JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

PHYTOCHEMICAL ANALYSIS AND FREE RADICAL SCAVENGING ACTIVITY OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF *Barleria lupulina Lindl*

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ABSTRACT:

The aim of the current study was to determine the preliminary phytochemical analysis and free radicals scavenging activity of Aqueous and ethanolic leaf extracts of *Barleria lupulina Lindl* . Aqueous and ethanolic leaf extracts of *Barleria lupulina Lindl* was used to find out the phytoconstituents and to evaluate the antioxidant activity against various free radicals. Phytochemical analysis reported that the presence of Flavonoids, Alkaloids, Tannin, Phenol, Saponins ,Protein and Sterol were highly found in ethanolic leaf extract.. The findings of the recent study suggest that ethanolic leaf extract of plant has high antioxidant activity against free radicals such as DPPH, Reducing power- FRAP , NO, H_2O_2 and ABTS. The IC_{50} value also calculated for the two extracts of plant drug and the result revealed that the AEBL & EEBL possess corresponding IC_{50} value with increased the percentage of inhibition of free radicals .

Keywords: *Barleria lupulina Lindl* , Phytochemicals, Free radicals – DPPH, FRAP,NO, H_2O_2 and ABTS, IC_{50} value .

INTRODUCTION

Natural herbal plant derived compounds have currently used in significant application for various diseases .Secondary metabolites are the natural bioactive compounds found in the medicinal plants.. These active compounds present in minute quantities in higher plants, include phenol, flavonoid, terpenoids, tannins, sterols, alkaloids etc[1]. Extraction mainly used pharmaceutically involves the separation of medicinally active principle of plant part from the inert compounds by using selective solvent components. During extraction methods, the solvent compounds diffuse into the solid plant material and solubilize compounds with similar polarity in nature [2].

Formation of free radicals during oxidative reduction reaction involving transfer of one electron, or when a covalent bond is affected and one electron from each pair remains with each group. Due to the influence of unpaired electrons , free radicals are considerably more active and involve in a number of pathophysiological functions such as includes cardiovascular disease ,atherosclerosis ,cancer, arthritis and diabetes [3]. The process of biological ageing might be due to the damage of cells by free radicals. Lipid peroxidation of cell membranes, and inactivate membrane bound enzymes are carried out by free radicals [4] . Antioxidant compounds which can reactive with free radicals and it can prevent the oxidation of other molecules and may therefore have health enhancing effects in the prevention of degenerative diseases [5]. It has been concluded that antioxidant activity of plants might be due to their phenolic compounds [6].

The recent studies on oxidative stress and its adverse effects on human health care system have become a problem of considerable interest [7]. The mechanism of oxidation is a natural metabolic reaction in cell, resulting in the formation of free radicals such as Hydrogen peroxide (H2O2) and Hypochlorous acid (HOCL) [8]. Generation of free radicals are mainly caused by Cigarette smoke, automobile exhaust, radiation, pesticides and air pollution. [9] . It plays a vital role in ageing, neurodegenerative disorders, , damage of cell membrane, DNA proteins and fats causing oxidative stress [10] . Recent studies have proved that plant based secondary metabolites phenols in medicinal and dietary plants can prevent the oxidative stress caused by Sunbeam and Oxygen. [11]

The number of natural medicinal plants are reported for free radical scavenging activity because of the presence of some important active principle such as Flavonoids , phenol, tannins, saponin , terpenoids etc [12] . The antioxidant potential of the plant and its compounds can be evaluated by using in vitro methods which involve the prevention of oxidation process and its damage by inhibiting of free radicals. and can be compared with synthetic antioxidant [13].

MATERIALS AND METHODS

Collection of Plant Material

The leaf part of plant *Barleria lupulina lindl* belongs to Acanthaceae family were collected from the surrounding area of Coimbatore. The collected plant was identified and authenticated by Botanical Survey of India (BSI) TNAU, Coimbatore and the voucher number is BSI/SRC/5/23/2017/Tech/1612.

