



## **GC-MS Analysis of Whole Plant Extracts of *Mecardonia procumbens* (Mill.) Small**

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### **ABSTRACT**

*The least concerned plant species Mecardonia procumbens (Mill.) Small of scrophulariaceae was subjected to preliminary and GC-MS analysis for the chemo-constituents in them. A total of three solvent for preliminary and three for GC-MS studies were taken and the in vivo and in vitro samples were tested and compared for their metabolites. The preliminary analysis revealed the presence of flavonoids, alkaloids, glycosides and coumarins and absence to tannin, saponin, terpenoids, fixed and volatile oils in the tested solvent extracts. The GC-MS analysis elucidated a total of 96 compounds from all the three tested solvent extracts and esteric, palmitic and lineolic acid compounds were common in all three of them. The study find these compound have commercial and therapeutic uses and can be further studied for their importance and purifications.*

**KEYWORDS:** Phytochemical, GC-MS, leaf extract, Solvents

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### **INTRODUCTION**

Medicinal plants serve as raw material for drugs which are effective and reasonable health care of people [1]. The advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety beyond being economical, effective and easy availability [2]. Research need in the field of medicinal plants is huge but are balanced by potential health benefits and enormous size of market. The medicinal plants are rich in secondary metabolites and essential oils for curative purpose. Predictably, 70-80% of people worldwide have confidence chiefly on tradition herbal medicines to come across their crucial primary health care needs. The universal demand for herbal medicine is not only enormous but growing. Various technologies have been adopted for enhancing bioactive molecules in medicinal plants. Biotechnological tools have an essential role in multiplication and genetic enhancement of the medicinal undergrowth by agreeing performances such as *in vitro* reinforcement and hereditary conversion. The therapeutics of a plant hides in the chemical substances that are active either directly or indirectly to the metabolism of a plant. The bioactive compounds include alkaloid, phenolics, tannin and flavanoid [3]. Secondary metabolites or secondary products are compounds survive in organisms which are inert in basic life processes while active in secondary, non essential roles. Metabolites are intermediates and products of metabolism [4]. The ability of nature to produce a wide array of structurally complex and diverse molecules has resulted in analyses of secondary metabolites. Studies on plant secondary metabolites are elevating over the last 50 years. Secondary products are used commercially for aroma, pigments, food flavors, pesticides and pharmaceuticals. Materialistic importance of these have resulted great interest in manufacturing and exploring possibilities of enhancing mass production by means of tissue culture technology in recent years. They establish high potent in recreation and stimulation. The drugs thus obtained have cold and hot potency.

*Mecardonia procumbens* (Mill.) Small is a least concerned medicinal plant of the family scrophulariaceae. The plant is least explored and it is traditionally used to heal wounds. The study aims to find out the medicinal potentiality of the plant by elucidating the chemical compounds present in them so that they can be processed in pharma industry.

## MATERIAL AND METHODS

### Preliminary analysis of *Lindernia antipoda* (L.) Alston

**TEST FOR FLAVONOIDS: Alkaline Reaction Test-** To 1 ml of crude extract few drops of dil. NaOH was added. The formation of yellow colour indicates the presence of flavonoids.

**Shinadow's Test-** To alcoholic extract, the addition of a few magnesium turnings and 1-2 drops of conc. HCl and boiling for five minutes if it leads to the formation of red colour shows the presence of flavonoids.

**TEST FOR ALKALOIDS: Dragendroff's Test-** To 5 ml crude extract 2 ml conc. HCl was added. The addition of 1 ml dragendroff's reagent produces an orange or red precipitate to indicate the presence of alkaloids.

**Hager's Test** - The plant extract was added with few drops of a saturated solution of picric acid, yellow precipitate confirms the presence of alkaloids.

**TEST FOR COUMARINS** - The extract was treated with 1 ml of 10% alcoholic NaOH. The presence of coumarin is indicated by the formation of a yellow colour.

**TEST FOR STEROIDS AND STEROLS-** To 2 ml chloroform, a few drops of conc.  $H_2SO_4$  added along the sides of the test tube. To it 5 ml of aqueous plant extract was added to obtain a red colour for the presence of steroids and sterols.

**Liebermann - Burchard Test** - A few drops of chloroform and 3 ml of glacial acetic acid to the extract, warmed and cooled. To that few drops of concentrated sulphuric acid were added along the sides of the test tube. The solution becomes red, then blue and finally turns bluish-green indicating the presence of steroids and sterols.

