Original Article

Extraction Of Phytochemicals Through Sequential Cold Maceration And Evaluation Of Total Polyphenol Content And Antioxidant Properties In Ailanthus Altissima Of Simaroubaceae Family

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Abstract

In the present study on the sequential cold maceration extraction of *Ailanthus altissima* (Simaroubaceae) leaves was performed. The methonolic, ethanolic and aqueous extracts were subjected to evaluation of preliminary phytochemical analysis, total polyphenol content (TPC) and DPPH antioxidant activity. The preliminary qualitative analysis of all three extracts shown presence of various secondary metabolites and almost all three extracts exhibited presence of flavonoids and terpenoids. The highest TPC quantity was observed in aqueous extract, In contrast to this, the lowest IC_{50} value was observed in ethanolic extract for DPPH antioxidant activity. All the three extracts were shown concentration dependent free radical scavenging activity. This work provides scientific supports for the high antioxidant activity; it may find potential applications in the development of natural herbicides and antioxidants for combating the oxidative stress in various disease conditions.

INTRODUCTION

The biological combustion of respiration results in production of harmful intermediates referred to as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are also produced during disturbances in metabolism of living organisms. The production of these ROS and RNS can lead to generation of oxidative stress which in turn damages the macromolecules in cells such as lipids, proteins and DNA. The oxidative stress can also cause aging, neurodegenerative disorders, and various ailments in humans. Hence, the equilibrium among anti oxidation and oxidation is supposed to be a precarious concept for sustaining a healthy biological system (S. Kumar et al., 2014). The development of chronic diseases, such as cancer, cardiovascular diseases (CVD), type-2 diabetes and hypertension involve production of free radicals in large quantity also leads to oxidative stress (Poljuha et al., 2017). It has been proved that there is an inverse relationship between the intake of some vegetables and fruits and mortality from age-related diseases, which could be partially ascribed to the presence of antioxidant compounds, importantly phenolic compounds, which are highly abundant hydrophilic antioxidants in the almost all plant based food sources and the most are active antioxidant compounds. These dietary antioxidants also stimulates cellular defences and helps to prevent the various biomolecules against oxidative damage (Albouchi et al., 2013). In addition, antioxidants have been used to improve shelf life of various products in food industry. The butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) are two conventional synthetic antioxidants widely used in various food industries; however, there is a need of replacing these conventional antioxidants with natural antioxidant compounds to reduce the potential risks associated with conventional antioxidants. Therefore, the identification and isolation of antioxidants from natural sources such as plants has received much attention, and several studies have been reported identification of natural antioxidant compounds. In addition, these naturally oxidant molecules are also can formulate into nutraceuticals which are efficient molecules to prevent the oxidative damage (Faller & Fialho, 2010). Fundamental biochemical processes of plants involved in the production of primary metabolites which play major role in growth, survival and reproduction of plants (Sulaiman & Balachandran, 2012). The secondary metabolites and their derivatives of plants are produced to combat pathogens, unfavourable environmental conditions and stress etc. and

these are diverse number of compounds with various physiological, biochemical and pharmacological activities such as hepatoprotective, anticancer effects, anti-allergic, diuretic and antioxidant etc. Phytochemicals like flavonoids, tocotrienols, carotenoids, alkaloids, tocopherols ascorbates, and phenols, are strong antioxidants and have imprinted out a significant role in the health care system. There is renewed interest in discover out new natural antioxidants from living system for application in food, pharmaceutical and cosmetic industries (Aryal et al., 2019).The composition, distribution and quantity of polyphenol compounds in plants vary significantly according to various intrinsic and extrinsic factors such as plant cultivar and genetics, soil physicochemical parameters and growing conditions, maturity state and post-harvest conditions. The polyphenols are one of the important compounds with antioxidant defence mechanism, with its synthesis stimulated under stress conditions, such as temperature alterations, UV exposure and pathogenic attacks (Singh et al., 2016) (Faller & Fialho, 2010).