Preparation of Plant Extract

The collected leaf part of *Barleria lupulina lindl* were cleaned, washed with water and reduced to coarse powder with a blender. 15 gm of powdered sample were extracted overnight with 150ml of water and ethanol separately in a shaker at room temperature for 48 hours to obtain aqueous and ethanolic extract. The respective extracts were filtered through Whatman No-1 paper, concentrated and dried under vacuum. The extracted samples filled in plastic bottle and stored at -20 C Until used.

Soxhlet Extraction

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance [14].

PHYTOCHEMICAL SCREENING

PRELIMINARY PHYTOCHEMICAL SCREENING

The Phytochemical Screening of the extracts was conducted using procedures described by Trease and Evans

DETECTION OF ALKALOIDS:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayers's Test:

Filtrates were treated with Mayers's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of Alkaloids.

Wagner's Test:

Filtrates were treated Wagner' reagent (Iodine in Potassium Iodide) Formation of a brown/ reddish precipitate indicates the presence of Alkaloids

Dragendroff' Test:

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's test:

Filtrates were treated with Dragendroff's reagent Hager's reagent (Saturated picric acid solution) presence of alkaloids confirmed by the formation of yellow coloured precipitate.

DETECTION OF CARBOHYDRATE:

Molisch's Test:

Filtrates were treated with 2 drops of alcoholic a- naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Benedict's Test:

Filtrates were treated with Benedict 's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test :

Filtrates were hydrolysed with dilute HCl, neutralized with alkali and heated with Fehling A & B solutions. Formation of red precipitate indicates the presence of reducing sugars

DETECTION OF SAPONINS:

Froth Test:

Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins.

Foam Test:

0.5 gm of extract was shaken with 2ml of water. If foam produced persists for ten minutes it indicates the presence of saponin.

DETECTION OF PHYTOSTEROLS:

Libermann Burchard's Test:

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride boiled and cooled. Concentration of Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

DETECTION OF PHENOLS:

Ferric Chloride Test:

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

DETECTION OF TANNINS:

Gelatin Test :

To the extract 1% gelatin solution containing sodium chloride was added. Formation of White precipitate indicates the presence of tannins.

DETECTION OF FLAVONOIDS:

Alkaline Reagent Test:

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test:

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

DETECTION OF PROTEINS:

Xanthoproteic Test:

The extracts were treated with few drops of conc,Nitric acid. Formation of yellow colour indicates the presence of proteins,

INVITRO ANTIOXIDANT ACTIVITY

DPPH radical Scavenging activity:

The antioxidant activity of aqueous and ethanolic extract of *Barleria lupulina Lindl* was evaluated by DPPH Scavenging assay [15] . For measuring the scavenging activity of DPPH free radicals about 0.1 mM solution of DPPH in ethanol was prepared and 1ml of this solution was added to 3ml of each extract at different concentration range (10,20,30,40,50µg/ml). After 30 minutes, the absorbance was measured at 517nm . The DPPH radical scavenging activity was measured and calculated using the following equation.

% radical scavenging activity= [(Ao- At) /Ao] X 100

Where Ao was the absorbance of the control and was the absorbance in the presence of the tested samples.

Reducing Power Assay- FRAP Assay:

The reducing power of the leaf extracts of *Barleria lupulina Lindl* was determined by Ferric reducing assay [16] **.** In brief, 1 ml of different concentration of extract (10,20,30,40,50µg/ml) in 1 ml of methanol were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of potassium ferricyanide ([K3Fe (CN)6, 1%].The tubes were incubated at 50 C for 20 minutes in water bath, cooled and 2.5 ml of trichloroacetic acid (10%) and 0.5 ml of 0.1% ferric Chloride (FeCl3) were added to each type .The absorbance of reaction mixtures was measured at 770nm .Increased absorbance of the reaction mixture on increasing the concentration of extracts indicated increased reducing power.

