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Phytochemical screening, *in-vitro* antioxidant and anti protease activities of various solvent extracts of *Muehlenbeckia platyclada* root (F. Muell.) Meisn

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Abstract
The objective of this study was focussed on phytochemical screening, *in vitro* antioxidant and antiprotease activities of various solvent extracts of *Muehlenbeckia platyclada* root. The roots were washed thoroughly, shade dried and coarsely powdered. The powdered material of *Muehlenbeckia platyclada* was successively extracted with hexane, chloroform and methanol using soxhlet apparatus. Preliminary phytochemical screening for carbohydrates, proteins, alkaloids, phytosteroids, flavonoids, glycosides, polyphenolics, saponins, and tannins were done by following standard procedure. *In vitro* antioxidant activities of three solvent extracts were assessed using DPPH, ABTS and total antioxidant capacity and flavonoids were estimated using aluminum chloride colourimetric assay. *In vitro* anti-protease activity of the root was evaluated using trypsin as enzyme and BAEE (N-benzoyl-L-arginine ethyl ester) as a substrate. The results showed that phytochemicals such as carbohydrates, proteins, flavonoids and glycosides, which present in the methanolic extract, were absent in hexane and chloroform extract of the root. The *in vitro* antioxidant and antiprotease activities of the *Muehlenbeckia platyclada* root clearly showed that the plant root has prominent antioxidant and protease inhibiting properties. From this work, it can be concluded that *Muehlenbeckia platyclada* root has the potential to be a powerful antioxidant and protease inhibitor.

Keywords: Oxidative stress, antioxidants, *muehlenbeckia platyclada*, flavonoids, alkaloids

1. Introduction

Oxidative stress is generally an imbalance between the excess formation of free radicals and the capacity of the body to neutralise their harmful effects using antioxidants. Oxidative damage is mainly caused by the enhanced production of free radicals. Free radicals are unstable molecules formed when an atom or molecule gain or lose electrons and they cause cellular and tissue damage. The oxygen generated free radicals such as hydroxyl, peroxyl, nitric oxide and superoxide radicals, are formed in living species both exogenously and endogenously. It can bring about oxidative stress to DNA, lipids and proteins and may cause severe conditions such as diabetes, ageing, cancer and degenerative disorders.

Antioxidants are substances that may defend our cells from free radicals, which have a major role in cancer, heart disease and other disorders are defined as one that delays oxidative stress to a target molecule [1]. Antioxidants reduce the free radicals due to their singlet oxygen quenchers and redox hydrogen donators [2-3]. Free radicals can be scavenged by natural as well as synthetic antioxidants [4]. The overuse of artificial antioxidants is replaced with natural antioxidants as they are regarded as protective devoid of toxicity [5]. In recent times, many scientists worked on herbal medicine for evaluating antioxidant compounds like flavonoids tannins and phenolics which got more attention for their probable role in preventing chronic disorders [6].

The significance of herbal medicine in drug discovery is known to humans that have been practised for exclusive disorders from the starting point of human history [7]. Traditional folk remedy from herbal medicine has continually promoted scientists to search for new medicinal drugs to expand wholesome lifestyles for animals and people [8]. Medicinal plants contain several phytochemicals inclusive of flavonoids, tannins, alkaloids, glycosides, saponins and polyphenolics. According to the World Health Organization (WHO), herbal medicine would be a satisfactory source to obtain a variety of drugs for preventing human diseases. Most of the individuals from developed countries use traditional medicines. However, such medicinal plants should be investigated to better understand their properties, safety, and efficiency [9].

In particular, using herbal medicine as an antioxidant, anti-inflammatory agent had been the been the target of the latest studies [10]. B *Muehlenbeckia platyclada* (F. Muell.) Meisn generally called ribbon-bush belongs to own family Polygonaceae is a medicinal plant used for the remedy of poisonous snake bites, fever, detoxification and fracture injuries [11]. The methanolic extract of this plant leaves was found to have effects on the production of human neutrophil superoxide anion and neutrophil elastase deliver when activated by formyl-L-methionyl-L-leucyl-L-phenylalanine. Besides, the plant has been traditionally used as antiulcerogenic, hypotensive, antihemorrhagic, sedative, diuretic, anti-inflammatory antirheumatic, abortive and anthelmintic agents [12]. But there is limited information about the pharmacological activities of the root material of the plant. Based on the ethanobotanical importance, the present work was focused on the phytochemical screening, *in vitro* antioxidant and antiprotease activities of various solvent extracts of *Muehlenbeckia platyclada* root.

