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ANTIOXIDANT ACTIVITY OF ETHYL ACETATE AND CHLOROFORM FRACTION OF *IPOMOEA PURPUREA* FLOWERS

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ABSTRACT

Ipomoea purpurea L., a plant of the convolvulaceae family, popularly known as morning glory, possesses numerous medicinal values. The present study aimed to explore the antioxidant activity and ethyl acetate and chloroform bioactive compounds of *Ipomoea purpurea* flowers. Antioxidant activity was determined using 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) ABTS (2, 2'-azinobis (3ethylbenzthiazoline-6-sulphonic acid), ferric reducing antioxidant power (FRAP), nitric oxide scavenging assay (NO), reducing power, hydroxy radical scavenging assay, superoxide radical scavenging (SOD), hydrogen peroxide radical assay, metal chelating activity as well as phosphomolypdenum and standard ascorbic acid (AA) assay. Based on the findings of this investigation, we can conclude that *Ipomoea purpurea* extract possesses various bioactive compounds and moderate antioxidant potentials, which may be a path to the discovery of traditional medicines and remedies for many critical diseases.

KEYWORDS

Antioxidant activity, DPPH, *Ipomoea purpurea* and Phytochemical screening.

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INTRODUCTION

The role of antioxidants is important in fixing the conditions such as heart, cancer, chronic inflammation and Alzheimer's diseases which arose due to the oxidative cell damage. Inflammation alone may initiate variety of diseases including vasculitis, lupus erythematous, glomerulonephritis, adult respiratory diseases syndrome, and arthritis. Oxidative stress or cell damage may also involves in many other kinds of chronic diseases such as ischemic diseases (stroke, heart diseases, intestinal

ischema), acquired immunodeficiency syndrome (AIDS), hemochromatosis, hypertension emphysema, gastric ulcers, organ transplantation, preeclampsia, neurological disorder (muscular dystrophy, Alzheimer's disease, Parkinson's disease), smoking-related diseases, and alcoholism[1]. Several edible plants play a vital role in different ethnic groups by providing fresh food, nutrition, and medicines. Various researches have been conducted to record ethnobotanical knowledge of wild edible plant species[2]. Many plant species have demonstrated significant pharmacological activities in animal and human studies, such as antioxidant, cytotoxic, anti-inflammatory, antiproliferative, analgesic, immunomodulatory, antimicrobial, hepatoprotective, hypoglycemic, hypotensive, hypolipidemic, diuretic, etc[2-5]. In addition, these plants have abundant flavonoids, alkaloids, phenols, saponins, anthraquinone, tannins, cardiac glycosides, steroids, etc. These bioactive compounds and therapeutic actions. *Ipomoea purpurea* L. (Convolvulaceae), known as morning glory, is an annual twiner with a thin and frail stem[6]. It is typically found in the untamed areas of the Chattogram Hill Tracts in Bangladesh. According to India's indigenous medicine system, it belongs to anti-psychotic, antioxidant, anti-cancer, antimicrobial, oxytocic, and anti-inflammatory activities. However, no ethnopharmacological study has been conducted on its flowers. Therefore, the present study aimed to explore the antioxidant activity and bioactive compounds of *Ipomoea purpurea*.

MATERIAL AND METHODS

Plant material- Identification and authentication

Ipomoea purpurea flower was selectively removed from the plant in and around areas of Pudussery, Palakkad, Kerala and identified by a plant taxonomist. BSI/SRC/5/23/2022/Tech/628.

Preparation of *Ipomoea purpurea* flower extract

Ipomoea purpurea flower was washed, dried in a hot air oven at 40°C and subsequently ground into powder in an electric grinder. Delipidation was performed with ethyl acetate and chloroform soxhalation was performed with 95% chloroform and ethyl acetate was evaporated in a rotary evaporator at 40-50°C under reduced pressure. The yield of the flower extract was around 13.5 % of dry weight.

Free Radical Scavenging Assays

The *in vitro* anti radical scavenging potential *Ipomoea purpurea* flower extract (100-500µg/ml) was determined using DPPH[7], ABTS[8], FRAP[9], Nitric oxide[10], Reducing power[11], hydroxy radical[12] superoxide scavenging[13], hydrogen peroxide[14], metal chelating activity as well as phosphomolypdenum assay[15,16].