Nitric oxide radical scavenging activity:

The antioxidant activity *of* aqueous and ethanolic extract of *Barleria lupulina lindl* was evaluated by NO radical scavenging assay [17] . Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in (5 mM) in Phosphate buffer saline (PBS) was prepared and mixed with 3 ml of different concentration (10,20,30,40,50µg/ml µg/ml).of each extract and incubated at 25 C for 2 hr. After incubation, 1 ml of Greiss reagent (1% Sulphanilamide, 2% H3PO4 and 0.1% napthylethylenediamine dihydrochloride) was added to each sample and the absorbance was measured at 546 nm using UV Spectrophotometer .The percentage of inhibition was measured using following formula:

Hydrogen Peroxide radical scavenging activity:

The antioxidant activity of aqueous and ethanolic extract of *Barleria lupulina Lindl* to scavenge hydrogen peroxide was determined by the method of Ruch etal [18,19] . The stock solutions of EDTA (1 mM) ,FeCl3 (10 mM), ascorbic acid (1 mM), H202 (10 mM) and deoxyribose (10 mM) were prepared in distilled deionized water. The assay was performed by adding 0.1 ml EDTA , 0.01 ml of FeCl3, 0.1 ml of H2O2, O.36 ml of deoxyribose, 1 ml of each extract of different concentration ((µg/ml) was mixed with 0.33ml of phosphate buffer (50 mM,pH 7.4) and 0.1 ml of ascorbic acid in sequence. The mixture was incubated at 37 C for 1 hr, about 1 ml portion of each incubated mixture was taken and mixed with I ml of

10% TCA and 1.0 ml of 0.5% TBA (in 0.025 M NaOH containing in 0.025 M NaOH BHA) to develop the pink chromogen. The hydroxyl radical scavenging activity of each extract was determined as percentage of inhibition of deoxyribose degradation and was calculated using the following equation.

ABTS radical scavenging activity:

The efficacy of leaf extracts of AEBL & EEBL to scavenge free radicals was determined using ABTS radical scavenging assay with minor modification [20] . The ABTS radical was generated by mixing (7 mM).ABTS stock solution with 2.45 Mm . Potassium persulfate and the mixture was left in the dark for 12-16 hours at room temperature. The resulting solution was diluted with distilled water to an absorbance of 0.70 at 730 nm.1 ml of different concentrations of seed extracts ((10,20,30,40,50 µg/ml) were added to 4ml of ABTS solution in labelled tubes and the tubes were incubated for 30 minutes followed by measuring the absorbance at 730 nm.

RESULTS:

PHYTOCHEMICAL SCREENING OF DIFFERENT SOLVENT EXTRACTS OF *Barleria lupulina Lindl*

Table- 1

S.No	Active compounds	Hexane	Chloroform	Ethyl Acetate	Ethanol	Water
1	Alkaloids	+	+	-	++	+
2	Flavonoids	-	+	+	++	+
3	Phenol	+	-	+	++	+
4	Carbohydrate	-	-	-	+	-
5	Saponin	-	+	-	+	+
6	Protein	-	-	-	+	+
7	Tannin	+	-	+	+	+
8	Phytosterol	+	+	-	++	+

In the current study, phytochemical analysis of *Barleria lupulina lindl* revealed that the presence of major secondary metabolites found in the plant (Table -1) Aqueous and Ethanolic extract of *Barleria lupulina Lindl* showed that the presence of Phenol, Flavonoid, Alkaloids, Phytosterols ,Saponins and Proteins .

ASSESSMENT OF ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging activity:

The percentage of scavenging effect of DPPH on aqueous and ethanolic extracts of *Barleria lupulina Lindl* with different concentration of 10,20,30,40,50 µg/ml and found dose dependent inhibitory antioxidant potential.. The IC_{50} values of aqueous and ethanolic extract were found to be 28 µg/ml and 25 µg/ml respectively.

