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ANTIOXIDANT ACTIVITY OF ETHANOL AND CHLOROFORM FRACTION OF *RUELLIA PROSTRATE* FLOWERS

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ABSTRACT

Ruellia prostrata belongs to Acanthaceae family and are perennial creepers with widespread medicinal uses, including analgesic and anti-inflammatory activity. The objective of this study was to determine anti-oxidant activity of ethanol and chloroform extracts from flowers parts of *Ruellia* species viz. *Ruellia prostrate*. The presence of these compounds could attribute to the potent anti-oxidant activity of *Ruellia prostrata* extracts. From this study, it was concluded that *Ruellia prostrata* could have anti-inflammatory activity. The antioxidant nature of the flower extract was proved from Free radical scavenging was determined by 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 assay. From the results obtained, *Ruellia prostrate flower* extract can be considered as a therapeutic agent for the treatment of free radical mediated diseases.

KEYWORDS

Ruellia prostrate, Phytochemicals, Free radicals and Antioxidant property.

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INTRODUCTION

India is widely known as the botanical garden of the world since it is the largest producer of medicinal herbs[1]. Medicinal plants are the richest bio-resource on drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs[2]. The use of medicinal plants as a source of relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east,

but it is doubtless an art as old as mankind[3]. Herbal medicine has been practiced worldwide and is now recognized by WHO as an essential building block for primary health care[4]. Although, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ) have been widely applied in food processing, they have been reassessed for their possible toxic and carcinogenic components formed during degradation[5]. In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and incidence of a number of human diseases[6,7]. *Ruellia prostrate* (Acanthaceae) is a perennial herb, commonly found as a weed throughout India. Reviewing the previous work of the *Ruellia prostrata* was found to have very little chemical and biological studies. This plant is widely distributed throughout India from extending to east Africa, central and peninsular India[8]. Anti-oxidants from plant materials play a significant role in termination of these free radicals, thereby protecting the body from these diseases. Herb is mainly known for its traditional use as an anti-inflammatory and anti-cancer against the epidermis of nasopharynx region and possesses wound healing properties[9,10]. Arthritis is an inflammatory disorder which manifests by destructive arthropathy and extra-articular manifestations, leading to severe disability and premature mortality[11]. The present study aimed at screening the phytoconstituents and anti-oxidant potential of Kenyan *Ruellia prostrata* to make this plant useful for the formulation of analgesic and anti-arthritic drug for the management of arthritis. *Rule prostate* is an indigenous medicinal plant, which present in moist, shady places throughout India. It is widely distributed in Arica, Srilanka, Pakistan and throughout India[12]. The plant is commonly known as bell weed and black weed[13,14]. The objective of this study was therefore to investigate the flower extracts of the *Rullia prostrata* for antioxidant activities as well as to determine the phytochemical contents.

MATERIAL AND METHODS

Plant material- Identification and authentication

Rullia prostrate 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/96.

Preparation of *Rullia prostrate* flower extract

Rullia prostrate 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 ethanol and chloroform (60-80°C) for overnight. Soxhalation was performed with 95% ethanol. Ethanol 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 *Rullia prostrate* flower extract (100-500µg/ml) was determined using DPPH[15], ABTS[16], FRAP[17], Nitric oxide[18], Reducing power[19], hydroxy radical[20] superoxide scavenging[21], hydrogen peroxide[22], metal chelating activity as well as phosphomolypdenum assay[23,s24].

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 test using SPSS version 16.

RESULTS AND DISCUSSION

Figure No.1 and Figure No.2 shows the effect *Rullia prostrate* flower extract ethanol 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 10.7, 6.8µg/ml and ABTS radicals having IC_{50} values of 8.2, 7.4µg/ml.

Figure No.3 and FigureNo.4 shows the effect of the FRAP power of the *Rullia prostrate* flower extract ethanol and chloroform with the increasing concentration was 13.1,12.4µg/ml. The scavenging

of nitric oxide by *Rullia prostrate* was increased concentration of 18.2, 16.2µg/ml of *Rullia prostrate* 50% of nitric oxide generated by incubation was scavenged.