**TEST FOR CARBOHYDRATES: Benedict's Test** - To 2 ml Benedict's solution five drops of the extract were added and boiled for 5 minutes. Red, Yellow or Green precipitate confirms carbohydrates in the plant.

**Fehlings test** - A mixture of 1 ml of Fehling solution A and 1 ml of Fehling solution B were added in a test tube and to it few drops of extract was added and boiled. Yellow or brown precipitate indicates the presence of carbohydrate.

**TEST FOR PROTEIN AND AMINO ACIDS : Ninhydrin Test** - The extract is treated with 0.2% ninhydrin reagent. The development of purple colour reveals the presence of proteins, peptides or aminoacids.

**Biurette test** - 2 ml of 10% NaOH added to an equal amount of extract and a few drops of 0.1%  $CuSO_4$ . Persistence of violet or pink colour in the sample is a confirmation for protein and amino acid.

**TEST FOR PHENOLS-** A few drops of alcohol and ferric chloride solution were added to the extract. The appearance of a blue or green colour indicates the presence of phenols.

**TEST FOR TANNINS: Gelatin Test** - The extract is dissolved in a minimum amount of water and filtered. To the filtrate, a 1% solution of gelatin is added. Precipitation by gelatin in glass.

**Braymer's test** - A few drops of 5% ferric chloride and 2 ml  $H_2SO_4$  was added to 2 ml of plant extract. Green precipitation is the indication for tannins.

**TEST FOR SAPONIN: Lather Test** - 1 ml of extract is diluted with 5 ml of distilled water and agitated. The formation of copious lather indicates the presence of saponins.

**Froth's Test** - Heat 5 ml of extract along with 5 ml of water. The formation of froth is the final point for the secondary metabolite presence.

**TEST FOR GLYCOSIDES-** The extract was mixed with a little amount of anthrone on a watch glass. One drop of concentrated sulphuric acid was added and made into a paste and warmed gently over a water bath. The presence of glycosides was indicated by dark coloration.

**Borntrager's Test** - Distilled water of 1 ml was added to 1 ml crude extract along with few drops of NaOH. The appearance of yellow colour shows glycosidic contents.

**TES FOR FIXED OILS AND FATS (SPOT TEST)** - A small quantity of extract is pressed separately between the filter paper. Formation of a grease spot indicates the presence of fixed oils and fats.

**TEST FOR VOLATILE OILS (SPOT TEST)** - A drop of extract is placed on Whatman's No. 1 filter paper. No trace of extract (complex evaporation) is the indication.

### GC MS METHODOLOGY

The GC-MS analyses were conducted in a QP-2020 (Schimadzu, Kyoto, Japan). An aliquot (1  $\mu$ L) of extract was injected into the GC-MS on a 30 m capillary column, with a film thickness of 0.25  $\mu$ m (Length 30 m, id 0.25 mm, Phase SH-Rxi-5Sil MS) using the following temperature program: initial oven temperature 50  $^{\circ}$ C, then 6  $^{\circ}$ C Rate /min to 250  $^{\circ}$ C and final hold at 280  $^{\circ}$ C for 2 min. Ion source temp: 200 $^{\circ}$ C. The gas chromatography facility was equipped with ION source detector connected to an integrator. The area under each peak was used for qualification. The GC-MS was operated under Electron impact ionization at 70 eV, using Helium gas. Identification of unknown compounds was made by probability-based matching, using the computer library within the NIST 14, Wiley8 system. Total GC running time was 40 minutes.

## RESULT AND DISCUSSION

The *in vivo* ethanolic whole plant extract of *Mecardonia procumbens* showed the presence of flavonoid, alkaloid, coumarin, carbohydrate, glycoside phenol, saponin and ninhydrin test for protein while steroids, tannin, fixed oil and volatile oils were absent. The *in vitro* ethyl acetate extract marked the presence of flavonoid in the alkaline test of *in vitro* sample, carbohydrate, tannin and glycoside. It marked absence for alkaloid, coumarin, steroid, protein, phenol, tannin, saponin, fixed oils and volatile oils. The petroleum ether extract showed significance to Fehling's test of carbohydrate, biuret test of protein and gelatin test of tannin.