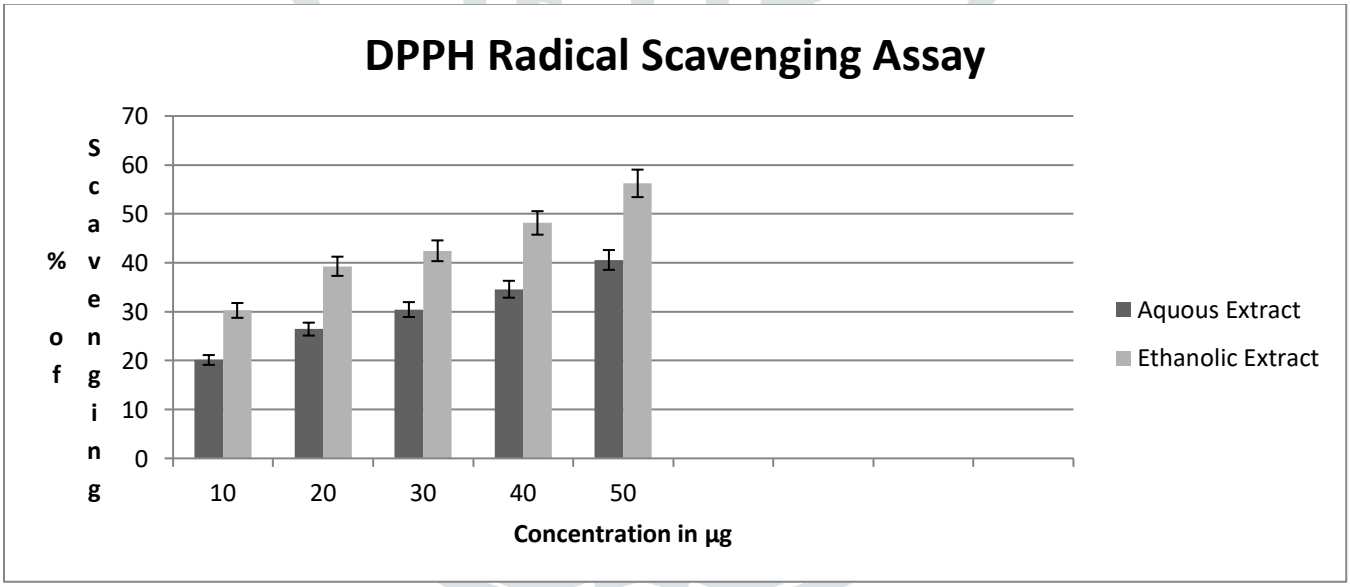

Figure-1

DPPH free radicals scavenging activity of AEBL & EEBL. All values were expressed in triplicate as mean ±SD

Reducing Power Assay – FRAP Assay:

The Reducing power assay showed that two extracts of *Barleria lupulina Lindl* from different concentration range (10,20,30,40,50 µg/ml) revealed that the percentage of reducing power assay in dose dependent manner. The IC_{50} values of aqueous and ethanolic extract were found to 44µg and 40 µg respectively.