2. Materials and Methods

2.1 Plant Collection

The roots of *Muehlenbeckia platyclada* was collected from M.S Swaminathan Research Foundation, Kalpetta, Wayanad. The roots were washed, shade dried and powdered.

2.2 Successive extraction of root material

The powdered material of *Muehlenbeckia platyclada* was extracted with hexane, chloroform and methanol using soxhlet apparatus and is stored for further *in vitro* assays.

3. Phytochemical screening of different solvent extracts of the root material

Phytochemical analysis was done to analyze the presence of phytochemicals found in different solvent extracts of *Muehlenbeckia platyclada* root [13-14].

4. *In-vitro* anti-oxidant property of various solvent extracts of root material

4.1 DPPH Radical Scavenging Assay

1,1- Diphenyl-2- picryl hydrazyl (DPPH) is a free radical scavenging assay for measuring the antioxidant activity of medicinal plants. In this assay, the reactive mixture contains 2.8 ml 100 μM DPPH in methanol and was added with 0.2 ml of different solvent extracts of root material at different concentrations. The mixture was incubated for 30 minutes and the optical density was recorded at 517 nm. Ascorbic acid is taken as standard and methanol without sample along with DPPH was taken as control [15].

% of Scavenging = (A control-A Test)/A control X 100

4.2. ABTS Radical Scavenging Assay

This assay generates the oxidation of ABTS (2, 2'-azinobis [3-ethylbenzothiazoline-6-sulphonate]) to a nitrogen-centred radical cation, ABTS. The stock solution contains 7.4 mM ABTS added with 2.6 mM potassium persulfate. The working solution is made by adding these solutions in equal amounts and allowed to react for 12 hrs in dark condition. The mixture was then diluted by adding 1ml ABTS solution with 60 ml methanol to get an absorbance of 1.1±0.02 at 734 nm. 150 μl of different concentrations of extracts of the root was allowed to react with 2850 μl of ABTS solution for 2 hours. The absorbance was taken at 734 nm using spectrophotometer [16]. IC50 values were also calculated.

4.3 Total antioxidant capacity (Phospho molybdenum assay)

Total antioxidant capacity is one of the important methods for determining the antioxidant activity of medicinal plants. The principle behind assay is the reduction of Mo (VI) to Mo (V) by the root extract to form a green coloured Mo(V) phosphate complex at acidic pH. The reagent solution (3 ml) which contains 0.6 M Sulfuric acid, 28 mM Sodium phosphate 4 mM Ammonium molybdate was added with 0.3 ml root extract. Incubate it for 90 minutes at 95 °C and was cooled. The optical density was taken at 695 nm using spectrophotometer [17].

4.4 Estimation of total flavonoid content.

The sample (0.5 ml) was added to 1.5 ml methanol, 0.1 ml 10% aluminum chloride, 0.1 ml 1M potassium acetate and 2.8 ml of distilled water. Incubated the mixture for 30 minutes and the absorbance was recorded at 415 nm. 10% aluminum chloride replaced by distilled water was taken as blank. Similarly, 0.5 ml of different solvent extracts of *Muehlenbeckia platyclada* reacts with aluminium chloride for estimating flavonoid content. The results were expressed as mg Quercetin equivalents/g sample. In this method, Quercetin was used as standard [18].

5. *In-vitro* anti protease activity

This assay was carried out using the spectrophotometric assay by Sigma Aldrich with slight modifications. Trypsin, the protease is used to find the anti-protease activity of different solvent extracts of root. The assay was done based on the hydrolysis of the substrate BAEE (N-benzoyl-L-arginine ethyl ester) at the ester linkage, which leads to an increase of optical density at 253nm. The mixture consisted of 200 μl trypsin, 200 μl tests and incubates for 10 min. The reaction is started by adding 3ml BAEE and absorbance was determined at 253 nm. Phenyl methyl sulphonyl fluoride (PMSF) is used as standard.

Inhibition (%) = (1-B/A) ×100.

Where A = Change in optical density of control, B = change in optical density of test solution.