Ailanthus altissima (Mill.) also referred as Swingle or Tree of Heaven belongs to the *Simaroubaceae* family which is an invasive deciduous tree. High production of seeds dispersed by wind, extremely fast growth (2 m/y), and a high regenerative capacity makes *A. altissima* easily dispersible and hard to control and therefore is considered one of the worst invasive plant species. A total of 18 alkaloids, 15 steroids, , 18 alkaloids, 15 steroids, 62 terpenoids, 15 , 30 aliphatic components, 7 flavonoids, several coumarins and, quassinoide ailanthone, organic acids and lignans, which are responsible for pharmacological properties of its extracts. A study found significant amount of phenolic content in *A. altissima* leaves, followed by stalks and stems. It makes sense to explore the possible use of invasive plant species since they grows easily (Caramelo et al., 2021; Mo et al., 2021). It is a prominent versatile tree since it is used in ethnic medicine for the treatment of amoebic dysentery, gastric diseases and colds. The antioxidant, antiviral, anti-inflammatory antimalarial, anti-fungal, anti-tuberculosis, cytotoxic, anti-proliferative, insecticidal anti-asthmatic and phosphodiesterase inhibitory activities have previously been reported for *A. altissima* (Albouchi et al., 2013) (Dudonné et al., 2009) (Faller & Fialho, 2010) (Sulaiman & Balachandran, 2012).

There is a scarcity of evidences about the secondary metabolites of *A. altissima* and a comprehensive study on its chemistry is certainly missing. This knowledge, however, is very crucial to explore the possible application areas for rational use of this species. Therefore, the present study is aimed to extract polyphenols from *A. altissima* leaves using sequential cold extraction and estimate the total polyphenol content (TPC) and antioxidant activity using DPPH free radical scavenging assay of all the extracts.

METHODOLOGY

Preparation of leaf extract: The Fresh leaves of *Alianthus altissima* (*Aa*) was collected and washed with running tap water and distilled water. The leaves were shade dried and made into fine powder using electronic blender. The cold maceration method was adopted to prepare the extract. 25 grams *Aa* leaf powder was dissolved in methanol and macerated with intermittent shaking for three days. The obtained solution was filtered using muslin cloth followed by What man No. 1 filter paper. The resulting residue was air dried and further extracted with 80% ethanol followed by distilled water. The obtained solutions were vacuum dried using rotatory evaporator and stored at 4^0C until further analysis.

Preliminary Phytochemical Investigation: The three extracts of *Aa* through cold maceration were subjected to qualitative phytochemical investigation to determine the bioactive compounds or phytochemicals such as Tannins, alkaloids, saponins, Cardiac glycosides, Steroids, terpenoids, flavonoids, Phlobatannins, Anthraquinones, reducing sugars and carbohydrates according to the Mishra et al., 2018.

Estimation of total Polyphenol Content (TPC): According to the Senguttuvan et al., 2014the three extracts were subjected to the estimation of TPC. In this method, 10mg of each dried plant extracts were taken and redissolved in 5% of dimethyl sulfoxide [DMSO] and the solution was filtered using What man No.1 filter paper. To this 1ml of FC reagent [1:10 V/V] was added and followed by addition of 7.5% sodium carbonate. The reaction solution was incubated at 37^0C for 2 hours and absorbance was read at 765nm using UV VIS spectrophotometer (Shimadzu UV – 2450). The gallic acid is used as standard.

Anti-Oxidant assay (DPPH free radical scavenging activity): The DPPH reagent was prepared by dissolving 1.3mg of DPPH (2'2' diphenyl 1'picrylhydrazyl) in 100ml of methanol and absorbance was adjusted to 0.600 – 0.650 at 517nm. Different concentrations of all three extracts (50-250 µg mL^{-1}) were dissolved in methanol and 2ml of DPPH reagent was added to them. The absorbance was read at 515nm using UV Vis spectrophotometer after 2 hours of incubation in dark chamber. The ascorbic acid was used as standard and methanol as blank. DPPH with methanol was considered as control. The anti-oxidant capacity was represented as percentage of radical scavenging activity (RSC) treated with plant extracts.

