Research Article

GC-MS Profiling and *in vitro* Assessment of Antioxidant and Neuroprotective Properties of Ethanolic and Acetone Extracts of *Oldenlandia corymbosa L.*

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ABSTRACT

To investigate antioxidant and neuroprotective activity of ethanolic (OCWE) and acetone extract (OCWA) of *Oldenlandia corymbosa plant* and identification of phytochemical constituents present in both extracts by GC-MS analysis. The antioxidant potential of *O. corymbosa* was evaluated by DPPH and ABTS assays. MTT assay was used to test the plant's neuroprotective effect on neuroblastoma cell lines. Phytoconstituents of said extracts have been quantified by GC-MS method. Ethanolic extract of plant showed lowest IC_{50} value (130.56µg/mL) compared to acetone extract (189.33µg/mL). More neuroprotective effect also exhibited by ethanolic extract. GC-MS analysis revealed the presence of numerous biologically active phytoconstituents in both extracts. On the basis of antioxidant and *in-vitro* neurotoxicity studies it is revealed that ethanolic extract of the plant showed more antioxidant and neuroprotective activity compared to the acetone extract and this may be due to the presence of antioxidant and anti-inflammatory fractions such as neophytadiene, squalene, stigmasterol and thunbergol.

Key words: *Oldenlandia corymbosa*, GC-MS analysis, Antioxidant, Neuroprotective, MTT assay.

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INTRODUCTION

Neuroinflammation is the process associated with the beginning of a number of neurodegenerative diseases, and it plays a key role in the pathogenesis and progression of Alzheimer's disease. Trauma, infection, oxidative agents, redox iron, tau oligomers, and Aβ all appear to trigger neuroinflammation. In fact, neuroinflammation causes an aberrant release of proinflammatory cytokines, which activate signaling pathways that cause brain tau hyperphosphorylation in residues that aren't changed in normal physiological conditions.[1]

Neurodegenerative disorders are incurable and result in the progressive deterioration and eventual death of nerve cells, posing a serious health risk. Many of our body's functions are affected by nerve illnesses, including balance, heart function, movement, speech, and even breathing. In this state the structure and activities of neurons in the central nervous system (CNS) are changed, resulting in decreased neuronal survival and increased neuronal death In comparison to other organs in the body, the CNS has a very complex system and there are currently no therapeutic medicines that can trigger

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neuronal regeneration in brain regions that have been injured or impacted.[2] Currently, researchers are focusing on pharmacologically active natural compounds with anti-inflammatory properties, making them a possible contender for treating various neurodegenerative diseases. On nutritional and botanical substances, several preclinical and clinical investigations have been conducted. Anti-inflammatory and neuroprotective phytochemicals like terpenoids, phenolic derivatives, alkaloids, glycosides, and steroidal saponins have been studied for their therapeutic potential in the treatment and prevention of neurodegeneration in Alzheimer's disease.[3]

Lipopolysaccharide is one of the chemical agent (LPS) which triggers inflammatory reactions in the body and causes tissue damage, and it's been linked to the development of a number of age-related neurodegenerative diseases. It's an endotoxin, a component of gram-negative bacteria's outer membrane, has been implicated in the pathophysiology of most of the endothelial cell injury and/or dysfunction associated with various disease states and also activates endothelial cells and causes apoptotic endothelial cell death.[4]

Phytochemicals such as flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, isocatechins, epicatechins, and others are utilized in clinical and preclinical studies and have a major scientific value due to the significant medicinal benefits of plant-derived medications.[5] *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.[6] *Oldenlandia corymbosa* reported to have immunoprotective activity and used in many traditional medicines to treat ulcers, bronchitis, pelvic and uterine inflammation. Aqueous extract of the plant contains arabinose, rhamnose, mannose, zylose glucose and galactose.[7] The present study was performed aiming at elucidating the antioxidant power and preventive effects of extracts of *O. corymbosa* against cytotoxicity induced by LPS on neuroblastoma cell lines.

MATERIALS AND METHODS

Plant Material

Whole plant of *O. corymbosa* was collected from Kottayam, Kerala. The plant was identified and authenticated by Dr. M.U. Sharief, The Scientist 'E' and 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 hr. Alcohol and acetone are completely removed under reduced pressure, and semi-solid extracts were obtained.[8]

GC-MS Analysis

Shimadzu GC-MS (Model Number: QP2010S) with GC –MS solutions software was used to carry out GC-MS profiling of plant extracts (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 50min. By comparing the average peak area to the total area relative percentage amount of each component was calculated.

In-vitro neuroprotective effect

MTT Assay Method

In-vitro Neuroprotective potential of selected extract of *Oldenlandia corymbosa* (ethanol and 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 (1µg/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 hr of incubation period were removed and MTT solution was added to all test and cell control wells again incubated for 4 hr. After removing the supernatant MTT solubilization solution (DMSO) was added and absorbance was measured by microplate reader at a wavelength of 540 nm.[9]

In-vitro Antioxidant Assay

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

DPPH and ABTS Radical Scavenging Assay

Antioxidant activities of ethanol and acetone extracts were determined by DPPH assay. Samples of different concentrations (12.5µg/mL to 200µg/mL) mixed with DPPH and this reaction mixture incubated at room

temperature in dark condition for 20 min, a control without test compound is also prepared. At 517 nm, the absorbance was measured, with ascorbic acid serving as a positive control.[10-11,12]

The antioxidant activity of ethanol and acetone extracts was determined using the ABTS test. Extracts of varied strengths were mixed with the ABTS solution. The produced radical monocation was reduced in the presence of antioxidants in the extract. The absorbance was measured at 734 nm and ascorbic acid is used as control.

