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Research Article

Gc-Ms analysis, *in-vitro* neuroprotective and antioxidant studies on ethanolic and acetone extract of oledenlandia corymbosa

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Abstract

The study was aimed to explore the phytochemicals present in the ethanol and acetone extracts of Oldenlandia corymbosa by GC-MS analysis and to determine the neuroprotective and antioxidant potential of both of the extracts. The GC-MS analysis of the ethanol and acetone indicated the presence of carotenoids, polyphenols, diterpenes, and fatty acids. Neuroprotective effect of ethanolic (OCWE) and acetone extract of *Oldenlandia Corymbosa* (OCWA) on IMR-32 Neuroblastoma cell lines were evaluated by MTT assay and the results showed that ethanol extract of the plant got more neuroprotective activity on cell lines. The antioxidant results showed that both of the extracts had almost similar DPPH and ABTS radical scavenging. However, the ethanol extract had more potent antioxidant power than the acetone extract. Study data suggest that whole plant of *Oldenlandia corymbosa* possesses neuroprotective and anti-oxidative activities and the best neuroprotective and anti-oxidant activity being exhibited by the ethanolic extract of the plant.

Keywords: Oldenlandia Corymbosa, GC-MS analysis, MTT assay, DPPH assay, ABTS assay antioxidant, Neuroprotective

INTRODUCTION

Oldenlandia corymbosa Linn.is a flowering plant belonging to the Rubiaceae family and is commonly found in India, tropical East Asia, Java Island and in Srilanka. It is commonly known as Parppatakapullu and is one of the chief ingredients in various ayurvedic preparations like Amritarishtam, Mahatiktaghrtam, Candanasavam, Jatyadi tailam (Das et al., 2019). *Oldenlandia corymbosa* reported to have immunoprotective activity and used in many

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traditional medicines to treat ulcers, bronchitis, pelvic and uterine inflammation. Aqueous extract of the plant contains arabinose, rhamnose, mannose, zylose glucose and galactose (Sivapraksam et al., 2014).

MATERIALS AND METHODS

Plant Material

Whole plant of Oldenlandia corymbosa was collected from Kottayam, Kerala. The plant was identified and authenticated by Dr. M.U. Sharief, The Scientist 'E' & Head of office, Botanical survey of India, Southern regional center, Coimbatore. Identification No.: BSI/SRC/5/23/2020/Tech/63.

Preparation of ethanol and acetone extract

Oldenlandia corymbosa plants (Whole plant) were washed several times with distilled water to remove complete impurities present in it. Then it is dried at room temperature and coarsely powdered and the powder was extracted with ethanol and acetone for 50 hours. Alcohol and acetone are completely removed under reduced pressure, and semi-solid extracts were obtained (Redfern et al., 2014).

GC-MS analysis

Shimadzu GC-MS (Model Number: QP2010S) with GC –MS solutions software was used to carry out GC-MS profiling of Oldenlandia corymbosa extract (ethanol and acetone). Chromatographic conditions: Elite-5 MS column(fused silica) of 30 mm length,0.25mm internal diameter and 0.25µ thickness was used. The carrier gas used was Helium at a flow rate of 1 ml /min and the injection volume of the sample was 1.0 microlitre. The oven temperature is maintained at 280°C. The total time taken for GC running was 50 min. By comparing the average peak area to the total area relative percentage amount of each component was calculated.

Invitro Neuroprotective Effect

MTT assay method: *In-vitro* Neuroprotective potential of selected extract of Oldenlandia Corymbosa (ethanol& acetone) was assessed using IMR-32 Neuroblastoma cells (purchased from NCCS Pune was maintained in Dulbecco's modified eagles media). After attaining sufficient growth of the cell line Lipopolysaccharide (1 µg/ml) was added to induce neuroinflammation and incubated for one hour, prepared extracts were added to the respective wells. The sample content in the well after 24 hours of incubation period were removed and MTT solution was added to all tests and cell control wells again incubated for 4 hours. After removing the supernatant MTT solubilization solution (DMSO) was added and absorbances were measured by microplate reader at a wavelength of 540 nm (Chang et al., 2001).

In-vitro antioxidant Assay

DPPH Radical Scavenging assays and ABTS assay were used to assess antioxidant potential of the extracts.

DPPH Radical Scavenging assay

Antioxidant activities of ethanol and acetone extracts were determined by DPPH assay. Samples of different concentrations (12.5 µg/mL to 200 µg/mL) mixed with DPPH and this reaction mixture incubated at room temperature in dark condition for 20 minutes, a control without test compound is also prepared. Absorbance was measured at 517 nm and as the positive control Ascorbic acid was used (Rajurkar & Hande, 2011).