6. Results

6.1 Phytochemical screening of different solvent extracts of the root material

The hexane, chloroform and methanolic root extract of *Muehlenbeckia platyclada* were screened for the presence of phytochemical compounds. The qualitative analysis of the methanolic root extract showed the presence of flavonoids, alkaloids, phytosterols, saponins, terpenoids and glycosides. But phytochemicals like carbohydrates, proteins, glycosides and flavonoids, which are present in methanolic extract, were absent in hexane and chloroform extract. The results of the analysis were represented in Table 1. The potential of these phytochemical constituents has high pharmacological activities such as antioxidant compounds of the medicinal plant for the care of health and protection from coronary heart problems, anti-mutagenic and anti-carcinogenic effects [19].

Table 1: Phytochemical analysis of *Muehlenbeckia platyclada* root

Phytochemicals	*Muehlenbeckia platyclada* root		
	Hexane extract	Chloroform extract	Methanolic extract
Carbohydrates	_	_	+
Proteins	_	_	+
Alkaloids	+	+	+
Phytosteroids	+	+	+
Flavonoids	_	_	+
Saponins	+	+	+
Tannins	_	_	_
Polyphenolics	_	_	_
Terpenoids	+	+	+
Glycosides	_	_	+

6.2 DPPH Scavenging activity

DPPH assay is one of the methods for determining the antioxidant activity of *Muehlenbeckia platyclada* root. Here the percentage of scavenging of hexane extract is 49.45± 0.05 at a maximum concentration of 1000 µg/ml whereas the chloroform extract showed inhibition of 81±0.07. But the methanolic extract of the root showed the highest significant radical scavenging effect of 92.72±0.134 on DPPH radicals at 1000 µg/ml. DPPH receive electron easily from antioxidants to become stable of diamagnetic nature.

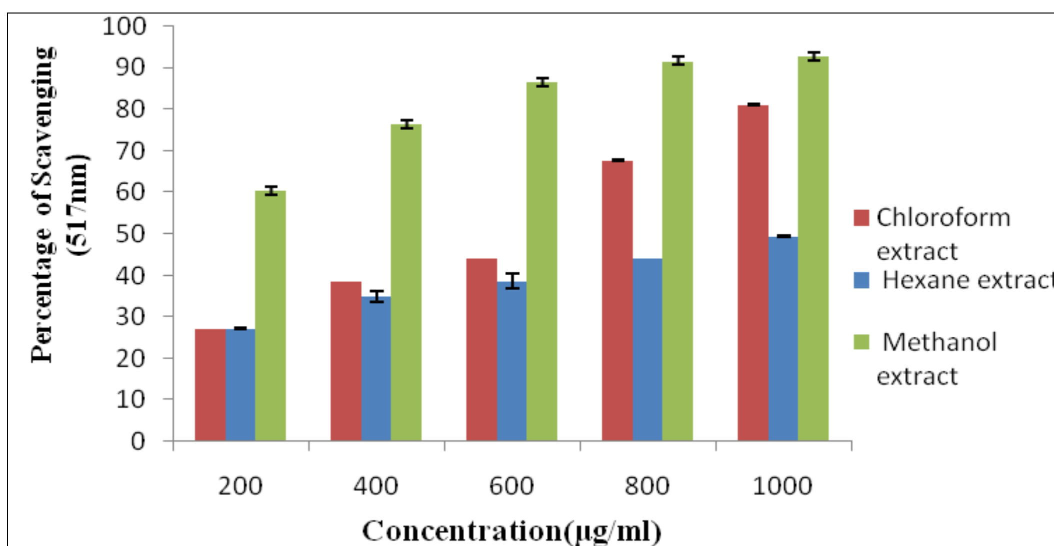

Fig 1: DPPH Radical Scavenging assay of *Muehlenbeckia platyclada* root

6.3 ABTS Radical Scavenging assay

It is one of the methods for evaluating the antioxidant property of medicinal plants. At a high concentration of 1000 µg/ml, the percentage of scavenging of hexane extract of the root sample is 47.12 ± 0.99 and the chloroform extract is 62.5±0.59 at the same concentration. But the methanolic extract of the root showed a significant radical scavenging effect of 81± 0.89 on ABTS radicals.

