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GC-MS profiling and antioxidant activity of *Mundulea sericea* (Willd) A. Chev – a potential plant with anti-mycobacterial activity

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Abstract

Mundulea sericea, a tree legume, is used as medicinal plant by tribal peoples of Andhra Pradesh for various ailments. *M. sericea*, have been reported to possess bioactivity such as insecticidal, anti-mycobacterial, antimicrobial and analgesic activity. However, there is little research on the potential antioxidant activity of this plant. Hence, the plant was selected for the present investigation. The present study was aimed to explore the phytochemcials present in the leaves of *Mundulea sericea* using preliminary phytochemical screening and GC-MS analysis. The leaf material was collected and shade dried at room temperature. They were made in to fine powder with an electric grinder. The dried powder material was extracted with methanol, ethanol, and Hexane. The leaves were further quantified for phenolics, flavonoids and total antioxidant activity. In the preliminary phytochemical screening, the leaves showed the presence of flavonoids, phenolics, glycosides, carbohydrates, oils and fat. GC-MS analysis showed a variety of compounds *i.e* around 23 compounds were identified on the basis of their molecular weight.

Key words : *Mundulea sericea*, tree legume, anti-mycobacterial, insecticidal, analgesic, GC-MS

Now a days, antioxidants derived from plants have been gaining more attention due to their therapeutic implications and fewer side effects[14]. Moreover, the published research literature reveals that the large number of plants possess antioxidant and medicinal properties[6]. In addition, investigation on few of these plants leads to the development of neutraceuticals, cosmetics and medicines[16,28]. However, scientific investigation on these plants for exploring their phytoconstituents was limited or rather scarce. Therefore examination of plants for their potential against the presence of several metabolites (primary and secondary),

antioxidants be responsible for the development of new drug from these plants and also to reconnoitre its potential against various ailments. *Mundulea sericea,* is one such medicinal plant used by the tribes of Koyas, Lambadas, Yerukalas and Chenchus for various ailments. The bark, seeds, leaves and root are also used as insecticides to kill cockroaches, houseflies, and caterpillars[24]. The insecticidal activity of *M. sericea* was reported by Langat *et al.,*[15] and Anjali *et al*.[1], anti-mycobacterial was reported by Chopra *et al*.,[4] antimicrobial by Mazimba *et al.,*[17] and analgesic activity by Iyer *et al.,*[10] is also reported. However, there is little research on the potential antioxidant activity of this plant. Hence, the plant was selected for the present investigation to examine the phytoconstituents from leaves of *M.sericea* and its potential antioxidative property.

Sample collection and extraction :

The plant materials were collected from our college campus. The plant materials were collected and shade dried at room temperature. They were made in to fine powder with an electric grinder. The dried powder material was extracted with methanol, ethanol, and Hexane. Mixture was kept for 72 hrs with frequent shaking (200 rpm) (ORBITEK) at room temperature. Then the extracts obtained were filtered using cheese cloth. The extract thus obtained was preserved in the refrigerator for further use.

The extracts were subjected to preliminary phytochemical analysis[2,8].

Estimation of phytochemicals and antioxidant activity :

Total soluble phenolics of the fraction were determined with Folin-Ciocalteu reagent using pyrocatechol as the standard[7]. Total soluble flavonoid content of the fractions was determined with aluminium nitrate using quercetin as the standard[9]. The total antioxidant capacity of the fractions was determined by phosphomolybdate method using α-tocopherol as the standard[11].

GC–MS analysis of M. sericea leaf extract:

M. sericea leaf extract were analyzed by GC–MS (Perkin Elmer, Turbomass Gold, Mass spectrometer) equipped with a flame ionization detector (FID) using a fused silica Rtx-2330 column (Restek made, 30 m 9 0.32 mm ID and 0.20 lm film thickness). The 19 injector port and detector temperatures were set up 230 and 250°C, respectively, N2 was used as the carrier gas. Initially, the column temperature was maintained at 120°C, followed by increasing it to 220°C over 20 min, and holding it at 220°C for 10 min. The FAME were identified by comparing their fragmentation patterns and retention times with authentic standards and also with the NIST library.