The ethanolic whole plant of *Mecardonia procumbens* delineated a total of 25 compounds of that 17 compounds were from *In vivo* sample and 15 compounds from the *in vitro* sample. Ethyl oleate, Glycidyl palmitate, Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester, Oleoyl chloride, 9,12-octadecadienoyl chloride, (z,z)-, 9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- and Glycidol stearate were present in both the samples.

The ethyl acetate extract of the plant samples contained 39 compounds in total of which 16 were from wild sample and 29 from the cultures sample. Glycidyl oleate, Glycidyl palmitate, Glycidol stearate, 9-octadecenoic acid (z)-, 9-hexadecenyl ester, (z)-, Oleyl oleate and 13-Tetradecen-1-ol acetate were present in both the samples.

The methanolic whole plant extract produced a total of 32 compounds with 15 constituents from wild and 24 from *in vitro* samples. 9-Octadecenoic acid, methyl ester, (E)-, Glycidyl palmitate, 9, 12-octadecadienoic acid, (2-phenyl-1, 3-dioxolan-4-yl) methyl ester, cis-, Glycidyl oleate, Glycidol stearate, 9,12 Octadecanoic acid, methyl ester and Methyl stearate were found in common.

The phytochemical screening of *Mecardonia procumbens* showed the presence of flavonoid, alkaloid, carbohydrate, and glycoside in three solvents similarly Basak *et al.*, [5] reported the presence of reducing sugar, flavonoids, alkaloids, steroids and saponins in *B. monnieri*. We have used ethanol, petroleum ether and ethyl acetate for preliminary analysis of the whole plant extract while Gupta and Jain [6] revealed the existence of alkaloids, tannins, saponins, phytosterols and steroids in all three solvents (methanol, ethanol and distilled water) from leafy extract. Patil *et al.*, [7] in preliminary phytochemical screening of the plant powder using various solvents (petroleum ether, ethanol, methanol and water) reported the presence of tannins, alkaloids, steroids, saponins, glycosides, flavonoids, resins, amino acids, carbohydrates, fats and fixed oils, protein and starch.

The GC-MS analysis of the three solvents ethanol, methanol and ethyl acetate extract of the whole plant revealed a total of 96 compounds that belongs to alkaloids, flavonoids, tannins. Jagtap *et al.*, [8] revealed 20 different acetogenins, 13 different alkaloids, terpenes, and kauranes from bark, fruit and seeds of *Annona squamosa*. Murthy *et al.*, [9] recorded twelve bacopa saponins from *Bacopa* samples using reversed phase high performance liquid chromatography (HPLC). Leaves of custard apple were reported 18 phenol-based compounds, mainly alkaloids and flavonoids [10]. Moghadamtousi *et al.*, [11] pronounced more than 100 acetogenins and it was found to be the major compound of *A. muricata*. Sun *et al.*, [12] first time reported the presence of phenol glycoside derivatives in the genus *Physalis* with an identification of a new phenol glycoside and four known compounds from the stems and leaves of *P. angulata*. Biba *et al.*, [13] reported flavanoids, coumarines, alkaloids, terpenoids in the seed extracts of petroleum ether, methanol, ethyl acetate, and chloroform of *A. squamosa*. Though the preliminary analysis has shown absence to a number of compounds the Glycidol stearate, 9, 12 Octadecanoic acid, Glycidyl oleate, and Glycidyl palmitate were seen in all extracts of the GC-MS analysis. These compounds are mainly esters, linoleic acid and palmitic acids. These compounds find their use in commercial production (soap, cosmetics) and in therapeutics of heart and blood diseases and enhance the vessels and arteries so as to enhance the smooth flow of blood. Esters have been used in rheumatism and also in stimulating central nervous system. Further studies on these compounds could enhance and help in exploring more compounds.

## CONCLUSION

The preliminary and GC-MS analysis of *Mecardonia procumbens* in various solvent extracts has revealed the major compounds and their uses. The compounds preliminary analysis indicated the existence of flavonoids, alkaloids, carbohydrate, glycoside and coumarins. The maximum number of compounds was found in the ethanolic extract. The GC-MS analysis showed the compound present in the solvents are medicinally beneficial and can be further elucidated for their potentialities.