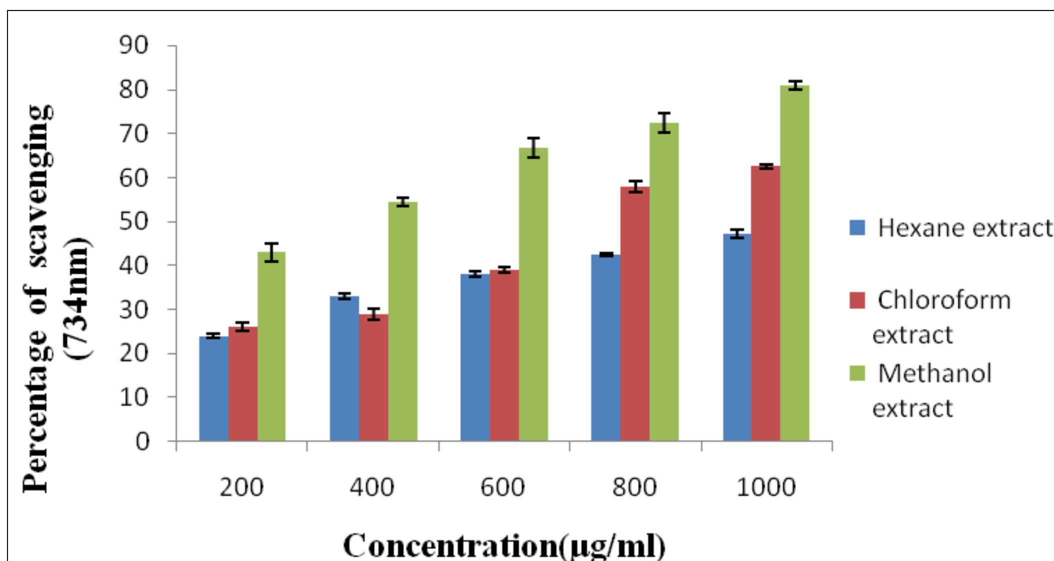

Fig 2: ABTS Radical Scavenging Assay of *Muehlenbeckia platyclada* root

6.4 Total antioxidant capacity (Phospho molybdenum assay)
Phosphomolybdenum assay was used for screening the antioxidant capacity of various solvent fractions of medicinal plants. Here the chloroform extracts of *Muehlenbeckia platyclada* root showed higher antioxidant capacity with increasing concentration when compared to methanol and hexane extracts of the sample. 200µg/ml of the chloroform extract of *Muehlenbeckia platyclada* root was equivalent to 110 µg/ml of the ascorbic acid standard. When related to the other extracts, chloroform extract showed higher antioxidant capacity.

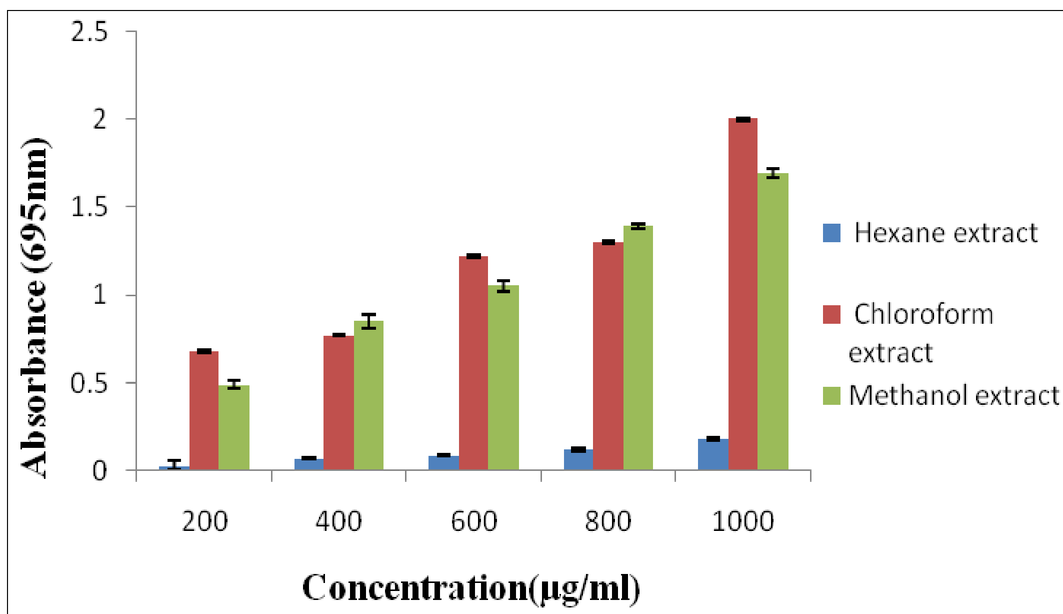

Fig 3: Total antioxidant capacity of *Muehlenbeckia platyclada* root

6.5 Estimation of total flavonoid content
From the standard plot of quercetin, the flavonoid of methanol extract was found to be 120 mg quercetin equivalents per gram of the root material. It may be noted that flavonoid contents were not deducted in hexane and chloroform extracts of the root material.