Statistical analysis :

All experiments were performed in triplicate and results were expressed as mean ± SE. Statistical analysis was carried out with (SPSS package version 17.10) using ANOVA followed by Tukey's test.

Table-1. Phytochemical Screening of different fractions of *Mundulea sericea*

S.No.	Phytochemical	Test performed	Msmle	Msele	Mshle
1	Alkaloid	Wagner's Test	-	-	-
2	Carbohydrate	Benedict's Test	+	+	+
3	Glycoside	Borntrager's Test	-	+	+
4	Saponins	Foam Test	-	-	-
5	Phytosterol	Salkowski's Test	-	-	-
6	Oil/fat	Stain test	-	-	-
7	Phenol	Ferric chloride Test	+	+	+
8	Flavonoids	Alkaline Reagent Test	+	+	+
9	Protein/aminoacid	Xanthoproteic Test	+	+	+

MSMLE – *M. sericea* Methanolic Leaf Extract; MSELE- *M. sericea* Ethanolic Leaf Extract; MSHLE - *M. sericea* Hexane Leaf Extract

+ - Presence of tested compound; - Absence of tested compound

Phytochemical attributes :

In the present study, the phytochemical screening of successive methanolic, ethanolic and hexane extracts obtained through maceration were further subjected to preliminary phytochemical screening followed by GC-MS analysis. The fractions were also used for the quantification of phenolics, flavonoids and total antioxidant activities.

The phytochemical analysis of *Mundulea sericea* leaves showed the presence of secondary metabolites like glycosides, protein, phenol compound, carbohydrates, oil/fat, flavonoid, etc. which are responsible for the enzymatic activity and regulate the metabolic pathway. The results of the preliminary phytochemical analysis were depicted in Table-1. Methanolic extract shows the presence of compounds like carbohydreates, phenol, flavonoids and proteins whereas Glycosides, fat, saponins and alkaloids were tested against the sample and found to be absent in the tested sample. Similarly, the ethanolic extracts of Mundulea sericea leaves showed the presence of glycosides, carbohydrates, proteins, fat, phenol and flavonoids. Phytosterol, alkaloid, saponins, were found to be absent among the fractions tested in the present study. The compounds obtained in the ethanolic fractions were also obtained in the methanolic extracts and these results lead to a conclusion that functional groups of the solvent have some impact on the extraction of compounds from the plant material. Hexane extract of Mundulea sericea leaves showed the presence of glycosides, carbohydtrates, proteins, flavonoids and phenol. The presence of glycosides was not observed in the other two extracts tested (ethanol and methanol extracts).

From the phytochemical screening results obtained (Table-1) it is evidenced that metabolites like phenolics, carbohydrates,

flavonoids, proteins and aminoacids are present in the hexane, methanol and ethanol fractions of leaf of *M. sericea*. Whereas, the presence of glycosides was observed in the ethanol and hexane fractions. Glycosides was not observed in the methanolic extract of leaf of *M. sericea*. Alkaloids, phytosterols and saponins were not detected in the fractions tested. The compounds identified through preliminary phytochemical screening are widely reported to have strong antioxidant and free radical scavenging activity[5,26]. Further, these phytoconstituents plays a prominent role in curing various ailments like fever, ache and cancer too[18,25]. The presence of flavonoids was observed in all the three fractions. The results show that the extracts can potentially be applied as an antioxidant, anti-inflammatory and anti-cancer agents, since flavonoids are the potential source of these agents[20]. The phytoconstituents identified in the preliminary phytochemical analysis namely flavonoids and phenolics are reported as the most widely distributed chemical compounds in the plant kingdom. The latent nature of these compounds like metal chelation and radical scavenging activity gained much attention of the recent scientific fraternity as a potent natural antioxidant.

The phytoconstituents like total phenolic, flavonoid content and total antioxidant acitivity were analyzed and depicted in Table-2.