ABTS assay

Antioxidant activities of ethanol and acetone were determined by ABTS assay. Extracts of different concentrations mixed with ABTS solution. Generated radical monocation was reduced in the presence of antioxidants present in the extract. Absorbance was measured at 734 nm. Ascorbic acid was used as standard (Massada, 1976).

RESULTS AND DISCUSSION

Identification of Components

Detection of phytoconstituents was done using National Institute Standard and Technology (NISTII) and WILEY 8 library. For characterizing phytoconstituents, parameters such as comparison of retention time and peak, computer matching, and the characteristic fragmentation patterns of the mass spectra were used (Adams, 2007; Cazes, 2004; Bhardwaj et al., 2020).

Phytochemicals detected from the chromatogram are shown in **Table 1** (ethanol extract) and (Tables 2 and 3) (acetone extract).The chromatograms (**Figures 1 and 2**) exhibited the presence of numerous biologically active compounds (Lozano et al., 2018).

In vitro neuroprotective Effects

The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope followed by MTT assay. The data obtained are given in the **table 4**. Percentage viability against concentration graphically represented in **figure 3**.

DPPH Radical Scavenging Assay

It is one of most popularly used assay method to detect antioxidant potential of herbal extracts. **Table 5 and figure 4** exhibited ethanol extract had got more antioxidant activity compared to ethanol extract of the plant. IC 50 Value from DPPH assay for ethanol extract is 130.56 µg/mL and acetone extract is 189.33 µg/mL.

Table 1. List of Chemical fractions identified in the Ethanol Extract of *Oldenlandia Corymbosa*.

Peak	Retention Time	Area	Area%	Name	Base m/z
1	16.784	1736537	1.53	4-ALLYL-1,2-DIMETHYOXY BENZENE	178.10
2	21.463	2473631	2.17	HEPTADECANE	57.05
3	26.712	7619606	6.69	NEOPHYTADIENE	68.05
4	26.858	3992898	3.51	HEXAHYDFARNESYLACETONE	58.05
5	27.586	2216814	1.95	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	82.05
6	28.399	3106963	2.73	1-AMINODECANE, N-TRIflUROACETYL	184.10
7	28.481	19021396	16.71	METHYLPALMITATE	74.05
8	29.254	4233864	3.72	HEXADECANOIC ACID	73.05
9	31.709	32147298	28.25	9,12-OCTADECADIENOIC ACID, METHYL ESTER	67.05
10	32.045	21380475	18.79	PHYTOL	71.05
11	32.264	4600472	4.04	METHYL STEARATE	74.05
12	35.738	1803139	1.58	METHYL MELISSICATE	74.05
13	36.241	2380057	2.09	4,8,12,16- TETRAMETHYLHEPTADECAN 4-OLIDE	99.05
14	38.691	1790455	1.57	GLYCEROL .BETA.PALMITATE	57.05
15	43.317	1704582	1.50	SQUALENE	69.05
16	45.557	3605707	3.17	9(11)-DEHYDROERGOSTERYL BENZOATE	251.15

Table 2. List of Chemical fractions identified in the Acetone Extract of *Oldenlandia Corymbosa*.

Peak	Retention Time	Area	Area%	Name	Base m/z
1	20.721	9,939,510.0	3.552	DECANE, 1-BROMO-2-METHYL	57.3163
2	21.306	9,573,032.0	3.421	TETRATETRACONTANE	57.3163
3	21.891	11,929,057.0	4.263	HEXATRIACONTANE	57.2465
4	22.467	16,370,365.0	5.850	TETRATETRACONTANE	43.2645
5	24.127	10,850,997.0	3.878	HEPTACOSANE	43.1946
6	25.008	6,647,038.0	2.375	ETHYL ISO-ALLOCHOLATE	43.1946
7	25.133	14,378,199.0	5.138	STIGMASTEROL	55.2897
8	25.288	6,993,453.0	2.499	1-HEPTATRIACOTANOL	55.2198
9	25.398	33,330,096.0	11.911	LANOSTEROL	55.2198
10	25.918	23,234,928.0	8.303	4,22-STIGMASTADIENE-3-ONE	55.2198
11	26.153	20,960,502.0	7.491	2,2-DIBROMOCHOLESTANONE	55.2198
12	26.748	11,069,512.0	3.956	THUNBERGOL	43.1946

Table 3. Phytocompounds were proven for their antioxidant and anti-inflammatory activity.