DPPH scavenging effect [%] = $A_0 – A_1/A_0$ X 100

Where A_0 = the absorbance of control

A_1 = the absorbance of sample

The Inhibition Concentration 50 [IC_{50}] value was determined using the formula IC_{50} [0.5-b/a].

RESULT AND DISCUSSION

Plant medicine or herbal medicine are practiced in many cultures and serves as source of potential therapeutic agents against various diseases and ailments of human body (Olivia et al., 2021). Phytochemicals are the secondary metabolites produced from different parts of the plants and have definite and targeted physiological action on human body (Dhanarasu

& Al-hazimi, 2011). The phytochemicals are natural, eco-friendly and with less or no side effects when compared to the synthetic modern medicines (Karki et al., 2021). Various bioactive compounds such as flavonoids, phenols, alkaloids, tannins, terpenoids, saponins exhibit pharmacological actions like anti-oxidant, anti-inflammatory, anti-microbial and anti-cancer properties (Karki et al., 2021) (B. Y. S. Kumar & Fathima, 2017). An enormous pool of bioactive compounds or secondary metabolites present in various plant species, but only a small fraction of these have been identified, isolated and examined. It is very important to develop and control the suitable screening methods for new compounds (Olivia et al., 2021) (Kanthal et al., 2014). In the present study, the qualitative phytochemical investigation found various phytochemicals of all the three extracts of *Ailanthus altissima* leaves which are represented in table 1. The methanol extract contains alkaloids, cardiac glycosides, terpenoids, and flavonoids. The ethanolic extract contains tannins, cardiac glycosides, terpenoids, flavonoids, anthraquinones, and carbohydrates. The aqueous extract contains alkaloids, saponins, steroids, terpenoids, and flavonoids.

S.No	Phytochemical constituents	Methanol	Ethanol	Aqueous
1	Tannins	-	+	-
2	Alkaloids (Mayer's test)	+	-	-
3	Alkaloids (Wagner's test)	-	-	+
4	Saponins	-	-	+
5	Cardiac glycosides	+	+	-
6	Steroids	-	-	+
7	Terpenoids	+	+	+
8	Flavonoids	+	+	+
9	Flavonoids (Shinoda's test)	-	-	+
10	Phlobatannins	-	-	-
11	Anthraquinones	-	+	-
12	Reducing sugars	-	-	-
13	Carbohydrates	-	+	-
+ = Positive, --= Negative				

Table 1: Qualitative phytochemical investigation of methanol, ethanol and aqueous extracts of *Ailanthus altissima* leaves.

The quantity and types of phytochemicals are different from one species to other species of plants. In recent decades, different phytochemicals bioactive compounds with pharmacological activities have been investigated depicted in table 2 (Ahmad et al., 2018).

Name of the phytochemical constituents	Biological activities and applications	References
Alkaloids	Anti-malarial, anti-cholinergic, anti-diabetic, anti-inflammatory, anti-oxidant, anti-depressant, hepatoprotective activity, nootropic, anti-psychotic, anti-hypertensive, anti-diuretic, anti-cancer, anti-stress, anti-arrhythmic, anti-microbial and anti-nociceptive.	(Debnath et al., 2018)
Anthraquinone glycosides	Anti-tumor, anti-inflammatory, anti-arthritic, anti-bacterial, anti-fungal, anti-oxidant and anti-malarial activities	(Diaz & Miranda, 2018)
Carbohydrates	Anti-coagulant, anti-thrombotic, anti-viral, anti-oxidant, anti-tumour, anti-inflammatory, hepatoprotective, radioprotective and immunomodulating activities	(Liu et al., 2015)
Cardiac glycosides	Antiviral, anti-tumour, and inhibit Na+, K+-ATPase activity and increase myocardial contraction	(Rodríguez et al., 1989)
Coumarins	Anti-cancer, anti-bacterial, anti-coagulant, anti-tuberculosis, anti-fungal, anti-inflammatory, and anti-cholinesterase activities	(Xu et al., 2015)
Flavonoids	Anti-oxidant, anti-inflammatory, antibacterial, anti-cancer, anti-diabetic, antiviral and hepatopancreatic activity	(Kočevar et al., 2007)
Phenols	Anti-oxidant, anti-bacterial, anti-diabetic, anti-hemorrhoidal, anti-rheumatic, anti-proliferative, anti-depressant and neuroprotective activities	(Tanase et al., 2019)
Saponins	Hypoglycemic activity, anti-zygotic, virucidal, anti-inflammatory, hyoplipidaemic, anti-fungal and anti-bacterial activities	(Desai et al., 2009)
Steroids	Hypocholesterolemic activity, anti-cancer activity, anti-inflammatory, anti-leishmanial, anti-microbial, immunomodulatory, neuroprotective, and anti-genotoxicity, anti-metastatic, trypanocidal, hypoglycaemic and cholinesterase inhibitory activities	(B. Gunaherath & Gunatilaka, 2014)
Tannins	Anti-carcinogenic, anti-viral, anti-cancer, anti-bacterial, anti-inflammatory, anti-oxidant, anti-diabetic, α-glucosidase and tyrosinase inhibitory activities	(Das et al., 2020)
Terpenoids	Broad spectrum anti-cancer compounds and with anti-inflammatory, anti-bacterial, anti-viral activities and also effective for the treatment of cardiovascular diseases, antimalarial, hypoglycaemic, anti-aging, immunoregulation and neuroprotective activities.	(Yang et al., 2020)