Figure No.5 shows the effect the reducing power *Rullia prostrate* flower extract ethanol and chloroform was increased in quantity of sample. The IC_{50} value of *Rullia prostrate* was 16.5, 14.3µg/ml respectively.

The results for hydroxyl scavenging assay are shown in Figure No.6. The concentrations for inhibition were found to be 27.6, 24.3µg/ml for the *Rullia prostrate* respectively.

Figure No.7 and Figure No.8 shows the effect of the superoxide scavenging activity of *Rullia prostrate* flower extract ethanol and chloroform showed superoxide scavenging activity (IC_{50}= 42.4, 40.8µg/ml), *Rullia prostrate* showed concentration dependent activity and the H_2O_2 scavenging effect at a concentration was 66.7, 64.2µg/ml.

Figure No.9 and Figure No.10 shows the effect of the metal chelating activity and phosphomolybdenum reduction of *rule prostrate* flower extract ethanol and chloroform to the quantity of the sample. The IC_{50} value of *Rullia prostrate* was 38.4, 36.2µg/ml and 67.7,66.2µg/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[25]. In the present study, *Rullia prostrate* 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 persulfate due to the radical scavenging activity of anti-oxidants present in the plants[26]. The change in intensity of the color is directly proportional to the antioxidant efficiency of the *Rullia prostrate* flower extract at a concentration of 100-500µg/ml, the extract significantly scavenged of DPPH radicals (IC_{50}= of 10.7, 6.8µg/ml, ABTS radicals (IC_{50}=8.2, 7.4µg/ml). Some previous studies have also reported that the reducing power may serve as a significant indicator of potential antioxidant activity[27]. 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[28]. The reducing power of the *Rullia prostrate* increases with the increasing concentration 13.1, 12.4µg/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. *Rullia prostrate* inhibited nitrite formation in a concentration dependent manner (100-500µg/ml). This may be due to the presence of antioxidant principles in the *Rullia prostrate* which complete with oxygen to react with nitric oxide. The scavenging of nitric oxide 18.2, 16.2µg/ml of *Rullia prostrate* of nitric oxide generated by incubation was scavenged.

The reducing power of the *Rullia prostrate* 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. 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 *Rullia prostrate* was 16.5, 14.3µg/ml.

Hydroxyl radical scavenging capacity of *Rullia prostrate* is directly related to its antioxidant activity. 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 27.6, 24.3µg/ml for the *Rullia prostrate* 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. *Rullia prostrate* flower extract exhibited a maximum of superoxide scavenging activity (IC_{50}= 42.4, 40.8µg/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. From the results, *Rullia prostrate* showed concentration dependent activity and the H_2O_2 scavenging effect at a concentration was 66.7, 64.2µg/ml.

Iron is an essential mineral for normal physiology, but an excess of it, may result in cellular injury. The chelating ability of ferrous ions by the *Rullia prostrate* 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 *Rullia prostrate* is present 42.4µg/ml and 40.6µg/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 *Rullia prostrate* was 38.4, 36.2µg/ml and 67.7,66.2µg/ml.