Total phenolic and flavonoid content :

Total phenolic content was estimated by using Folin-Ciocalteu reagent and the results were given in the Table-2. Total phenolic content of the different fractions of *M. sericea* were solvent dependent and expressed as µg pyrocatechol equivalent. The content of the total phenolics in the fractions decreased in the order of methanol > ethanol > hexane fractions. Total phenolic content was estimated by using Folin-Ciocalteu reagent. Total phenolic content of the different fractions of *M. sericea* were solvent dependent and expressed as µg pyrocatechol equivalent. The content of the total phenolics in the fractions decreased in the order of methanol > ethanol > hexane fractions. The methanolic extract showed the highest phenolic content (163.88±0.88 µg). However, 107.86±0.053µg of phenolics was observed and reported earlier on the aqueous methanolic extract of leaf of *M. sericea*[13]. From the obtained results, it might be concluded that the quantity of the phytochemicals may vary depends upon the environmental condition they grow.

The fractions' combined flavonoid concentration was calculated as g of quercetin equivalent. The methanolic fraction of *M. sericea* showed highest amount of flavonoids among the fractions tested. The content of total flavonoids in the fractions decreased in the order of methanol > ethanol>hexane fractions (Table-2). The quercetin equivalent was used to express the overall flavonoid content in the fractions.

The methanolic fraction of *M. sericea* showed highest amount of flavonoids among the fractions tested. The content of total flavonoids in the fractions decreased in the order of methanol > ethanol > hexane fractions. The highest quantity of flavonoids were observed in the methanolic extract (192.02±0.4), whereas, 44.53 ±0.156 µg of rutin/mg of flavonoids was reported from the methanolic

Table-2. Total antioxidant activity (phosphomolybdate method) and total phenolic and flavonoid contents of different fractions of *Mundulea sericea* (Willd) A. Chev leaves

S.No	Fractions	Total antioxidangt acitivity (µg vitamin E equivalent/100µg)	Total phenolic content (µg pyrocatechol equivalent/mg)	Total flavonoid content (µg quercetin equivalent/mg)
1	Methanol	156.46±0.03[a]	163.3±0.88[a]	192.02±0.4[a]
2	Ethanol	131.66±0.33[b]	147.66±0.88[b]	112.01±0.6[c]
3	Hexane	127.23±0.36[c]	132.50±0.28[c]	114.33±0.13[b]

Values are expressed as mean±SE of 20 replicates. Values within a column followed by different letters are significantly different ($P<0.05$).

extracts by Khyade *et al.*,[13]. Khoudja *et al.,*[12] reported that the quantity of phenolics and flavonoid compounds vary depends upon the solvents used for extraction and environmental condition where the plant grows. This was reflected in the present study too, the results obtained were in contradictory to the report of Khyade *et al.,*[13].

Total antioxidant content :

Total antioxidant content was estimated using phosphomolybdate assay. Since the total antioxidant capacity is represented in ascorbic acid equivalents, the phosphomolybdate technique is precise and measurable. Among the fractions tested, the methanolic fraction contains the highest quantity of antioxidants (156.46±0.03 AsA equivalent/ 100 µg) followed by ethanolic (131.66±0.33 AsA equivalent/ 100 µg) and hexane fractions (127.23±0.36 AsA equivalent/ 100 µg). The antioxidant activity increased in the order of methanolic fraction > ethanolic fraction > hexane fraction (Table 2). The quantity of antioxidants and its potential were mainly because of the presence of phenolics and flavonoids in the plant material. This was reported by many authors and was reflected in the present study too[12,21,23].