RESULTS

Gas Chromatography–mass Spectrometry (GCMS) Analysis

GC-MS chromatograms (Figure 1, 2) revealed the presence of numerous biologically active phyto-constituents in the plant. The major constituents present in ethanol extract are 9, 12- Octadecadienoic acid (28.25%), Phytol (18.79%), Methyl palmitate (16.71%), Neophytadiene (6.69%), Methyl stearate (4.04%), Squalene (3.50%). In the acetone extract are: Lanosterol (9.19%), 4,22-stigmastadiene-3-one (8.303%), Tetratetracontane (5.850%), Sigmasterol (5.138%), Thunbergol (3.956%).

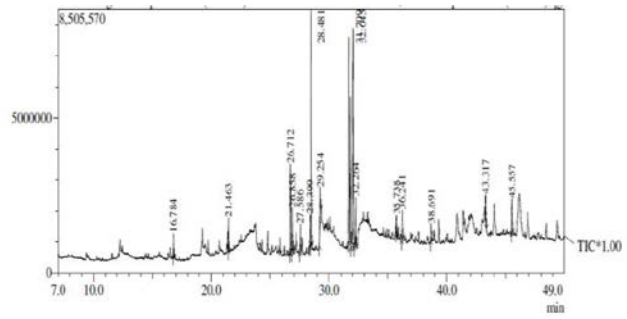

Figure 1: GC-MS chromatogram of Ethanol extract of *Oldenlandia corymbosa.*

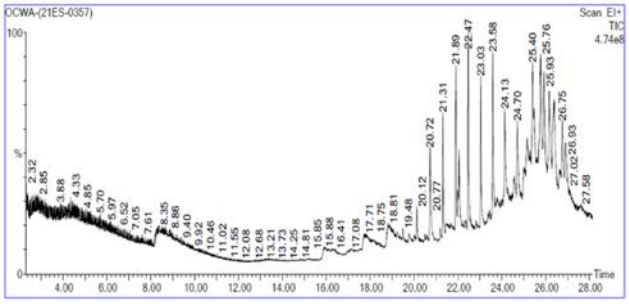

Figure 2: GC-MS chromatogram of Acetone extract of *Oldenlandia corymbosa*

DPPH and ABTS radical Scavenging Activity

The results of the plant extracts' DPPH radical scavenging activities are shown in Figure 3. The ethanol extracts demonstrated the strongest DPPH radical scavenging activity, with an IC_{50} value of 130.56g/mL, compared to the acetone extract's IC_{50} value of 189.33g/mL. Radical scavenging activity of both extracts was in a dose-dependent manner.

The ABTS radical decolorization activities of plant extracts against the respective concentrations are presented in Figure 4. At a concentration of 200 g/mL, the highest activity was noted in ethanol extract at 60.21% and in acetone extract at 57.34%. The IC_{50} value noted for ethanol extract is 132.83 g/mL and for acetone extract is 146.27 g/ml.

In-vitro neuroprotective effects

The protective effects of extracts on cell viability against LPS-induced cell damage were assessed using the MTT assay. As shown in Figure 5, cell viability in the non-treated cells was assigned as 100%, whereas in the LPS-treated cells, the cell viability was reduced to 54.61±0.01%. However, the cell viability was increased to 77.80±2.323% by acetone extract and 85.91±2.499% by ethanol extract.

Percentage cell viability against concentration

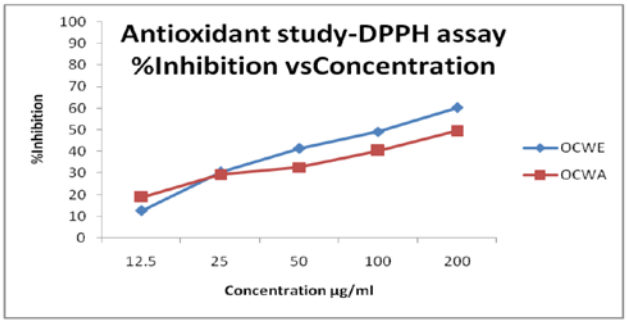

Figure 3: Line graph showing % of inhibition of ethanol and acetone extract against DPPH

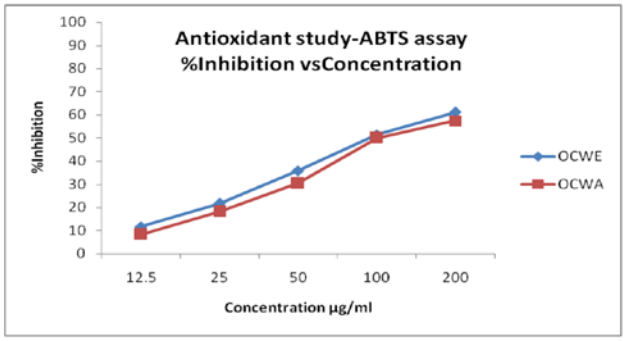

Figure 4: Line graph showing % of inhibition of ethanol and acetone extract against ABTS.