Statistical analysis

All the assays were carried out in triplicate. Experimental results are expressed as mean ± standard deviation. The results were analyzed using one-way analysis of variance and the group means were compared using Duncan's multiple range tests using SPSS version 16.

RESULTS AND DISCUSSION

Figure No.1 and Figure No.2 shows the effect *Ipomoea purpurea* flower extract ethyl acetate and chloroform on the DPPH and ABTS radicals present in the reaction mixtures. The extract at a concentration of 100 -500µg/ml, significantly scavenged of DPPH radicals with an IC_{50} value of 8.4,6.9µg/ml and ABTS radicals having IC_{50} values of 13.4, 10.5µg/ml.

Figure No.3 and Figure No.4 shows the effect of the FRAP power of the *Ipomoea purpurea* flower extract ethyl acetate and chloroform with the increasing concentration was 17.2,13.2µg/ml. The scavenging of nitric oxide by *Ipomoea purpurea* was increased concentration of 19.5, 15.6µg/ml of *Ipomoea purpurea* 50% of nitric oxide generated by incubation was scavenged.

Figure No.5 shows the effect the reducing power *Ipomoea purpurea* flower extract ethyl acetate and chloroform was increased in quantity of sample. The IC_{50} value of *Ipomoea purpurea* was 20.4, 18.7µg/ml respectively.

The results for hydroxyl scavenging assay are shown in Figure No.6. The concentrations for

inhibition were found to be 36.2, 30.5µg/ml for the *Ipomoea purpurea* respectively.

Figure No.7 and Figure No.8 shows the effect of the superoxide scavenging activity of *Ipomoea purpurea* flower extract ethyl acetate and chloroform showed superoxide scavenging activity (IC_{50}= 41.6, 34.9µg/ml), *Ipomoea purpurea* showed concentration dependent activity and the H_2O_2 scavenging effect at a concentration was 34.2, 31.8µg/ml.

Figure No.9 and Figure No.10 shows the effect of the metal chelating activity and phosphomolybdenum reduction of *Ipomoea purpurea* flower extract ethyl acetate and chloroform to the quantity of the sample. The IC_{50} value of *Ipomoea purpurea* was 57.4, 48.9µg/ml and 52.5, 45.2µg/ml.

Discussion

The antioxidant property of plant confers their free radical scavenging potential their bioactive components and to understand the mechanism of action of their phytoconstituents[17]. In the present study, *Ipomoea purpurea* flower extracts scavenge DPPH and ABTS radicals in a concentration dependent manner. The amount of DPPH which is reduced may be estimated by observing a decrease in absorbance at 517nm. ABTS assay involves reduction of the color intensity of ethanolic solution containing pre-formed radical monocation of ABTS which is generated by oxidation of ABTS with potassium per sulfate due to the radical scavenging activity of anti-oxidants present in the plants[18]. The change in intensity of the color is directly proportional to the antioxidant efficiency of the *Ipomoea purpurea* flower extract at a concentration of 100- 500µg/ml, the extract significantly scavenged of DPPH radicals (IC_{50}= of 8.4,6.9µg/ml), ABTS radicals (IC_{50}=13.4, 10.5µg/ml).

Some previous studies have also reported that the reducing power may serve as a significant indicator of potential antioxidant activity[19]. In this study, we used a FRAP assay because it is quick and simple to perform, and the reaction is reproducible and linearly related to the molar concentration of the antioxidant and FRAP assay was used by several authors for the assessment of antioxidant activity of various food product samples[21,22]. The reducing power of the *Ipomoea purpurea* increases with the increasing concentration 17.2,13.2µg/ml.

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological process including neurotransmission, vascular homeostasis, antimicrobial and antitumor activities. However, excess production of NO is associated with several diseases[23] inhibited nitrite formation in a concentration dependent manner (100-500µg/ml). This may be due to the presence of antioxidant principles in the *Ipomoea purpurea* which complete with oxygen to react with nitric oxide. The scavenging of nitric oxide 19.5, 15.6µg/ml of *Ipomoea purpurea* of nitric oxide generated by incubation was scavenged.