GC-MS analysis :

The methanolic fraction of leaf material of *M. serricea* was subjected to GC-MS analysis and the results were shown in the Fig. 1 and Table-3. GC-MS spectrum show the presence of compounds like, myo-inositol, Trimethylsilyl 23- acetyloxy-3,6,9 -heptaoxa-tricosan-1-oate and Trimethylsilyl 20-acetyloxy-3,6,9 acetate. Myo- inositol is a carbohydrate whereas Trimethylsilyl 20-acetyloxy-3,6,9 acetate and Trimethylsilyl 23-acetyloxy-3,6,9 -heptaoxatricosan-1-oate are TMS derivatives of fatty acids. The other compounds observed in the GC-MS spectrum were octosilane, 2-Methyl-7-phenylindole, Benzene, 2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl-, Propanenitrile, 3-(5- diethylamine, 3-Quinolinecarboxylic acid, 1,2-Benzisothiazol-3-amine tbdms, Anthracene, 2- Ethylacridine, Tetrasiloxane, 3'-Chlorooxanilic acid isopropyl ester, Diethylmalonic acid and 8- Chloro-5-quinolinecarboxylic acid.

Table-3. GC-MS analysis of *Mundulea sericea* (Willd) A. Chev leaves

S. No.	Compound	Molecular formula	Molecular Weight
1	Myo-Inositol,4-C-methyl	$C_7H_{14}O_6$	194.18246g/mol
2	Trimethylsilyl 23-acetyloxy-3,6,9-heptaoxatricosan-1-oate	$C_{21}H_{42}O_{11}Si$	498.6g/mol
3	Trimethylsilyl 20-acetyloxy-3,6,9 acetate	$C_{18}H_{38}O_{10}Si$	454.58452g/mol
4	Octasiloxane,	$C_{18}H_{54}O_7Si_8$	607 g/ mol
5	2-Methyl-7-phenylindole	$C_{15}H_{13}N$	207.3 g/l
6	Benzene, 2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl-	$C_{16}H_{28}OSi$	264.48 g/mol
7	Propanenitrile, 3-(5-diethylamin...	$C_{13}H_{22}N_2O$	222.33 g/mol
8	Fumaric acid, 4-heptyl tridecyl .	$C_{24}H_{44}O_4$	396.6 g/mol
9	Cyclobarbital	$C_{12}H_{16}N_2O_3$	236.27 g/mol
10	2-(Acetoxymethyl)-3-(methoxycarb...	$C_{17}H_{14}O_4$	282.29 g/mol
11	3-Quinolinecarboxylic acid	$C_{10}H_7NO_2$	173.17 g/mol
12	1,2-Benzisothiazol-3-amine tbdms	$C_7H_6N_2S$	150.2 g/mol
13	Anthracene	$C_{14}H_{10}$	178.23g/mol
14	2-Ethylacridine	$C_{15}H_{13}N$	207.27 g/mol
15	2,4-Cyclohexadien-1-one, 3,5-bis..	$C_{14}H_{21}NO$	219.32 g/mol
16	Tetrasiloxane,	$H_{10}O_3Si_4$	170.42 g/mol
17	Benzamide, N-heptyl-N-octyl-4-br.	$C_{23}H_{39}NO_2$	361.6 g/mol
18	3'-Chlorooxanilic acid isopropyl ester	$C_{11}H_{12}ClNO_3$	241.67g/mol
19	Diethylmalonic acid,	$(C_2H_5)_2C(COOH)_2$	160.17 g/mol
20	Phen-1,2-diol, 4-fluoro-5-aminoacetyl-, dimethyl ether	$C_{10}H_{12}FNO_3$	213.21 g/mol
21	Propanenitrile, 3-(5-diethylamin...	$C_{13}H_{22}N_2O$	222.33 g/mol
22	8-Chloro-5-quinolinecarboxylic acid	$C_{10}H_6ClNO_2$	207.61 g/mol
23	1,1-Dichloro-3-(1-adamantyl)-2-propyne	$C_{13}H_{16}Cl_2$	243.17 g/mol

Early, GC-MS analysis of aqueous methanolic extract of *M. sericea* was reported by Mazimba *et al.*,[9]. They observed compounds like caryophyllene and cadina-3,9-diene whereas, these compounds were not observed in the present study. The contradiction is due to the difference in the nature of the extracts used. Mazimba *et al*.,[17] used aqueous methanolic extract whereas methanol extract was used in the present investigation. Some of the