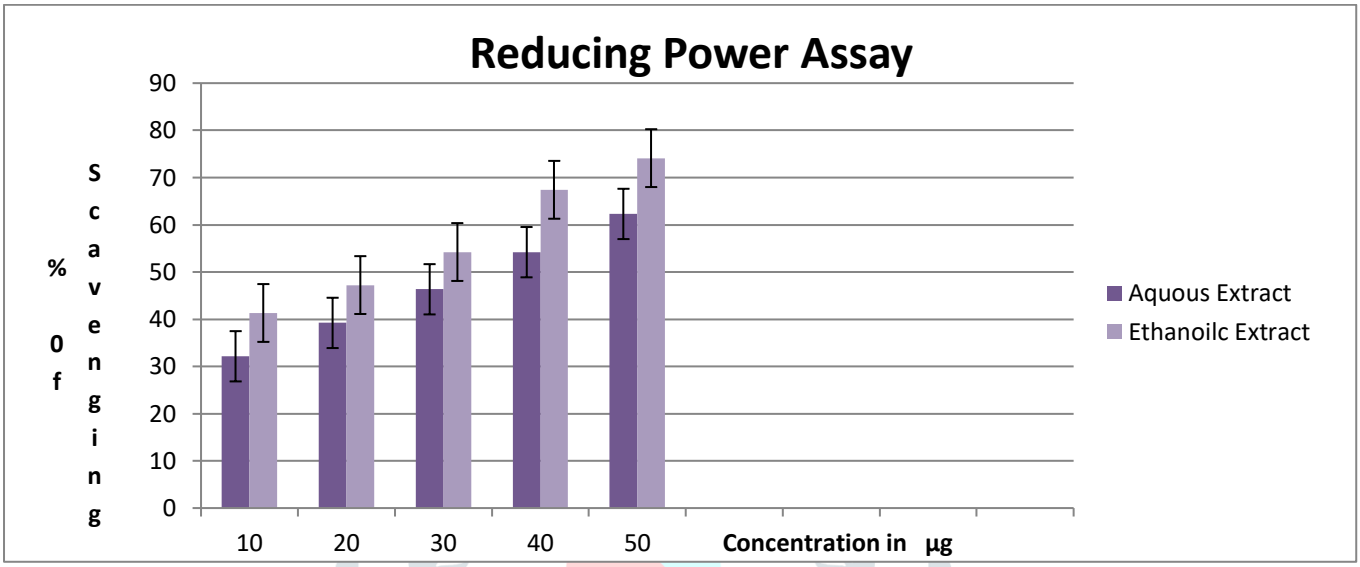

Figure-2

Reducing power activity of AEBL &EEBL . All values were expressed in triplicate as mean ±SD

Nitric Oxide Radical Scavenging Assay:

In this recent study ,the two different extracts of *Barleria lupulina Lindl were* checked for inhibitory effect of nitric oxide production and revealed that best result in the arresting of nitric oxide radical generation in a dose dependent manner. IC $_{50}$ values of aqueous and ethanolic extract were found to be 22 µg/ml.and 20 µg/ml respectively.

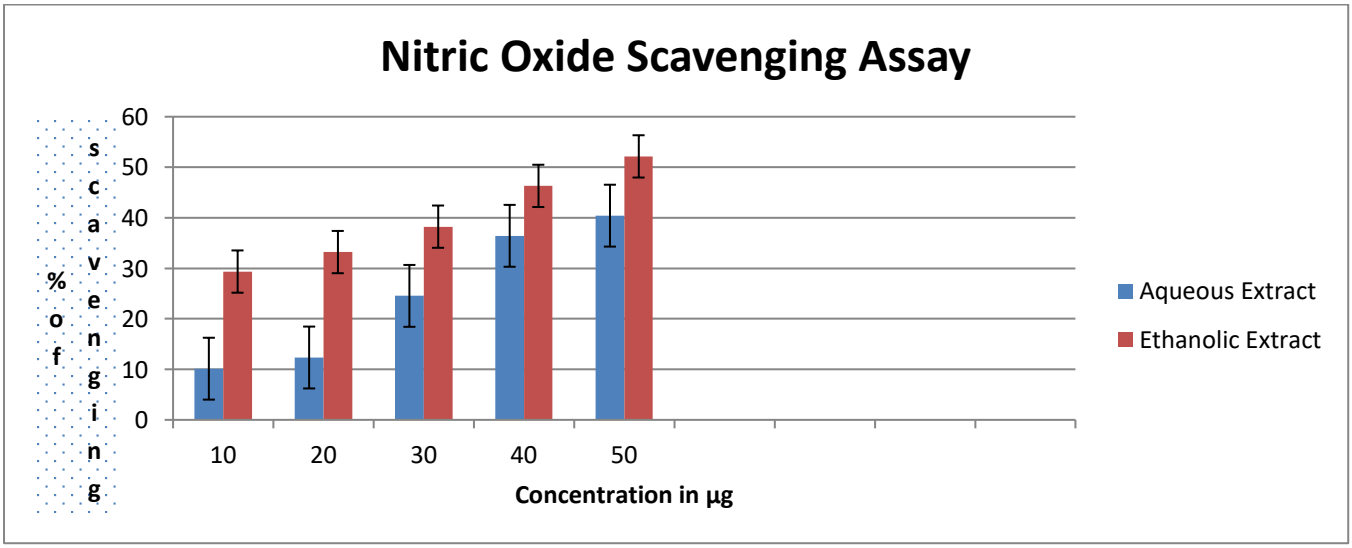

Figure-3- Nitric Oxide free radicals scavenging activity of AEBL & EEBL . All values were expressed in triplicate as mean ±SD

Hydrogen Peroxide Scavenging Assay:

The hydrogen peroxide radical scavenging activity revealed that two extracts of *Barleria lupulina lindl* from different concentration range 10,20,30,40,50 µg/ml showed the percentage of inhibition on hydroxyl radical activity in dose dependant. The IC_{50} values of aqueous and ethanolic extract were found to 42µg and 40µg .