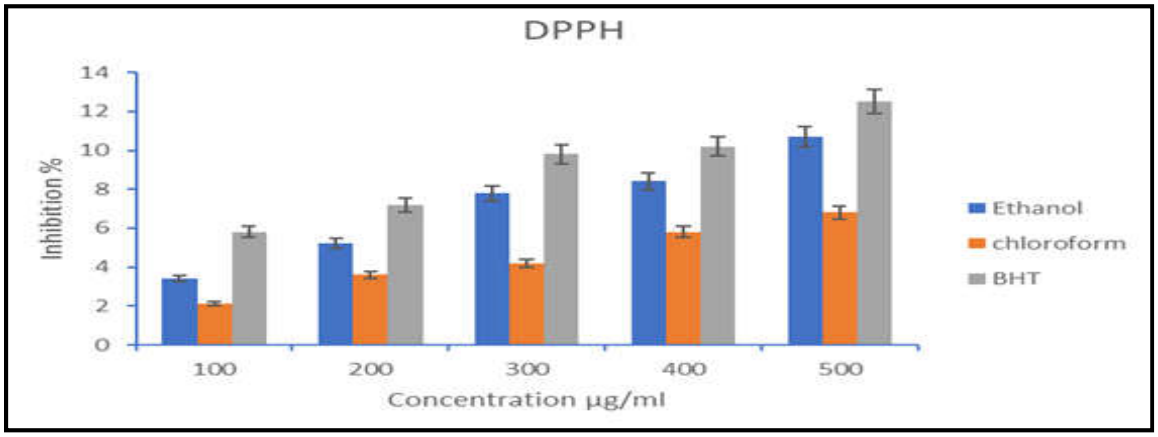

Figure No.1: Shows the DPPH effect of ethanol and chloroform flower extract of *Rullia prostrate* flower extract

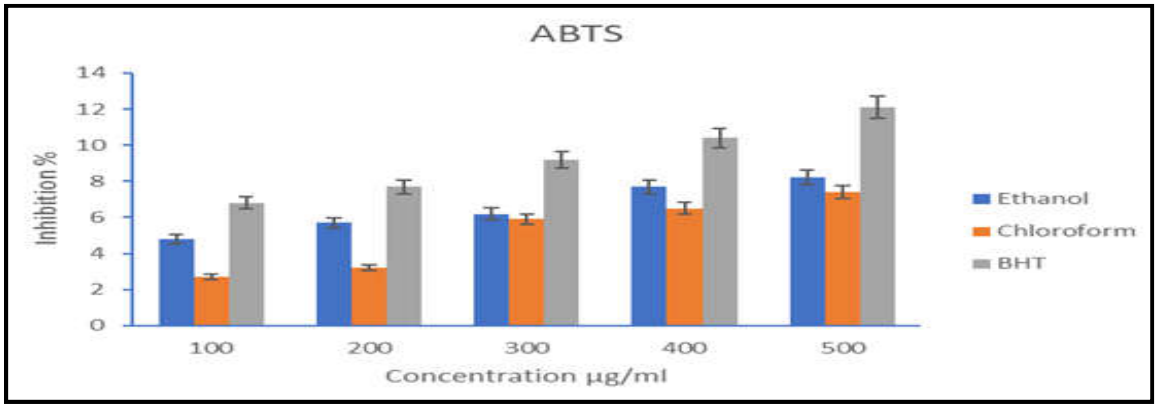

Figure No.2: Shows the ABTS effect of ethanol and chloroform flower extract of *Rullia prostrate* flower extract

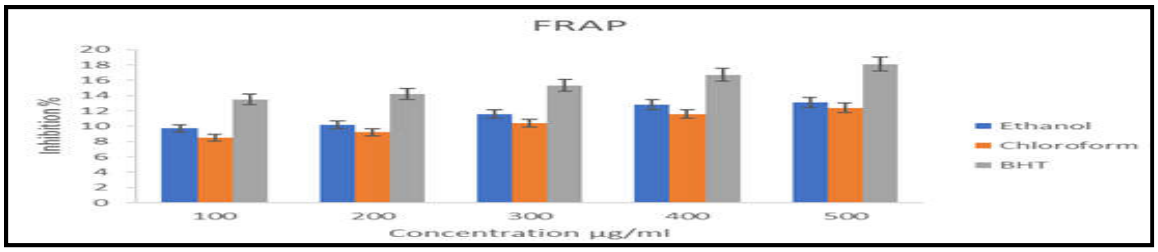

Figure No.3: Shows the FRAP effect of ethanol and chloroform flower extract of *Rullia prostrate* flower extract

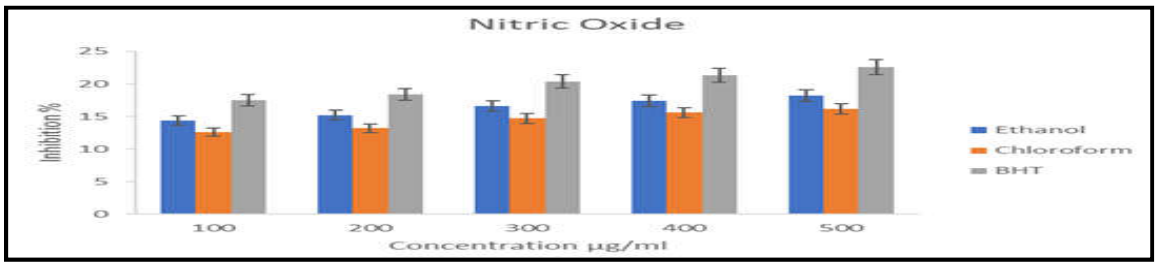

Figure No.4: Shows the Nitric oxide effect of ethanol and chloroform flower extract of *Rullia prostrate* flower extract

