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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF ETHANOLIC AND METHANOLIC EXTRACTS OF *BARLERIA BUXIFOLIA* L.

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ABSTRACT: The present investigation was carried out to evaluate the antimicrobial and antioxidant potential of ethanolic and methanoic leaf extract of *Barleria buxifolia*. The antimicrobial activity was tested against four bacteria and two fungal. Among the four different bacteria tested, in the case of *Streptococcus pyogenes* the zone of inhibition is higher (18 mm) in 60µg/ml in ethnolic and methanolic concentration. Antifungal actvity was tested against two stains, in this *Candida albicans* the zone of inhibition was higher in 60µg/ml concentration (09 mm). The extract was effective on all the four bacteria and two fungal. The higher percentage of activity in DPPH was observed in 1000µg/ml (61.72%) followed by 800µg/ml (49.27%). More absorbance (0.54) was observed in the concentration of 1000µg/ml followed by (0.43) absorbance in 800µg/ml in methanolic concentration of FRAP activity. Antioxidant activity and reducing power of solvent extracts was found to be dose dependent manner.

Key words: Antimicrobial, Antifungal, Antioxidant, Inhibition, *Barleria buxifolia*,

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INTRODUCTION

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter (Grover and Yadav., 2002). The value of medicinal plants to the mankind is very well proven. It is estimated that 70% to 80% of the people worldwide rely chiefly on the traditional health care system and largely on herbal medicines (Shanley, 2003). India harbours about 15 percent (3000 - 3500) medicinal plants, out of 20 000 medicinal plants of the world. About 90 percent of these are found growing wild in different climatic regions of the country (Farombi, 2003). South Indian tribes are blessed with rich biological diversity of plants and a high degree of traditional knowledge about medicinal plants and their uses for various ailments of human being. The Kolli hill of Eastern Ghats lies in Tamil Nadu is well known for its rich biological diversity of plants particularly of medicinal and aromatic plants. It is also the traditional hill country, the friendly land of malayali tribes and a part of the erstwhile kingdom of Valvil Ori and having a total geographical area of 28,293 ha. Presently, the area is approximately 51% agricultural land, and 44% forest land (Abu-Rabia, 2005; Kumar, 2001).The usage of medicinal plants is increasing worldwide. According to the World Health organization (WHO), approximately 80percent of the world's population currently uses herbal medicines directly as daily drinks, or extract with easily accessible liquids such as water etc. (Julsing Matthys *et al* 2007).

Over 50 percent of all modern clinical drugs are synthesized from natural product origin (Stuffness m, Douros J 1982). Natural products play in important role in drug development in the pharmaceutical industry (Baker *et al* 1995). There are many reports on the use of medicinal plants in traditionally used by either tribal people or indigenous population (Ignacimuthu *et al* 1998). A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties. Antioxidants are those substances which possess free radical chain reaction breaking properties. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems (Halliwell, 1994).

Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidences suggest that antioxidant reduces the risk of chronic diseases, including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risks (Hamasaki, 2006).

In spite of the rapid developments in scientific technology and the better understanding of the chemistry of natural products, only few plants used in different traditional systems of medicine, a few hundred have so far been examined in depth for their chemical constituents and its activity. The plant kingdom thus represents an enormous reservoir of pharmacologically valuable molecules yet to be discovered. The present study deals with the activity of the plant extracts of *Barleria buxifolia*, tested for the antimicrobial properties, and antioxidant activity by DPPH, FRAP and ABTS were studied.

MATERIALS AND METHODS

Source of plant materials

Barleria buxifolia were collected from Kolli hill is situated at an ever-so-pleasant altitude ranging from 1000 to 1300 m above mean sea level in the Namakkal district of Tamil Nadu state, South India. Some part of the eastern portion of the hill lies in the Perambalur district. Kolli hill (Kollimalai in Tamil) has an area of 282.92 sq. km16. It stretches 29 km from north to south and 19 km from east to west. Kolli hill is a part of the Talaghat stretch and eastward of the hill lies in Pachamalai. Collected plant specimen was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St' Joseph's College, Tiruchirapalli, Tamil Nadu, India and The Voucher specimen (IPH-16) was deposited in Entomology lab, Arignar Anna Government Arts College, Musiri, Tamil Nadu, India.