Compound name	Nature of compound	Activity established
Neophytadiene	Diterpene	Antiinflammatory (Lozano-Grande et al., 2018)
Squalene	Triterpene	Antioxidant (Kaur et al., 2011)
Stigmasterol	Sterol	Antipyrectic, Antiinflammatory (Ka et al., 2005)
Thunbergol	Diterpene	Natural antioxidant

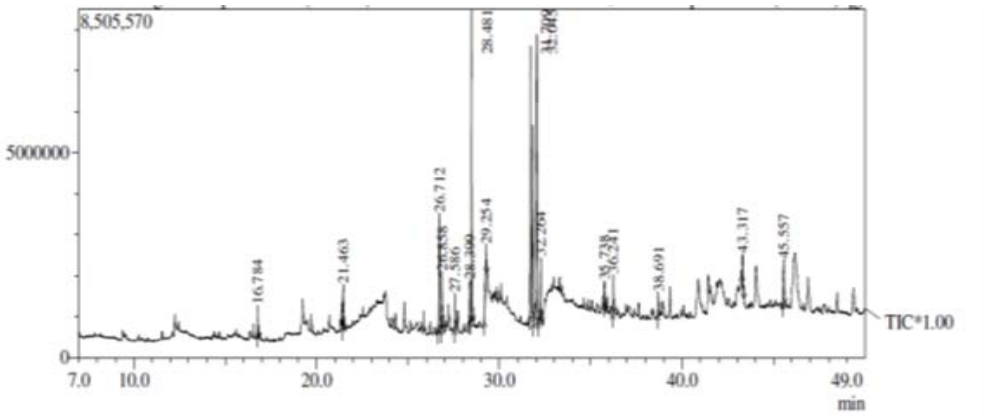

Figure 1. Chromatogram of Ethanol Extract of *Oldenlandia Corymbosa*.

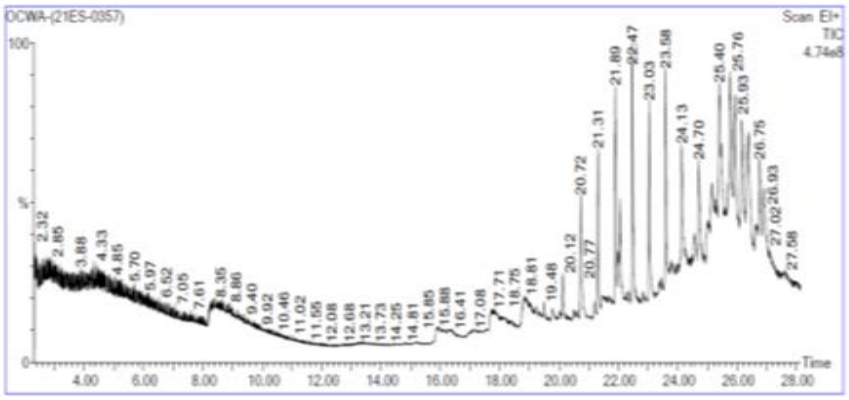

Figure 2. Chromatogram of Acetone Extract of *Oldenlandia Corymbosa*.

Table 4. PercentageViability of cells by MTT assay.

SampleConcentration (µg/mL	ViableCells (%) CONTROL	ViableCells (%)LPS	ViableCells (%)OCWA	ViableCells (%)OCWE
CONTROL	100	-----	-----	-----
LPS		54.61±0.01	-----	-----
6.25	-----	-----	61.11±2.179	55.36±1.605
12.5	-----	-----	61.31 ± 2.271	59.52±1.297
25	-----	- ----	66.31±2.232	66.10±2.118
50	-----	-----	69.69±2.535	76.06±1.757
100	-----	-----	77.80±2.323	85.91±2.499

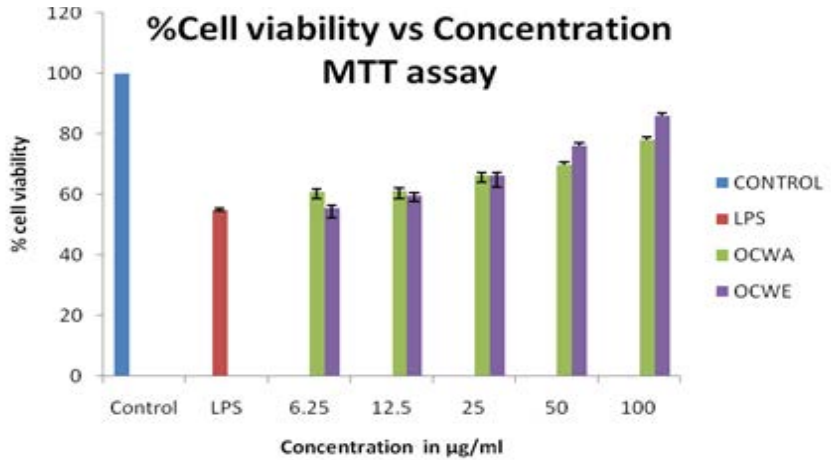

Figure 3. Percentage cell viability against concentration.