Table 2: Different phytochemical constituents and their pharmacological activities

Estimation of total polyphenol content (TPC)

Polyphenol compounds are the most abundant secondary metabolites widely distributed in the plant kingdom and are one of the significant antioxidant molecules. These polyphenols have major role in protecting plants from pathogens, herbivores and abiotic stress conditions. However, these are also involved in the regulation of plant cell growth and cell division (Binns et al., 1987). The polyphenols exhibit antioxidant activity by various mechansism such as donating H atoms to the free radicals generated in the cells and scavenging other ROS and RNS such as OH• , HOCl, N2O3, ONOOH and NO2 •. Mostly the di and polyphenols, can react with O2 •¯ or by binding transition metal ions (especially iron and

copper), which resulting in forms poorly active in endorsing free radical reactions and therefore can also affect with the uptake of metals from the diet (Bangou et al., 2012).

The polyphenolic content in any plant extracts is expressed as Gallic acid equivalent per gram (GAE /mg). In the present study the total polyphenol content of the methanolic extract 59.88 mg/100mg of GAE, ethanolic extract 49.11 mg/100mg of GAE and aqueous 70.12 mg/100mg of GAE which is high among all the other extracts (Fig 1(a) & (b)). A study reported 119.84 mg GAE/mg total phenolics of *Ailanthus altissima* leave. In this study it was found that only 70.12 µg of GAE/mg total phenols in the aqueous extract at maximum (Mo et al., 2021). Phenolic profiles of populations from different geographical areas significantly differ. Also, the extraction process of differs from *Luís et al., 2012* ours and this could cause the discrepancy as well.