Preparation of plant extracts

The collected leaves were shade dried at room temperature (27±2°C) for about 15 days. The dried leaves were powered using an electric blender and the powder (200gm) was loaded in the Soxhlet apparatus. Initially, ethanol (1000ml) was poured into a bottom flask, and heated gently to obtain hexane crude extract of the selected plant. Within 12 hours of seven cycles of extraction will be made to enable completeextraction. Then the crude extract was transferred to an amber bottle to avoid light exposure and photochemical reactions. Later methanol will be successively used for their respective extract. All the extracts were individually condensed *in vaccuo*, by rotary vacuum evaporator (Superfit - PBU 6 model). The condensed crude extracts were kept in hot air oven at 40°C, to get solvent free crude extract. Then these extracts will be stored at 4°C in a refrigerator.

Test microorganisms

The test organisms used in the present investigation were clinical isolates *viz., Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia*. The human fungal pathogens like *Candida albicans* and *Trichoderma viride,* which were obtained from Department of Microbiology, Hindustan college of arts and science Coimbatore. The bacteria and the fungal cultures were maintained on nutrient agar medium and potato dextrose agar (PDA) medium respectively.

Growth and Maintenance of Test Microorganism for Antimicrobial Studies

The bacteria and fungal cultures were maintained on a nutrient broth (NB) at37°C and fungus was maintained on Potato dextrose agar (PDA) at 28°C.

Preparation of Inoculum

The gram positive bacteria *Streptococcus pyogenes*, *Staphylococcus aureus* and *gram negative bacteria E. Coli, Klebsiella pneumoniae* were pre-cultured in nutrient broth over night in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm). The fungal inoculums *Candida albicans, Trichoderma viride*, were prepared from 5 to 10 days old culture grown in Potato dextrose agar medium.

The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with a spectrophotometer (595nm) to obtain a final concentration of approximately 10^5 spores/ml.

Antibacterial Activity (Anonymous, 1996)

The anti-bacterial activity was tested by the well diffusion method. Different concentration of the extracts (100 µg/ml) was prepared by reconstituting with methanol. The test microorganisms were seeded into the respective medium by spread plate method 10 µl (10 cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper wells (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Streptocycline (10 µg) used as standard for antibacterial test. The antibacterial assay plates were incubated at 37°C for 24 hrs. The diameters of the inhibition zones were measured in mm.

Antifungal Activity (Taylor *et al.*, 1995)

The antifungal activity was tested by the well diffusion method. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper wells (5 mm in diameter) impregnated with 100 µg concentrations of the synthesized silver nanoparticles were placed on test organism-seeded plates. Streptocycline (10 µg well 1) used as positive control. The activity was determined after 72 hours of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

DPPH RADICAL SCAVENGING ACTIVITY (Shimada *et al*., 1992)

Various concentrations of ethanol and methanol extracts of the sample (4.0 ml) were mixed with 1.0 ml of methanol solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as control. The percentage of inhibition in DPPH radical scavenging activity was calculated as follows;

% Inhibition = A0- A1/A0 X 100

$$\% \text{ Inhibition} \frac{\text{Absorabance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} X100$$

FRAP ASSAY (Benzie and Strain, 1996)