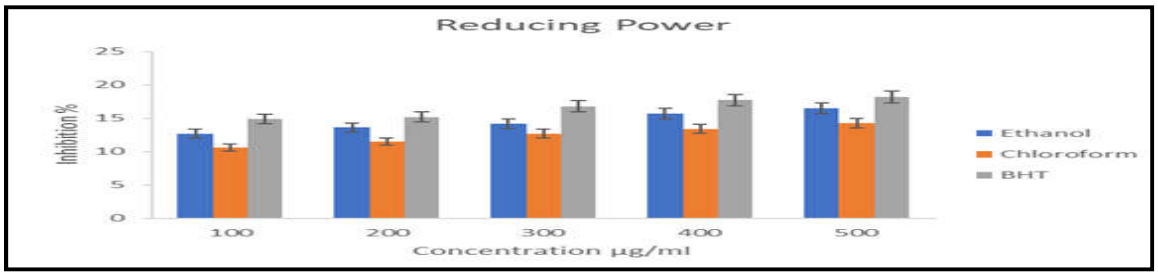

Figure No.5: Shows the Reducing power effect of ethanol and chloroform flower extract of *Rullia prostrate* flower extract

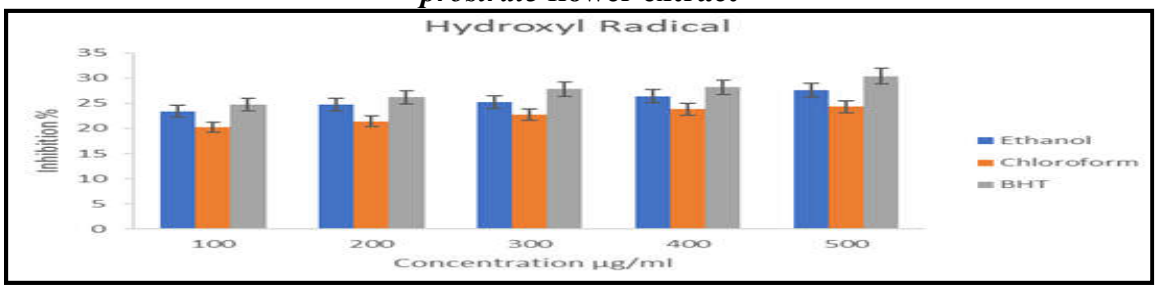

Figure No.6: Shows the Hydroxyl radical effect of ethanol and chloroform flower extract of *Rullia prostrate* flower extract

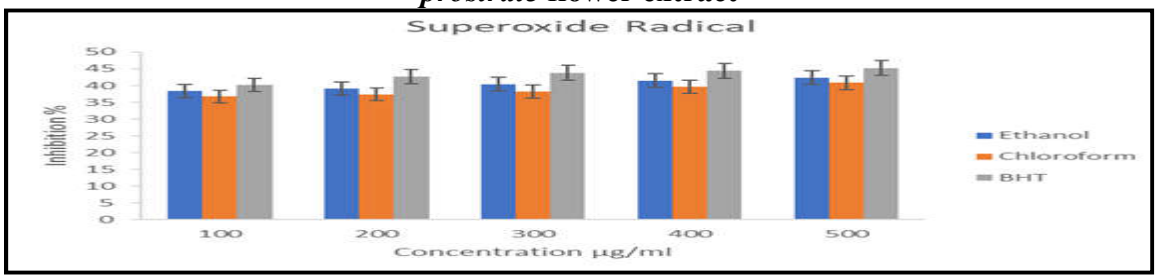

Figure No.7: Shows the superoxide radical effect of ethanol and chloroform flower extract of *Rullia prostrate* flower extract