**Table no. 1: Phytochemical screening of *In vivo* and *In vitro* whole plant extract of *Mecardonia procumbens* (Mill.) Small.**

SECONDARY METABOLITE	NAME OF THE TEST	ETHANOL		ETHYL ACETATE		PETROLEUM ETHER	
		<i>In vivo</i>	<i>In vitro</i>	<i>In vivo</i>	<i>In vitro</i>	<i>In vivo</i>	<i>In vitro</i>
FLAVONOID	Alkaline reaction test	+	+	-	+	-	-
	Shinoda test	+	-	-	-	-	-
ALKALOID	Dragendorff's test	+	-	-	-	-	-
	Hager's test	+	-	-	-	-	-
COUMARINS	Alkaline test	+	+	-	-	-	-
STERIODS AND STEROLS	Chloroform test	-	-	-	-	-	-
	Liebermann Burchard test	-	-	-	-	-	-
CARBOHYDRATES	Benedicts test	+	+	-	+	-	-
	Fehling's test	+	+	+	+	+	+
PROTEIN AND AMINOACID	Ninhydrin test	+	-	-	-	-	-
	Biurette test	-	-	-	-	+	+
PHENOL	Ferric chloride test	+	-	-	-	-	-
TANNIN	Gelatin test	-	-	-	-	+	+
	Braymer's test	-	+	+	+	-	-
SAPONIN	Lather test	+	-	-	-	-	-
	Froth test	+	-	-	-	-	-
GLYCOSIDES	Anthrone reagent test	+	+	+	+	-	-
	Borntrager's test	+	+	+	+	-	-
FIXED OILS AND FATS	Filter paper test	-	-	-	-	-	-
VOLATILE OILS	Filter paper test	-	-	-	-	-	-

**GC-MS RESULTS****Table no. 2: GC-MS Analysis for Ethanolic whole plant extract of *Mecardonia procumbens* (Mill.) Small**

S.NO	COMPOUND NAME	IN VIVO	IN VITRO
1	N-propyl 9,12-octadecadienoate	+	-
2	Butyl 9,12-octadecadienoate	+	-
3	Ethyl oleate	+	+
4	Glycidylpalmitate	+	+
5	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	+	+
6	9-octadecenoic acid (z)-, 2,3-dihydroxypropyl ester	+	-
7	Oleoyl chloride	+	+
8	9,12-octadecadienoyl chloride, (z,z)-	+	+
9	9,12-octadecadienoic acid (z,z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	+	-
10	Glycidyleleate	+	-
11	9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	+	+
12	Glycidolstearate	+	+
13	Lupeol	+	-
14	2-hydroxy-3-[(9e)-9-octadecenoyloxy]propyl (9e)-9-octadecenoate	+	-
15	Oleoyl chloride	+	-
16	9-octadecenoic acid (z)-, 2,3-dihydroxypropyl ester	+	-
17	Neophytadiene	+	-
18	Phytol	-	+
19	9,12-Octadecadienoic acid, ethyl ester	-	+
20	2,3-dihydroxypropyl elaidate	-	+
21	2,4(1h,3h)-pyrimidinedione, 5-.beta.-d-ribofuranosyl-	-	+
22	Z-9-pentadecenol	-	+
23	3-methyl-cis-3a,4,7,7a-tetrahydroindan	-	+
24	Octadecanoic acid, 2,3-dihydroxypropyl ester	-	+
25	5-[(5-amino-4h-1,2,4-triazol-3-yl)methyl]-1h-1,2,4-triazol-3-amine #	-	+
Total No. Of Compounds:- 25 ( <i>In vivo</i> -17, <i>In vitro</i> -15)			