The total antioxidant potential of the sample was determined using the ferric reducing ability of the plasma FRAP assay as a measure of antioxidant power. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue coloured Fe II-tripyridyl triazine compound from colourless oxidized Fe III form by the action of the electron donating antioxidants. The stock solution of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, 20 mM FeCl3.6H2O and 0.3 M acetate buffer (pH 3.6) was prepared. The FRAP reagent contained 2.5 ml TPTZ solution, 2.5 ml ferric chloride solution and 25 ml acetate buffer. It was freshly prepared and warmed to 37°C. Then, 900 µl FRAP reagent was mixed with 90 µl water and 30 µl test sample/methanol/distilled water/standard antioxidant solution. The reaction mixture was then incubated at 37°C for 30 minutes and the absorbance was recorded at 593 nm. An intense blue coloured complex was formed when ferric tripyridyl triazine (Fe3+-TPTZ) complex were reduced to ferrous (Fe2+) form. The absorption at 540 nm was recorded. The calibration was plotted with absorbance at 593 nm Vs concentration of ferrous sulphate in the range 0.1 mM both aqueous and ethanol solutions. The concentrations of FeSO4 were in turn plotted against the concentration of standard antioxidants L-ascorbic acid.

$ABTS^+$ RADICAL SCAVENGING ACTIVITY (Giao *etal*., 2007)

$ABTS^+$ decolourisation assay involves the generation of the $ABTS^+$ chromophore by the oxidation of $ABTS^+$ with potassium persulphate. It is applicable for both hydrophilic and lipophilic compounds. The scavenging activity of the leaf extracts on $ABTS^+$ radical cation was measured at 734 nm. The reaction was initiated by the addition of 1.0 ml of diluted $ABTS^+$ to 10 µl of different concentrations (50 - 250 µg / ml) of leaf extract and also to 10 µl of ethanol as a control. Ascorbic acid was used as positive control. The absorbance was read at 734 nm after 6 minutes and the percentage inhibitions were calculated. The inhibition was calculated according to the equation as mentioned below:

$$\% \text{ Inhibition} \frac{\text{Absorabance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} X100$$

RESULT AND DISCUSSION

The antimicrobial activity of of *Barleria buxifolia* ethanol and methanol leaf extracts against various microbial strains with respect to various concentrations (µg/ml) was presented in the tables 3-6. Similaly the antioxidant potential f B. buxiflia are shown in tables 7-9. The zone of inhibition of test concentrations was compared with the standard concentration of control (Streptocycline 30µg/ml). Plate 1A- E showed significant results of different concentration of the extract and the control.Among the four different bacteria used, in the case of *Streptococcus pyogenes* was found to be more susceptible to the extract and the zone of inhibition is higher (18 mm) in 60µg/ml concentration than the control (08 mm), followed by 40µg/ml concentration (16 mm) and (15mm) in 20µg/ml concentration. Also in *Staphylococcus aureus* (14mm) observed in 60µg/ml concentration against the control (07 mm), followed by 40µg/ml concentration (13 mm) and (12mm) in20µg/ml concentration. In *E. coli* the zone of inhibition is higher in 60µg/ml concentration (10 mm) against its control (08 mm) **(**Plate 1E**)** followed by 40µg/ml concentration (7.5 mm) and (6.5 mm) 20µg/ml concentration. In the case of *Klebsiella pneumonia* the zone of inhibition is higher (15 mm) in 60µg/ml concentration against the control (06 mm), followed by 40µg/ml concentration (09mm) and (08mm) in20µg/ml concentration. Earlier reports of seval workers have been noted that the plants do posses the remarkable antibacterial activity and antioxidant activity. To quote few, basil (*Ocimum* spp.), mint (*Mentha* spp.), rosemary (*Rosmarinus officinalis*), lavender (*Lavandula* spp.), and Baikal skullcap (*Scutellaria baicalensis*), are known to contain relatively high levels of phenolics and have demonstrated antioxidant activity (Zheng and Wang, 2001; Shao *et al*., 2004; Waisundara, 2010).

The present results confirmed the presence of scavenging activity for the *Barleria buxifolia* leaf extract. Figure 1-3 represents the reducing power of ethanolic and methanolic extracts *B. buxifolia*. In this study, the absorbance was increased with the increasing concentration of ethanolic leaf extract. Which is due to the reducing power of the extract. An increasing in the absorbance revealed the reducing power of extract. The antioxidant activities have been reported to be the concomitant development of reducing power (Yang et al., 2002). This positive results of our study on antioxidant activity are in justification with the medicinal importance of plants as naturally occurring antioxidants.