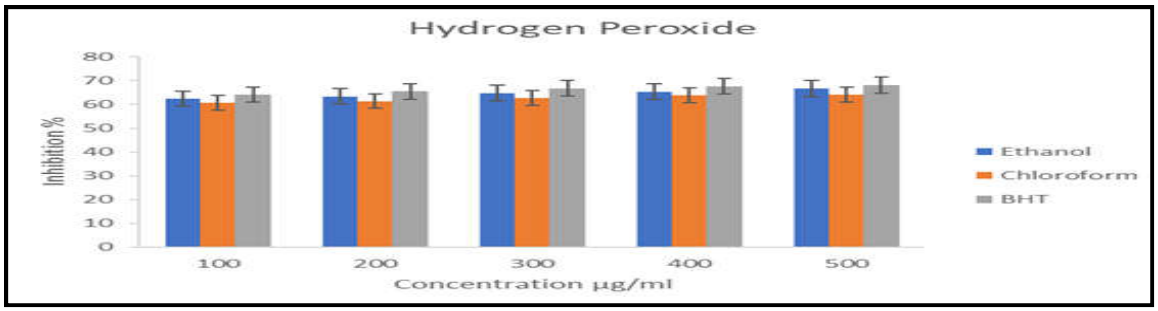

Figure No.8: Shows the hydrogen peroide effect of ethanol and chloroform flower extract of *Rullia prostrate* flower extract

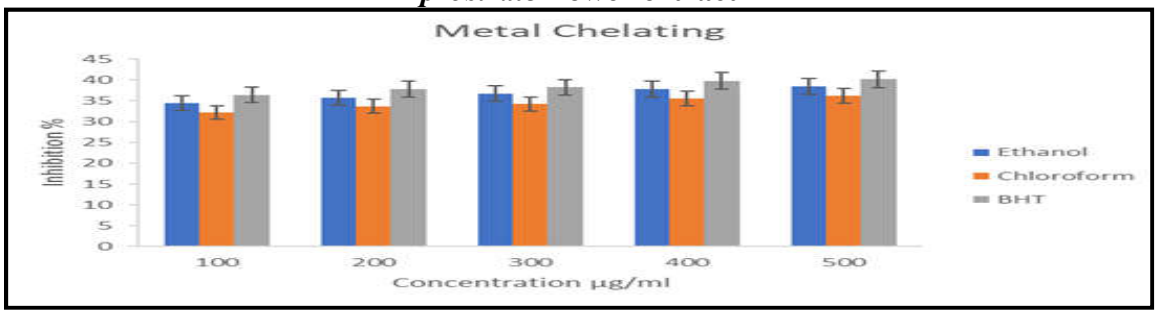

Figure No.9: Shows the Metal chelating effect of ethanol and chloroform flower extract of *Rullia prostrate* flower extract

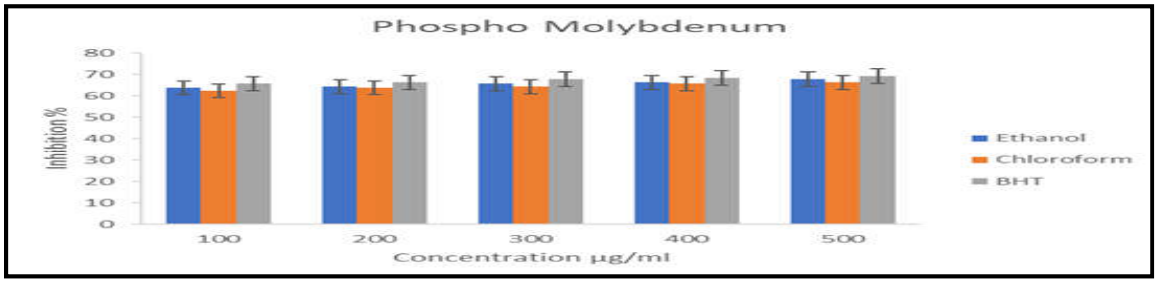

Figure No.10: Shows the Phospho molybdenum effect of ethanol and chloroform flower extract of *Rullia prostrate* flower extract

CONCLUSION

Ruellia prostrate 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 of this study.

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