The reducing power of the *Ipomoea purpurea* was evaluated by the transformation of Fe^{3+} to Fe^{2+} through electron transfer ability, which serves as a significant indicator of its antioxidant activity. Reductions are also reported to react with certain precursors of peroxide, thus preventing peroxide formation[24]. The presence of antioxidant substances in the compound samples causes the reduction of the Fe^{3+} ferric cyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700nm. The IC_{50} value of *Ipomoea purpurea* was 20.4, 18.7µg/ml.

Hydroxyl radical scavenging capacity of *Ipomoea purpurea* is directly related to its antioxidant activity[25]. This method involves *in vitro* generation of hydroxyl radicals using Fe^{3+} /ascorbate/EDTA/ H_2O_2 system using Fenton reaction. The concentrations for inhibition were found to be 36.2, 30.5µg/ml for the *Ipomoea purpurea* respectively.

Superoxide radicals generated *in vitro* by the system was determined by the NBT photo reduction method. The decrease of absorbance at 560nm with the plant extract indicates the consumption of superoxide anion in the reaction mixture[26]. *Ipomoea purpurea* flower extract exhibited a maximum of

superoxide scavenging activity (IC_{50}=41.6, 34.9µg/ml).

Hydrogen peroxide is a weak oxidizing agent that inhibits the oxidation of essential thiol (-SH) groups directed by few enzymes. It can probably react with Fe^{2+} and possible Cu^{2+} ions to form hydroxyl radicals[27]. From the results, *Ipomoea purpurea* showed concentration dependent activity and the H_2O_2 scavenging effect at a concentration was 34.2, 31.8µg/ml.

Iron is an essential mineral for normal physiology, but an excess of it, may result in cellular injury[28]. The chelating ability of ferrous ions by the *Ipomoea purpurea* was estimated by the method.

Ferrozine can quantitively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. The metal chelating activity of *Ipomoea purpurea* is present 57.4, 48.9µg/ml.

The phosphomolybdenum method is based on the reduction of M_0 (VI) to M_0 (V) by the antioxidant compounds and the formation of green phosphate/M_0 (V) complex with the maximal absorption at 695nm. The IC_{50} value of *Ipomoea purpurea* was 52.5, 45.2µg/ml.

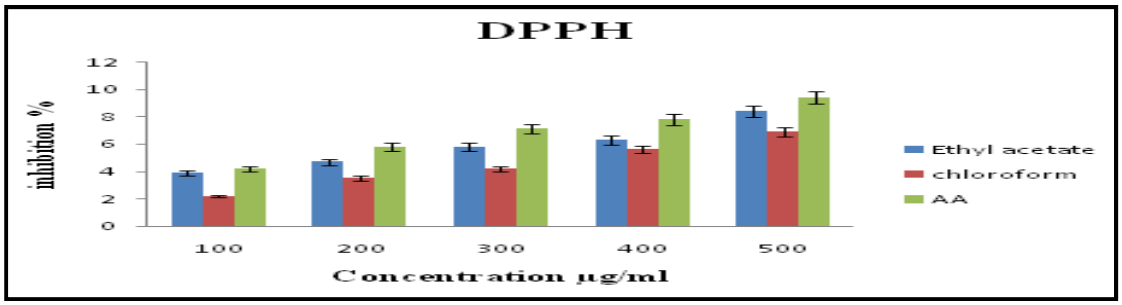

Figure No.1: Shows the DPPH effect of ethyl acetate and chloroform flower extract of *Ipomoea purpurea* flower extract

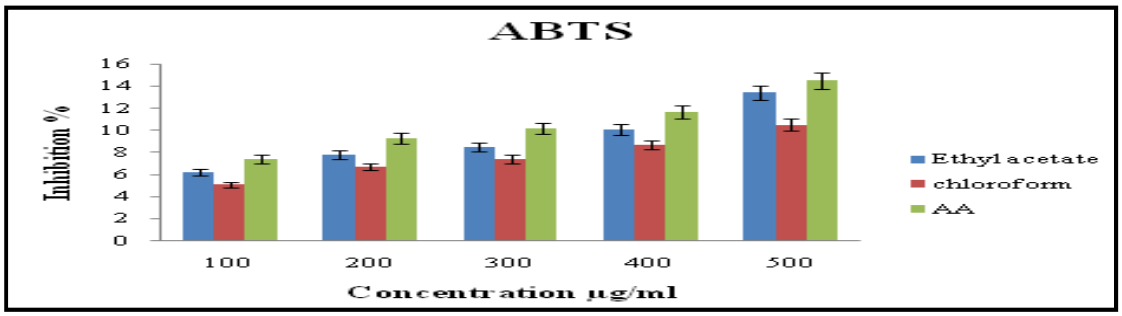

Figure No.2: Shows the ABTS effect of ethyl acetate and chloroform flower extract of *Ipomoea purpurea* flower extract