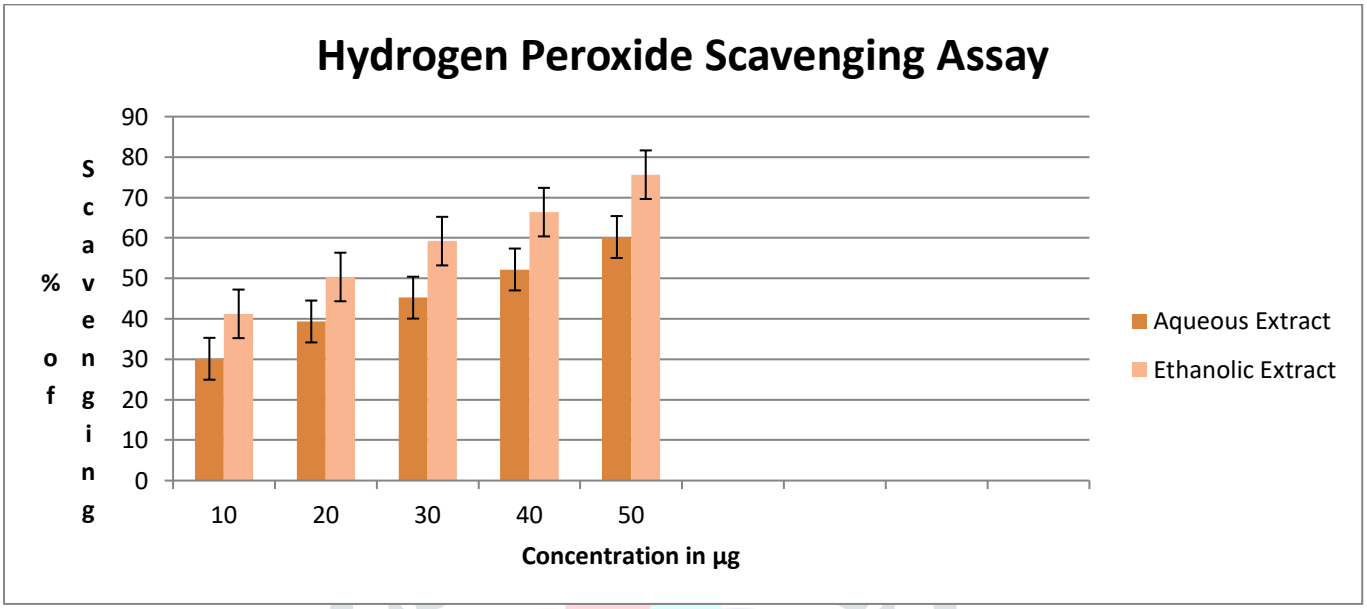

Figure-4

H_2O_2 free radicals scavenging activity of AEBL & EEBL . All values were expressed in triplicate as mean ±SD.

ABTS Radical Scavenging Assay:

The ABTS assay revealed that two extracts of *Barleria lupulina* from different concentration range 10,20,30,40,50 µg/ml showed that the percentage of ABTS assay in dose dependent manner. The IC_{50} values of aqueous and ethanolic extract were found to 47µg and 43 µg .