Table 5. DPPH Assay of extracts of *Oldenlandia Corymbosa*.

Concentrations (µg/mL)	Scavenging of DPPH free radical (% activity of ethanol extract)	Scavenging of DPPH free radical (% activity of acetone extract)
12.5	12.57	18.84
25	30.40	29.34
50	41.33	32.65
100	49.20	40.36
200	60.21	49.43

ABTS Assay Method

From the ABTS study also it is clear that ethanol extract has got more antioxidant potential compared to acetone extract. **Table 6 and Figure 5**. IC 50 Value from ABTS assay for ethanol extract is 1328.38 µg/mL and acetone extract is 1462.17 µg/Ml.

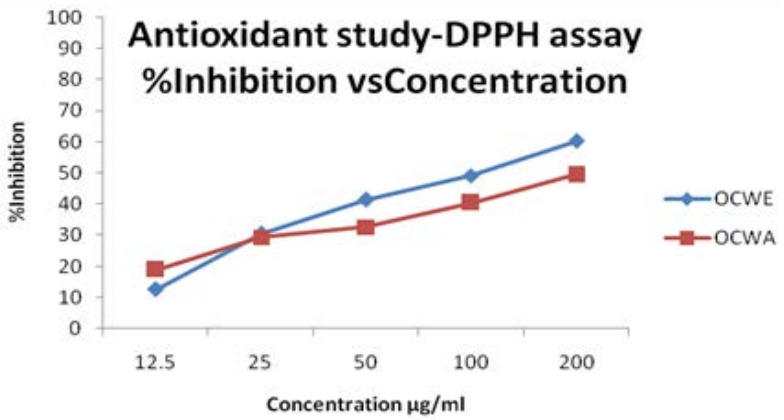

Figure 4. Antioxidant activity by DPPH assay.

Table 6. ABTS Assay of extracts of *Oldenlandia Corymbosa*.

Concentrations (µg/mL)	Scavenging of ABTS assay (% activity of ethanol extract)	Scavenging of ABTS assay (% activity of acetone extract)
12.5	11.72	8.29
25	21.68	18.12
50	35.78	30.33
100	51.42	49.88
200	61.01	57.34

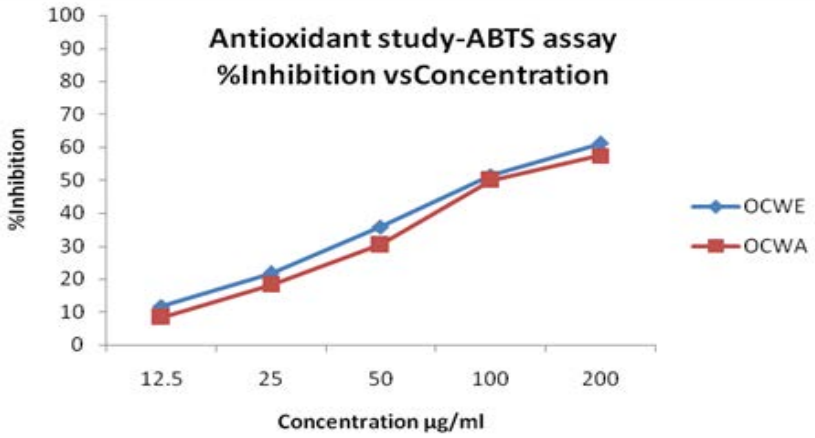

Figure 5. Antioxidant activity by ABTS assay.

CONCLUSION

In the present work attempts were made to scientifically validate and standardize the antioxidant and neuroprotective potential of Oldenlandia corymbosa. GC-MS analysis revealed that the plant is rich in phytoconstituents having antioxidant and anti-inflammatory activity. The antioxidant activity against DPPH and ABTS free radicals showed ethanol extract had got more antioxidant activity. Invitro neuroprotective activity against IMR-32 cell lines exhibited by both plant extract this may be due to the presence of anti-inflammatory fractions such as neophytadiene, squalene, stigmasterol and thunbergol. Further studies on these plants can result in the development of newer drug entities that can be most efficiently used in neuroinflammation mediated neurodegenerative diseases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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