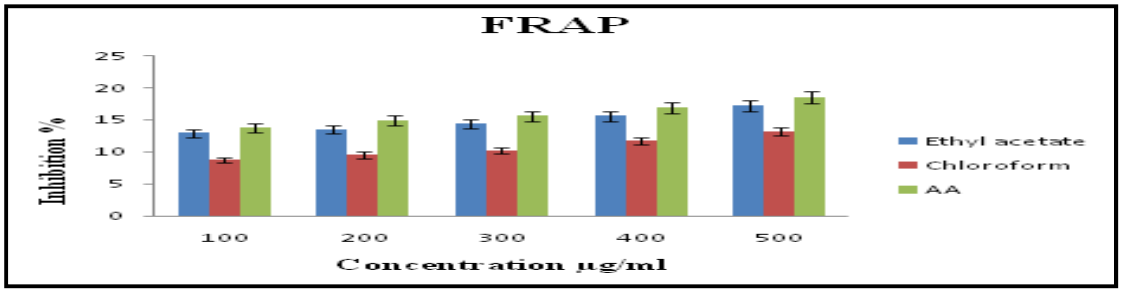

Figure No.3: Shows the FRAP effect of ethyl acetate and chloroform flower extract of *Ipomoea purpurea* flower extract

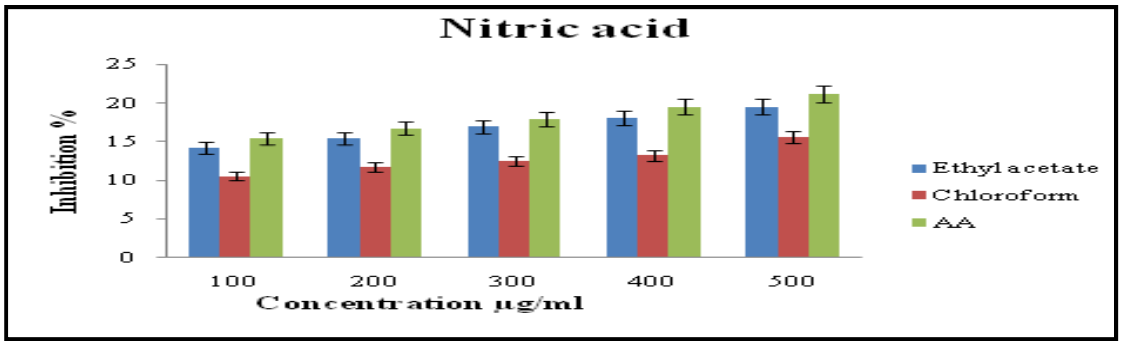

Figure No.4: Shows the Nitric oxide effect of ethyl acetate and chloroform flower extract of *Ipomoea purpurea* flower extract

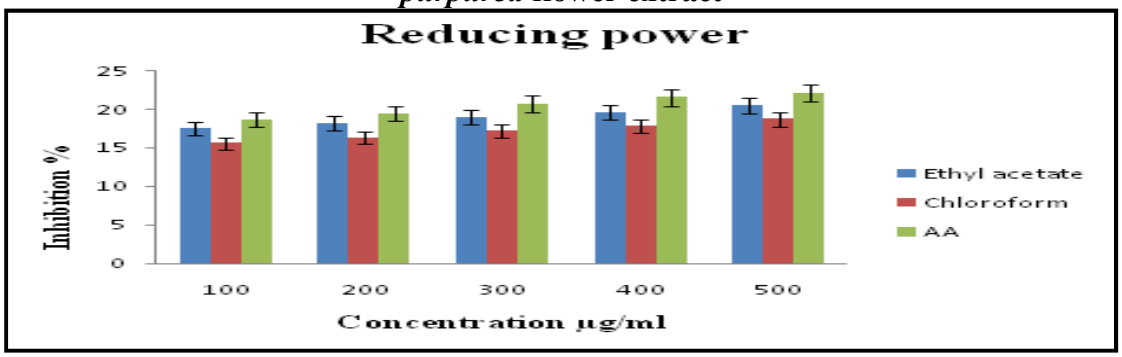

Figure No.5: Shows the Reducing power effect of ethyl acetate and chloroform flower extract of *Ipomoea purpurea* flower extract