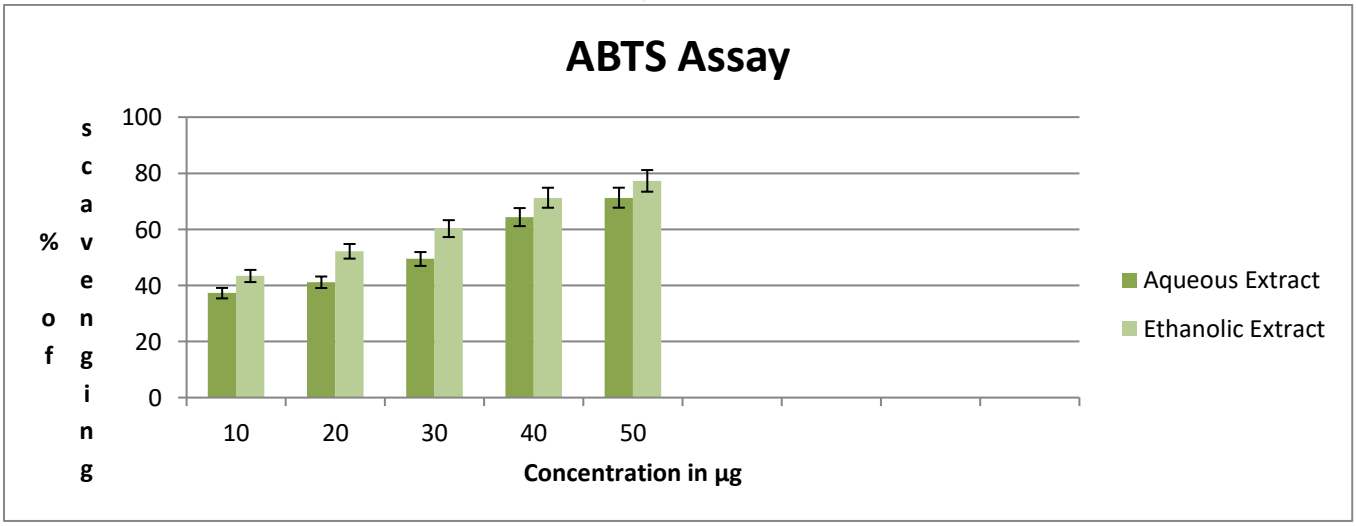

Figure-5

ABTS free radicals scavenging activity of . AEBL & EEBL . All values were expressed in triplicate as mean ±SD.

The Antioxidant activity results revealed that the aqueous and ethanolic extracts of *Barleria lupulina* have potent activities as evidenced by the IC_{50} values obtained in DPPH (28 µg/ml and 25 µg/ml) Reducing power assay (44 µg/ml and 40 µg/ml) Nitric oxide radical scavenging activity(22 µg/ml.and 20 µg/ml) H_2O_2 (42 µg/ml and 40 µg/ml) and ABTS (47 µg/ml and 43µg/ml). **(Fig 1-5)**

DISCUSSION

In the current study, phytochemical analysis of *Barleria lupulina* revealed that the presence of major secondary metabolites found in the plant **(Table -1**) Aqueous and Ethanolic extract of *Barleria lupulina* showed that the presence of phenol, flavonoids, alkaloids. Tannin, saponin and proteins Ethanolic extract found to be better than aqueous extract which may be due to the better solubility of the secondary metabolites found in organic solvents

In the present study , the free radical scavenging activity of AEBL & EEBL were determined by DPPH, ,Reducing power -FRAP, NO, H_2O_2 and ABTS assay. The result revealed that ethanolic extract exhibited good radical scavenging activity when compared to aqueous extract., Lipid peroxidation and enormous biological damage caused by hydroxyl free radicals which was scavenged by the antioxidant present in the plant extract.

CONCLUSION

Invitro antioxidant activity of the aqueous and ethanolic extract of *Barleria lupulina* was evaluated. The free radicals such as DPPH, Reducing power assay- FRAP NO, H_2O_2 and ABTS exhibited significant scavenging activity. This may be due to the presence of phenol, flavonoids, saponin, alkaloids, tannin, protein and phytosterol which was confirmed by preliminary phytochemical analysis . The result revealed that aqueous and ethanolic extract of *Barleria lupulina* having potential antioxidant activity and it can be considered for scavenging free radicals. I t can be benefited for prevention and treatment of various diseases. This plant drug has excellent antioxidant activity it can be selected for further research study based on cancer.

ACKNOWLEDGEMENT

We are highly grateful thank to the Management and Principal of Rathnavel Subramaniam College of Arts and Science (Autonomous),Sulur ,Coimbatore Tamil Nadu for providing us the infrastructure to carry out this research work. It is declared that there is no commercial or financial conflict of interest.

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