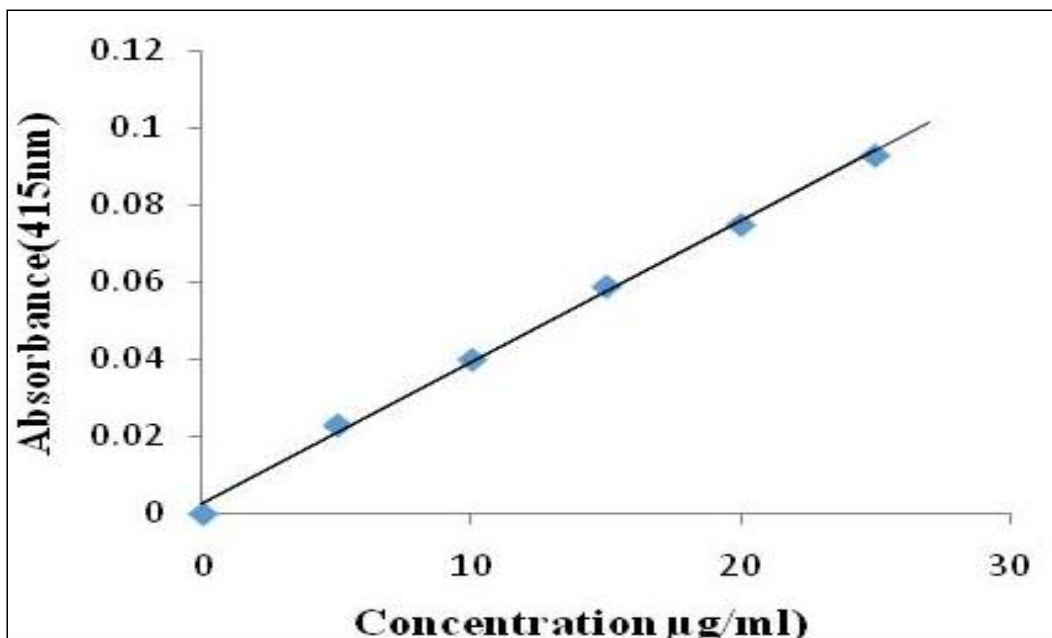

Fig 4: Determination of total flavonoid content- Calibration curve of quercetin

6.6 *In- vitro* anti protease activity
Anti protease activity was done using hexane, chloroform, and methanolic bark extracts of *Muehlenbeckia platyclada* root. In this study, the hexane extract of *Muehlenbeckia platyclada* root showed 43% inhibition whereas chloroform extract showed 53.89% inhibition. But methanolic extract showed 64% inhibition at a concentration of 500µg/ml. Phenyl methyl sulfonyl fluoride (PMSF), the standard showed inhibition of 55.19% at a suitable concentration of 100µg/ml.

