis vinifera* Using GC-MS. *Int J Toxicol Pharmacol Res*, 9(2): 113-126.

Kandasamy S, Sahu SK, Kandasamy K 2012. In Silico studies on fungal metabolite against skin cancer protein (4, 5-Diarylisoxazole HSP90 Chaperone). *ISRN Dermatology*, 2012: 1-5.

Kim J, Jayaprakasha GK, Patil BS 2014. Obacunone exhibits anti-proliferative and anti-aromatase activity in vitro by inhibiting the p38 MAPK signaling pathway in MCF-7 human breast adenocarcinoma cells. *Biochimie*, 105: 36-44.

Krishnamoorthy K, Subramaniam P 2014. Phytochemical profiling of leaf, stem, and tuber parts of *Solenaam plexicaulis* (Lam.) Gandhi Using GC-MS. *Int Sch Res Notices*, Article ID 567409.

Li Y, Jigang P, Meng G et al. 2019. The anti-proliferation, cycle arrest and apoptotic inducing activity of Peperomin E on prostate cancer PC-3 cell line. *Molecules*, 24: 1472.

Lin MG, Yu DH, Wang QW et al. 2011. Secolignans with antiangiogenic activities from *Peperomia dindygulensis*. *Chemistry & Biodiversity*, 8(5): 862-871.

Magaldi S, Mata ES, Hartung de CC et al. 2004. Well diffusion for antifungal susceptibility testing. *Int J Infect Dis*, 8(1): 39-45.

Malathi K, Anand A, Sudha R 2016. Ethyl Iso-allocholate from a Medicinal Rice Karungkavuni inhibits Dihydropteroate Synthase in *Escherichia coli*: A molecular docking and dynamics study. *J Pharm Sci*, 78(6): 780-788.

Narayanamoorthi V, Vasantha K, Rency RC et al. 2015. GC-MS determination of bioactive components of *Peperomia pellucida* (L.) Kunth. *Bioscience Discovery*, 6(2): 83-88.

Noori S, Hassan ZM, Yaghmaei B et al. 2013. Antitumor and immune modulatory effects of salvigenin on tumor-bearing mice. *Cellular Immunology*, 286(1-2): 16–21.

Okhale SE, Amupitan JO, Ndukwe IG et al. 2013. Synthesis and antibacterial activity of 7-deacetoxy-7 á hydroxygedunin. *Afr J Pure Appl Chem*, 7(4):157-163.

Osuntokun OT 2019. Bio-guided isolation, chemical purification, identification, antimicrobial and synergistic efficacy of extracted essential oils from stem bark extract of *Spondias mombin* (Linn). *Int J Biochem Mol Biol*, 4(4): 135-143.

Othman L, Ahmad S, Roula M et al 2019. Antimicrobial activity of polyphenols and alkaloids in Middle Eastern plants. *Front Microbiol J*, 10: 911.

Russo EB, Marcu J 2017. *Cannabis* pharmacology: The usual suspects and a few promising leads. *Adv Pharmacol*, 80: 67-134.

Shaaban MT, Ghaly MF, Fahmi SM 2021. Antibacterial activities of hexadecanoic acid methyl ester and green synthesized silver nanoparticles against multidrug resistant bacteria. *J Basic Microbiol*, 61(6): 557-568.

Shia YS, Yan Z, Hao TL et al. 2020. Limonoids from Citrus: Chemistry, anti-tumor potential, and other bioactivities. *J Funct Foods*, 75: 104213.

Stein SE 1990. National Institute of Standards and Technology (NIST), Mass Spectral Database and Software. *Version 3.02*. Gaithersburg, USA, 1990.

Sunita A, Ganesh K 2017. Gas Chromatography-Mass Spectrometry (GC-MS) determination of bioactive constituents from the methanolic and ethyl acetate extract of *Cenchrus setigerus* Vahl (Poaceae).*The Pharma Innovation Journal*, 6(11): 635-640.

Tyagi T, Agarwal M 2017. GC-MS analysis of invasive aquatic weed, *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) Solms. *Int J Curr Pharm Res*, 9(3): 111-117.

Ugbogu EA, Okezie Emmanuel, Miracle EU et al. 2022. The ethnobotanical, phytochemistry, and pharmacological activities of *Psidium guajava* L. *Arab J Chem*, 15(5): 103759.

Wang QW, Yu DH, Meng GL et al. 2012. Antiangiogenic polyketides from *Peperomia dindygulensis* Miq. *Int J Pharm Pharm Sci Molecules*, 17: 4474-4483.

Wu JL, Na L, Toshiaki H et al. 2006. Bioactive Secolignans from *Peperomia dindygulensis*. *J Nat Prod*, 17: 4474-4483.

Yang D, Michel L, Chaumont JP et al. 1999. Use of Caryophyllene oxide as an antifungal agent in an invitro experimental model of onycomycosis. *J Mycopathologia*, 148(2): 79-82.

Zilani MNH, Islam MA, Biswas P et al. 2021. Metabolite profiling, anti-inflammatory, analgesic potentials of edible herb *Colocasia gigantea* and molecular docking study against COX-II enzyme. *J Ethnopharmacol*, 281: 114577.

Paper received for publication in January, 2023
Paper accepted for publication in September, 2023