**Table no. 3: GC-MS Analysis for Ethyl Acetate whole plant extract of *Mecardonia procumbens* (Mill.) Small**

S.NO	COMPOUND NAME	IN VIVO	IN VITRO
1	Glycidylpalmitate	+	–
2	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	+	–
3	9,12-Octadecadienoyl chloride, (Z,Z)-	+	–
4	9-Methyl-10,12-hexadecadien-1-ol acetate	+	–
5	Glycidyloleate	+	+
6	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	+	–
7	E-2-Methyl-3-tetradecen-1-ol acetate	+	–
8	11-hexadecynal	+	–
9	Glycidylpalmitate	+	+
10	Glycidol stearate	+	+
11	9-octadecenoic acid (z)-, 9-hexadecenyl ester, (z)-	+	+
12	Oleyloleate	+	+
13	13-Tetradecen-1-ol acetate	+	+
14	3-Chloropropionic acid, heptadecyl ester	+	–
15	Methyl (12e)-12-[(2,4-dinitrophenyl)hydrazono]dodecanoate \$\$ methyl 12-[(2,4-dinitrophenyl)hydrazono]	+	–
16	Trichloroacetic acid, undec-2-enyl ester	+	–
17	9-Octadecenoic acid, methyl ester, (E)-	–	+
18	4-Octadecenoic acid, methyl ester	–	+
19	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-	–	+
20	Phytol isomer	–	+
21	9,12-Octadecadienoic acid (Z,Z)-	–	+
22	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester \$\$ 2-hydroxy-1-(hydroxymethyl)ethyl pal	–	+
23	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	–	+
24	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	–	+
25	Oleoyl chloride	–	+
26	Oleic anhydride	–	+
27	Gamolenic acid	–	+
28	I-Propyl 7,10,13,16-docosatetraenoate	–	+
29	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	–	+
30	2-hydroxy-3-[(9e)-9-octadecenoyloxy]propyl (9e)-9-octadecenoate	–	+
31	1,8,11,14-heptadecatetraene, (z,z,z)-	–	+
32	Methyl 2-hydroxy-octadeca-9,12,15-trienoate	–	+
33	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	–	+
34	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	–	+
35	9,12-Octadecadienoyl chloride, (Z,Z)	–	+
36	Methyl 7,11,14-eicosatrienoate	–	+
37	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	–	+
38	Oleoyl chloride	–	+
39	Octadecanoic acid, 2,3-dihydroxypropyl ester	–	+
<b>Total No. of Compounds:- 39 (In vivo-16, In vitro-29)</b>			

**Table No. 4: GC-MS Analysis for Methanolic Whole Plant Extract of *Mecardonia procumbens* (Mill.) Small**

S.NO	COMPOUND NAME	IN VIVO	IN VITRO
1	9-Octadecenoic acid, methyl ester, (E)-	+	+
2	Glycidylpalmitate	+	+
3	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester, 2-hydroxy-3-(palmitoyloxy)propyl palmitate.	+	-
4	9,12-octadecadienoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	+	+
5	Glycidyloleate	+	+
6	9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	+	-
7	Glycidolstearate	+	+
8	Toxaphen	+	-
9	Pregnane-3,17,20-triol, cyclic 17,20-(methylboronate), (3.alpha.,5.beta.,20s)-	+	-
10	2-oxo-1-(3-oxo-butyl)-cyclohexanecarboxylic acid ethyl ester	+	-
11	Cholestan-24-one, 3,6-bis(acetyloxy)-5-hydroxy-, (3.beta.,5.alpha.,6.beta.)- methyl 5.alpha.-hydroxy-6-me	+	-
12	Distearin	+	-
13	Hexadecanoic acid, methyl ester	+	-
14	9,12 Octadecanoic acid, methyl ester	+	+
15	Methyl stearate	+	+
16	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-, [1r-(1.alpha.,2.beta.,5.alpha.)]-	-	+
17	9,10-epoxyoctadecan-1-ol	-	+
18	Oleic acid, propyl ester	-	+
19	(1r,3e,7e,11r)-1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	-	+
20	6,11-hexadecadien-1-ol	-	+
21	Oleoylchloride	-	+
22	Oleic anhydride	-	+
23	2-hydroxy-3-[(9e)-9-octadecenoyloxy]propyl (9e)-9-octadecenoate #	-	+
24	1,8,11,14-heptadecatetraene, (z,z,z)-	-	+
25	Butyl 9,12,15-octadecatrienoate	-	+
26	9,12,15-octadecatrien-1-ol	-	+
27	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	-	+
28	Stigmasta-5,22-dien-3-ol, (3.beta.,22e)-	-	+
29	Octadecanoic acid, 2,3-dihydroxypropyl ester	-	+
30	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	-	+
31	9,12-Octadecadienoic acid (Z,Z)-	-	+
32	Cis-9,10-Epoxyoctadecan-1-ol	-	+
<b>Total No. Of Compounds:- 32 (In vivo-15, In vitro-24)</b>			

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**CONFLICT OF INTEREST**

The authors have no conflict of interest

**AUTHOR CONTRIBUTIONS**

Deepa K- Contributed in conducting experiment, collecting and analysing data, paper preparation; Dr. Jahirhussain G- Research supervisor and Research design.

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