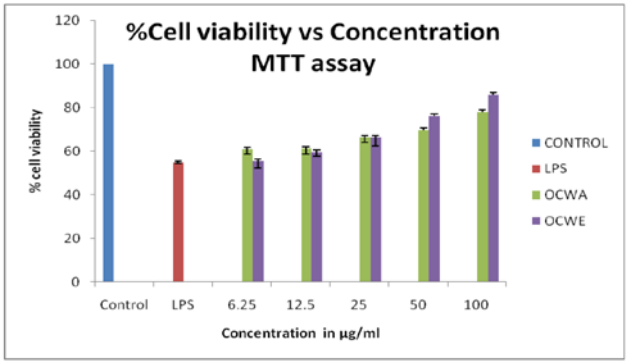

Figure 5: Bar graph showing % cell viability at different concentrations of ethanol and acetone extracts-MTT assay.

DISCUSSION

The present study described the antioxidant and anti-neuroinflammation activities of *O. corymbosa*. To find out the antioxidant property of plant, we first evaluated the free radical scavenging activities of ethanol and acetone by DPPH and ABTS assays. Both extracts exhibited dose-dependent radical scavenging activity. Antioxidants are thought to work by scavenging free radicals to prevent lipid oxidation. Antioxidants, or lipid peroxidation inhibitors, are crucial not just for food safety but also for the preservation of live cells from oxidative damage.[13] The DPPH and ABTS tests were utilized to evaluate the scavenging free radical activities. When compared to acetone extract, the ethanol extract of *Oldenlandia corymbosa* (OCWE) had the highest DPPH and ABTS radical scavenging activities (OCWA). From the interpolation of the curves displayed for percentage inhibition against the respective concentrations, the extract's concentration required to bring 50 percent scavenging (IC_{50}) was computed. The IC_{50} value indicates that the tested preparation contains 50% of the inhibitor's content, which is sufficient to provide the desired activity. In order to achieve the required function, it's critical to precisely quantify the ideal concentration of the compounds that must be delivered. The IC_{50} values for ethanol and acetone extracts from the DPPH assay are 130.56 g/mL and 189.33 g/mL, respectively and IC_{50} values for ethanol and acetone extracts from the ABTS assay are 132.83 g/mL and 146.27 g/mL, respectively. According to both antioxidant studies, the ethanol extract of the plant has a higher antioxidant capacity than the acetone extract.

Treatment with ethanol and acetone extracts of *O. corymbosa* raised the lowered cell viability percentage (54.610.01%) following Lipopolysaccharide (LPS) induction on neuroblastoma cell lines (IMR-32) by 85.91±2.499% and 77.8±02.32%, respectively. LPS is a powerful innate immune stimulator that causes macrophages to produce cytokines and inflammatory mediators like Nitric oxide (NO) and Prostaglandin E_2 (PGE2), and these, in turn, results in a reduction of cell viability.[14] From the data obtained, it is clear that higher cytoprotection was also exhibited by ethanol extract than acetone extract.

Table 1: List of compounds that have been proven for their anti-inflammatory and antioxidant activity.

Compound Name	Nature of Compound	Activity Established
Neophytadiene	Diterpene	Antiinflammatory[15]
Squalene	Triterpene	Antioxidant[16]
Stigmasterol	Sterol	Antiinflammatory[17]
Thunbergol	Diterpene	Natural antioxidant[18]

Phytochemical analysis by GC-MS of ethanolic and acetone extract of *O. corymbosa* revealed the presence of phytoconstituents like 9,12-Octadecadienoic acid, Phytol, Methyl palmitate, Neophytadiene, Methylstearate, Squaline, Lanosterol, 4,22-stigmastadiene-3-one, Tetra tetracontane Stigmasterol and Thunbergol. Table 1 represents those compounds reported to have antioxidant and anti-inflammatory activities. These chemicals are thought to be the key contributors *to O. corymbosa's* antioxidant and anti-inflammatory properties. However, it is unclear if plant's cytoprotective impact is due to a single molecule or the combination of multiple constituents, and more research is needed.

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. *In-vitro* 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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road, Salem for providing the necessary facility to accomplish the work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

ABBREVIATIONS

GCMS: Gas chromatography-mass spectroscopy; **DPPH:** 2, 2-diphenyl-1-picrylhydrazyl-hydrate; **ABTS:** 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)**; IC50:** Half maximal inhibitory concentration; **MTT:** 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; **OCWE:** *Oldenlandia corymbosa* whole plant ethanol extract; **OCWA:** *Oldenlandia corymbosa* whole plant acetone extract; **nm:** nano meter; µG**:** micro gram; **mL:** mili litre; **g:** gram; **mg:** mili gram; **mL/min:** mili litre/ minute; **min:** minute

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