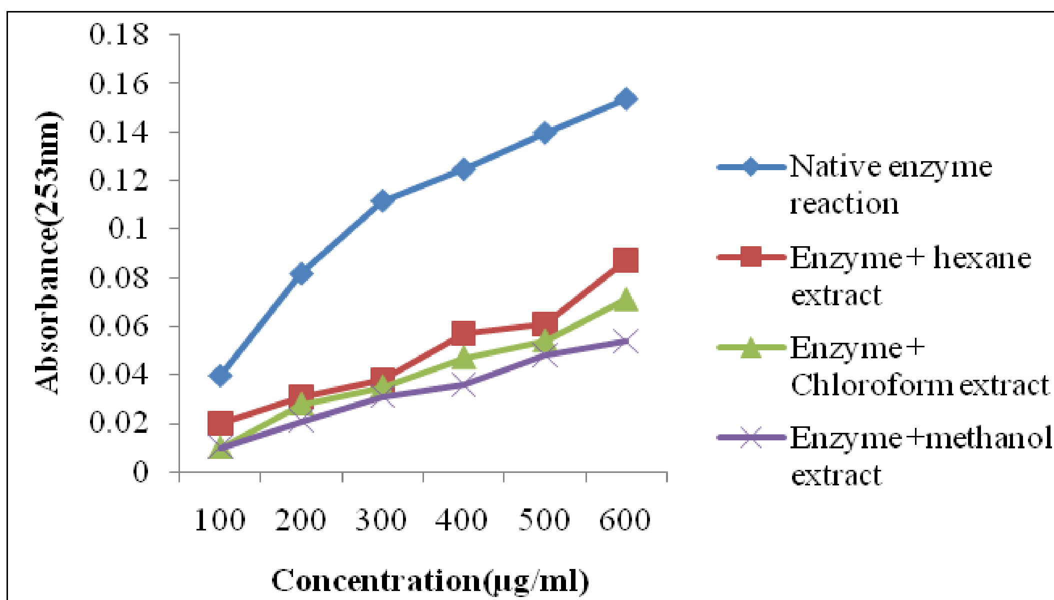

Fig 5: *In vitro* anti protease activity of *Muehlenbeckia platyclada* root

7. Discussions

DPPH receive electron easily from antioxidants to become stable of diamagnetic nature. DPPH is stable nitrogen centred synthetic free radicals that accept electrons from antioxidants present in the root sample, which was measured using a spectrophotometer at 517 nm. They produce violet colour in methanol solution, which was reduced to a yellow coloured diphenyl picryl hydrazine when different solvent extracts of *Muehlenbeckia platyclada* root was added in different concentration. Here the methanolic extract of the root showed the highest activity with an IC50 value of 165.67µg/ml.

The ABTS assay is based on the capacity of the antioxidants present in the plant sample to scavenge the long-lived radical cation ABTS+, which results in a reduction in optical density at 734nm. The scavenging of ABTS radicals increased in a concentration-dependent manner in the *Muehlenbeckia platyclada* root. Here the methanolic extract of the root showed the highest activity with an IC50 value of 367µg/ml.

An increase in absorbance indicates the higher antioxidant activity of the plant. The chloroform extracts reduced Mo(VI) to Mo(V) in the presence of natural antioxidants present in *Muehlenbeckia platyclada* root and green phosphate is formed with maximal absorption at 695 nm. This can be described by the fact that electron transfer from antioxidants depends on the antioxidant structure [20].

Flavonoids are polyphenolic compounds positively related to antioxidant activity and also have large chemical and biological activities against microbes, ulcers, cancer, inflammation, etc. This assay is based on the fact that aluminum chloride formed acid-stable complexes with the C-4 keto groups and with either C-3 or C-5 hydroxide group of flavones and flavonols. As it is the first report of *Muehlenbeckia platyclada* root against oxidative stress, phytochemical screening should be done to detect the active phenolic and flavonoid components.

The proteases are drug targets inflammation and the methanolic extract of the root has a major role in regulating protease activity. Proteases are involved in inflammation and tissue injury. Serine proteases from neutrophils are associated with inflammatory conditions like pulmonary emphysema, which was characterized by the destruction of alveoli leads to the up normal function of the lungs. Here the methanolic extract of root inhibits proteases at a suitable concentration.

8. Conclusions

In this study, *Muehlenbeckia platyclada* root was tested to analyze the presence of phytochemical constituents, their antioxidant and antiprotease activities. Successive extraction of the root was done using Soxhlet apparatus and the extract was partitioned using hexane, chloroform, and methanol. The analysis of different solvent extracts of the root material showed the presence of several phytochemical constituents. The antioxidant activity was measured by DPPH, ABTS, and Phosphomolybdenum assay, which was proven to be high. The flavonoid present in the root sample was also estimated. Finally, the results in this study prove that the *Muehlenbeckia platyclada* root material contains flavonoids, phytosteroids, terpenoids, and glycosides, which are the perfect sources of antioxidants. The findings of these studies suggested that *Muehlenbeckia platyclada* root could have a major role as a therapeutic agent in reducing oxidative stress-related disorders. The work regarding the isolation of active compounds responsible for antioxidant capacity will be carried out in the future.

9. Abbreviations

DPPH: 1, 1- Diphenyl-2- picryl hydrazyl; ABTS: 2, 2'-azinobis [3-ethylbenzothiazoline-6-sulphonate], BAEE: (N-benzoyl-L-arginine ethyl ester); PMSF: Phenyl methyl sulphonyl fluoride; Mo: Molybdenum; WHO: World Health Organization.

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11. Author's contributions

Jenson Jacob designed and directed the project and wrote the manuscript. Meenu john, Rohitha P and Arsha Krishna and Adithya babu performed the experiments and analyzed the data. All authors have read and approved the manuscript.

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13. Availability of data and materials

The data that support the findings of this study are available

from the corresponding author upon request.

14. Conflict of interest

The authors declare that there was no conflicting interest.

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