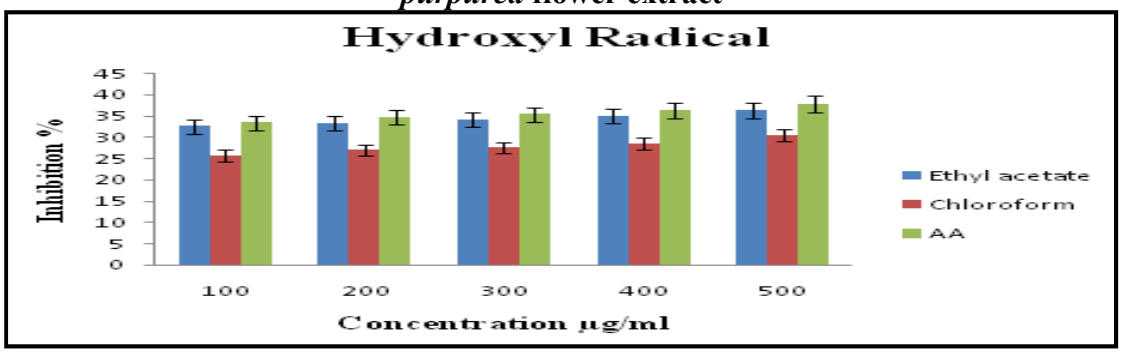

Figure No.6: Shows the Hydroxyl radical effect of ethyl acetate and chloroform flower extract of *Ipomoea purpurea* flower extract

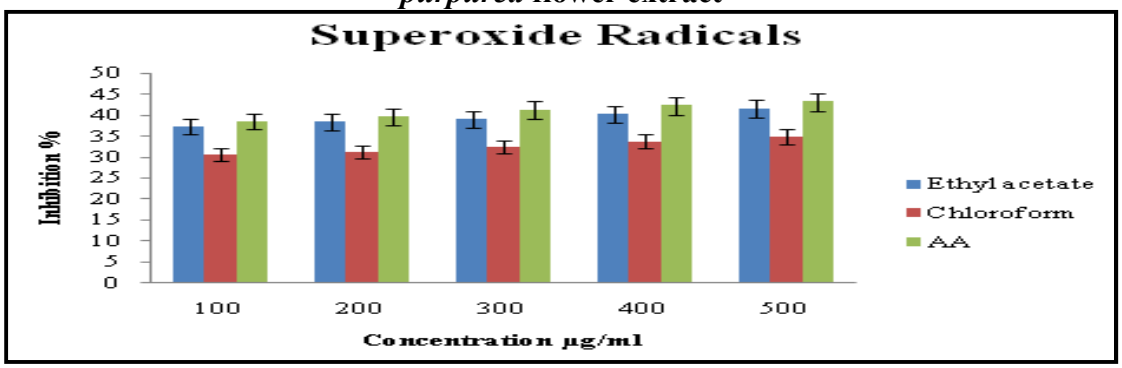

Figure No.7: Shows the superoxide radical effect of ethyl acetate and chloroform flower extract of *Ipomoea purpurea* flower extract