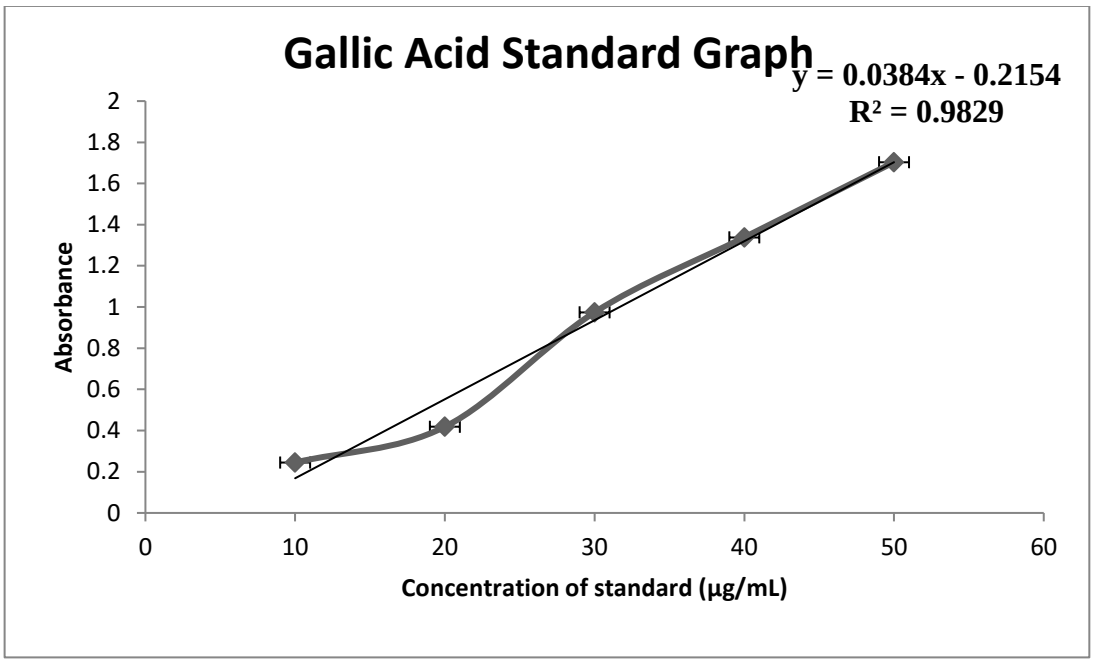

Figure 1 (a): Gallic acid standard graph

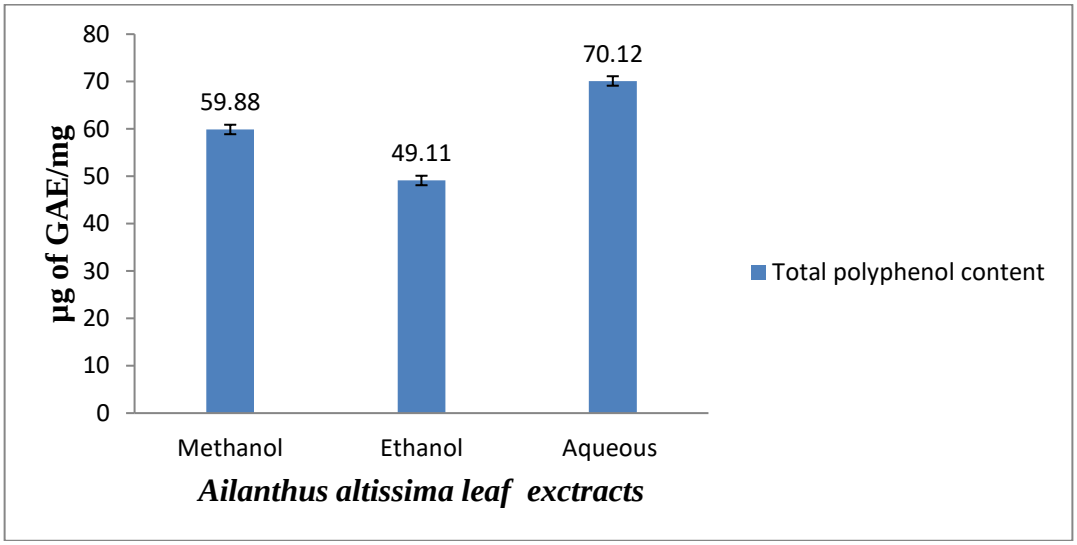

Figure 1(b): Total polyphenol content of *Alianthus altissima* leaf extracts

Antioxidant activity-DPPH free radical scavenging activity

DPPH assay predominantly depends on the capacity to donate hydrogen atoms. DPPH is commercially available nitrogen free radicals have the capacity to donate hydrogen. Free radicals play a role in damaging tissues in numerous chronic pathologies, such as cardiovascular diseases and cancer among others (Dorman et al., 2003). The DPPH free radicals are similar to the free radicals generate within the living cells, Hence most of the *in vitro* studies to evaluate antioxidant capacity of natural antioxidant compounds such as plant secondary metabolites are done with DPPH. The *in vitro* DPPH reduction with natural capacity usually estimates at 515 nm (Juan et al., 2005). The free radical scavenging property of DPPH can be observed as a change from purple to yellow color when a DPPH electron binds to a radical scavenger forming reduced DPPH-H (Cai et al., 2003). DPPH results are usually expressed as IC_{50} (half maximal inhibitory concentration) value and the lower the value the efficient in the antioxidant capacity. As shown in Fig. (Fig 2(a)-2(d)), In the present study all extracts showed a concentration-dependent scavenging activity and the highest antioxidant activity