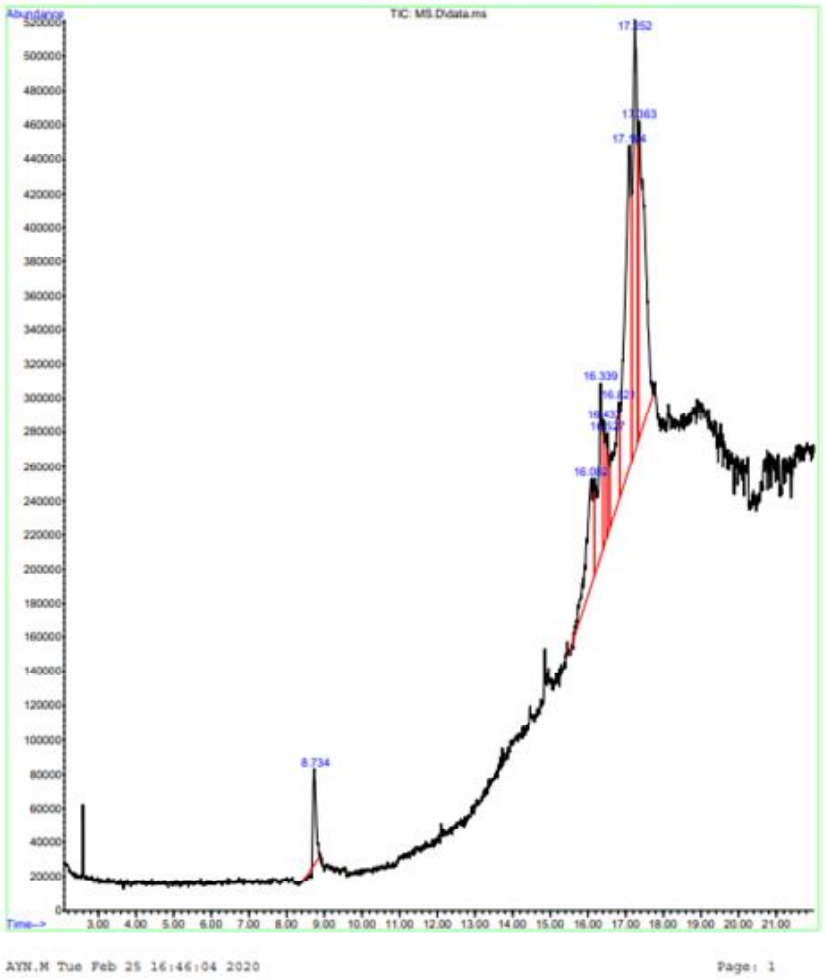

Fig. 1. GC-MS profiling of methanolic extract of leaf of *Mundulea sericea.*

compounds identified in the present investigation through GC-MS analysis of *M. sericea* were reported to have medicinal properties. They were, myo-inositol, Cyclobarbital, 3-Quinolinecarboxylic acid, Anthracene and Cyclohexadien. Myo-inositol is used for the treatment of diabetes and cancer. Its deficiency leads to intestinal lipodystrophy[3]. Cyclobarbital, this compound is used to treat insomnia[19]. 3-Quinolinecarboxylic acid, is an intermediate for the synthesis of quinoline based drugs is also present in the methanolic extract of *M. sericea*[27]. Anthracene, another compound detected through GC-MS analysis was reported to be used in the treatment of Psoriasis[22]. Cyclohexadien, used in the polymer synthesis of breast implants and tetrasiloxane, is used in the cosmetic industry (https://go.drugbank.com/).

Twenty three bioactive compounds were identified from the leaf material of *M. sericea* using GC-MS analysis. Total flavonoid, phenolics and antioxidant activity were also analysed and was found to be high in the methanolic extract when compared to ethanolic and hexane extracts. Earlier reports on this plant reported the presence of several similar bioactive principles in the aqueous methanolic and hexane extracts. The obtained results wage to show attention on further research like antimicrobial, anticytotoxic activity and so on.

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