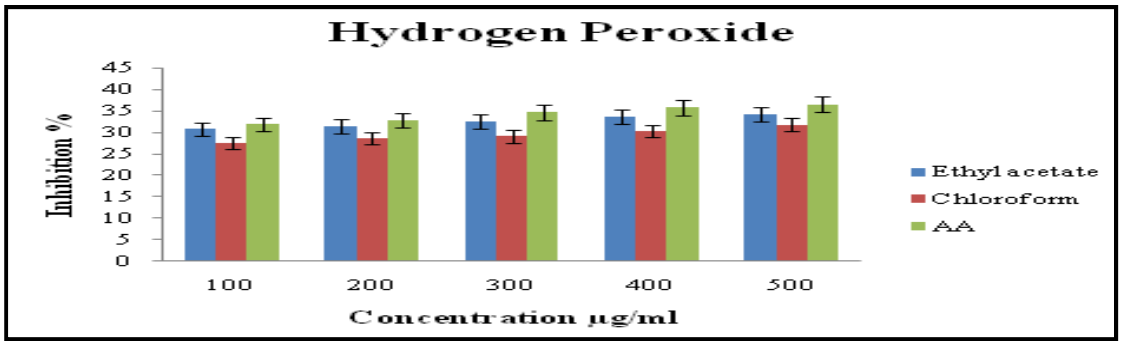

Figure No.8: Shows the hydrogen peroide effect of ethyl acetate and chloroform flower extract of *Ipomoea purpurea* flower extract

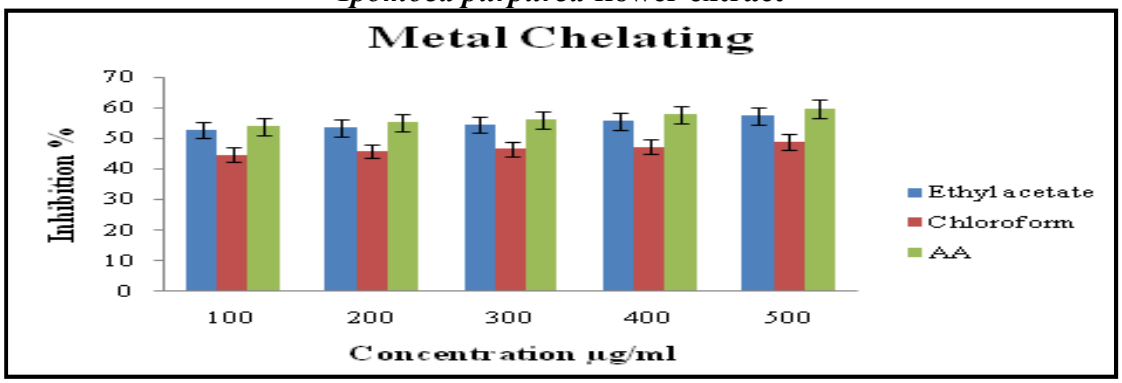

Figure No.9: Shows the Metal chelating effect of ethyl acetate and chloroform flower extract of *Ipomoea purpurea* flower extract

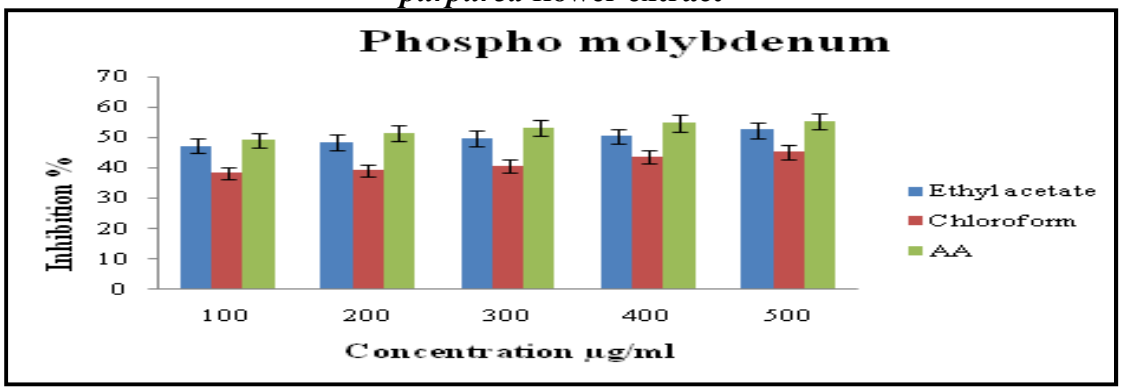

Figure No.10: Shows the Phospho molybdenum effect of ethyl acetate and chloroform flower extract of *Ipomoea purpurea* flower extract

CONCLUSION

Ipomoea purpurea species contain flavonoids and phenolic compounds which act as the primary anti-oxidants or free- radical scavengers. The presence of these compounds could be attributed to the potent anti-oxidant activity useful for the formulation of analgesic and anti-arthritic preparations.

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CONFLICT OF INTEREST

There is no conflict of interest among all authors in this study.

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