was shown by both methanolic and ethanolic extracts (Fig 2(a)-2(d)). In this study, we found that DPPH IC_{50} value of 134.83 µg mL^{-1} for methanolic, IC_{50} value of 26.13 µg mL^{-1} for ethanolic and IC_{50} value of 109.27 µg mL^{-1} for aqueous extracts. Among all the extracts only ethanolic extract shown lower IC_{50} value, which indicates less quantity of extract is required to show anti-oxidant capacity. These findings were comparable to the previously reported IC_{50} DPPH values of methanolic extracts of *Ailanthus altissima* whole plant (112.83 µg mL^{-1}) by Alam et al. (2012). Similarly, *Malla et al., 2014* also found lower IC_{50} value for ethanolic leaf extract (21.22 µg mL^{-1}) of *Ailanthus altissima* leaves.

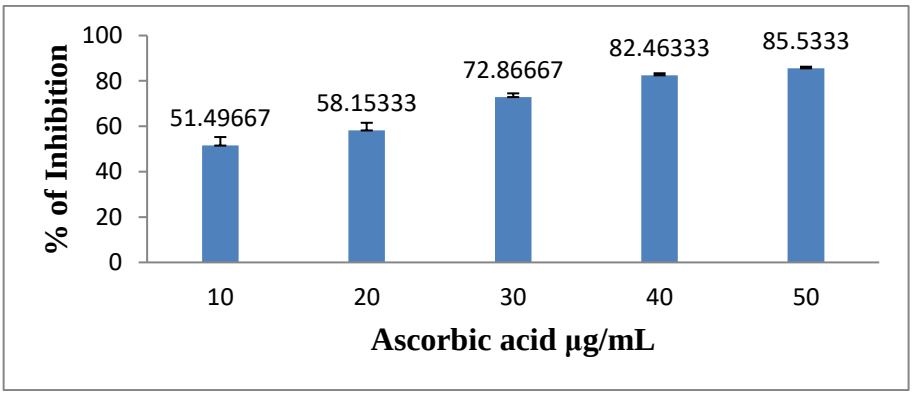

Figure 2(a): Ascorbic acid standard graph for DPPH assay

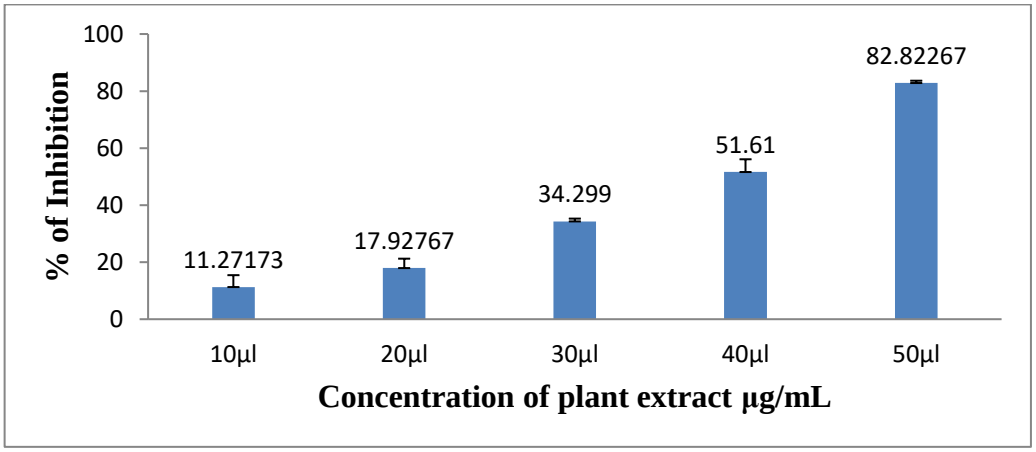

Figure 2(b): DPPH free radical scavenging activity of methanolic extract

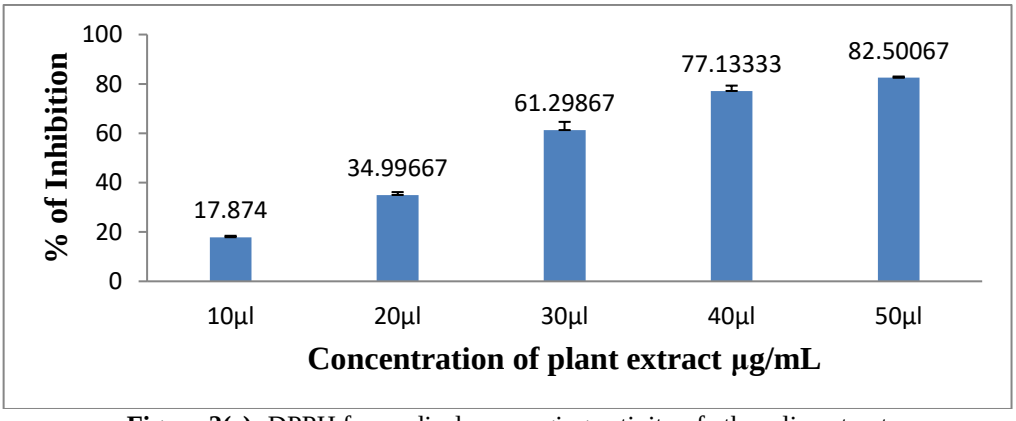

Figure 2(c): DPPH free radical scavenging activity of ethanolic extract

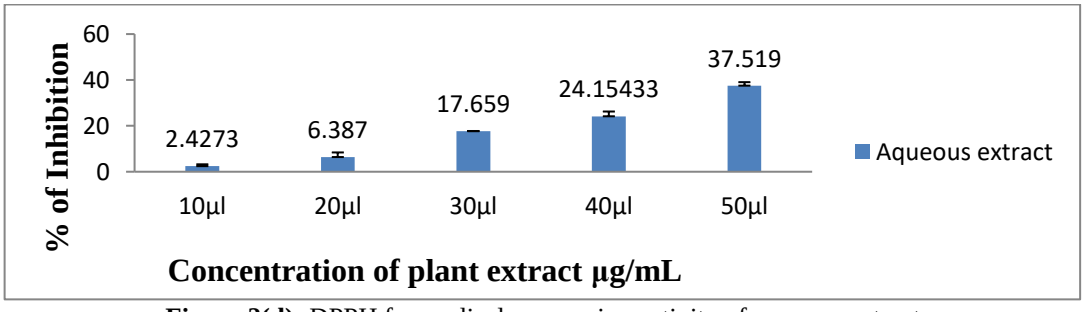

Figure 2(d): DPPH free radical scavenging activity of aqueous extract

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that the three extracts of *Ailanthus altissima* leaves prepared using cold maceration contains large amount of phenolic compounds, exhibited high polyphenolic content and free radical scavenging activity. A high correlation was found among the total phenolic content and the free radical scavenging activity using *in vitro* antioxidant model (DPPH) in this study. These *in vitro* assays indicates that these three extracts are vary in their antioxidant capacity, and are good source of antioxidants, which might be useful in preventing the progress of various oxidative stress conditions in living systems. Hence, more queries will be addressed in future studies aiming these varieties to discover the potential and isolation of bioactive compounds accountable for such activities and as chemo preventive and therapeutic agents.

DECLARATIONS

Ethics approval and consent to participate

Not Applicable

CONSENT FOR PUBLICATION

Not Applicable

COMPETING INTERESTS

The authors declared no competing interest

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AUTHORS CONTRIBUTIONS

PVN were involved in supervision and conceptualization of project. All other authors involved in investigation, resources and writing the original draft manuscript and also involved in data curation, editing and reviewing of the manuscript.

ADDITIONAL INFORMATION

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