In a variety of medicinal plants, Djeridane *et al.,* (2006) and Katalinic *et al*. (2006) have demonstrated a linear correlation between the content of total phenolic compounds and antioxidant capacity. Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants due to their scavenging activity are useful for the management of those diseases. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts (Suresh *et al.,* 2008). The 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical was widely used as the model system to investigate the scavenging activity of several natural compounds such as extract of plants in a relative short time. (Kadam Balaji *et al* 2013). DPPH, the natural antioxidants may have free-radical scavengers, reducing agents, potential complexes of pro oxidant metals, quenches of singlet oxygen. (Ebadi 2002). The antioxidant activities have been reported to be the concomitant development of reducing power. (Yang et al 2002).

In the present study, a set of pharmacognostical parameters was conducted on *Barleria buxifolia* leaf exracts. These studies revealed the presence of various important bioactive compounds and proved that the plant leaves can also be of medical importance. Understanding the anti-oxidative mechanisms of the plant *Barleriabuxifolia* and extraction methodologies could lead to the development and improvement of medicinal plant materials for human health.

Table 1. Composition of Nutrient agar medium

Substance	Quantity
Peptone	5.0 g
Beef extract	3.0 g
Agar	15.0 g
Distilled water	1000 ml
p^H	7.0

Table 2. Composition of PDA medium

Substance	Quantity
Potato	200.0 g
Dextrose	20.0 g
Agar	15.0 g
Distilled water	1000 ml
pH	6.2

Table 3. Antibacterial activity of ethanol extract of *Barleria buxifolia* against selected human bacterial pathogens

S.No	Pathogenic bacteria	Ethanol extract Zone of inhibition (mm)			Standard (Streptocycline) 10 µl
		20 µl	40 µl	60 µl	
1.	*Streptococcus pyogenes*	15	16	18	08
2.	*Staphylococcus aureus*	12	13	14	07
3.	*Escherichia coli*	6.5	7.5	10	08
4.	*Klebsiella nemoniae*	08	09	15	06

Table 4. Antifungal activity of ethanol extract of *Barleria buxifolia* against selected human fungal pathogens

S.No	Pathogenic fungus	Zone of inhibition (mm)			Standard (Streptocycline)
		20 µl	40 µl	60 µl	
1.	*Candida albicans*	6.5	07	08	08
3.	*Trichoderma viride*	08	07	08	08

Table 5. Antibacterial activity of methanol extract of *Barleria buxifolia* against selected human bacterial pathogens

S.No	Pathogenic bacteria	Zone of inhibition (mm)			Standard (Streptocycline)
		20 µl	40 µl	60 µl	
1.	*Streptococcus pyogenes*	13	14	18	08
2.	*Staphylococcus aureus*	12	14	15	07
3.	*Escherichia coli*	07	07	09	8.5
4.	*Klebsiella nemoniae*	09	12	15	07

Table 6. Antifungal activity of methanol extract of *Barleria buxifolia* against selected human fungal pathogens

S.No	Pathogenic fungus	Methanol extract Zone of inhibition (mm)			Standard (Streptocycline)
		20 µl	40 µl	60 µl	
1.	*Candida albicans*	08	08	09	7.5
3.	*Trichoderma viride*	07	7.5	08	08

Table 7. Table: Antioxidant activity of *Barleria buxifolia* -DPPH assay

S. No.	Extracts tested	% inhibition				
		200 (µg/ml)	400 (µg/ml)	600 (µg/ml)	800 (µg/ml)	1000 (µg/ml)
1	Ethanol extract	8.55 ± 0.26	17.02 ± 0.34	34.49 ± 0.59	43.52 ± 0.89	52.16 ± 0.46
2	Methanol extract	13.76 ± 0.67	25.18 ± 0.03	37.18 ± 0.50	49.27 ± 0.17	61.72 ± 0.80
3	Ascorbic acid	21.67 ± 0.21	41.85 ± 0.80	63.14± 0.76	70.23 ± 0.83	77.23 ± 0.29

The experiment was conducted in triplicates (n=3)

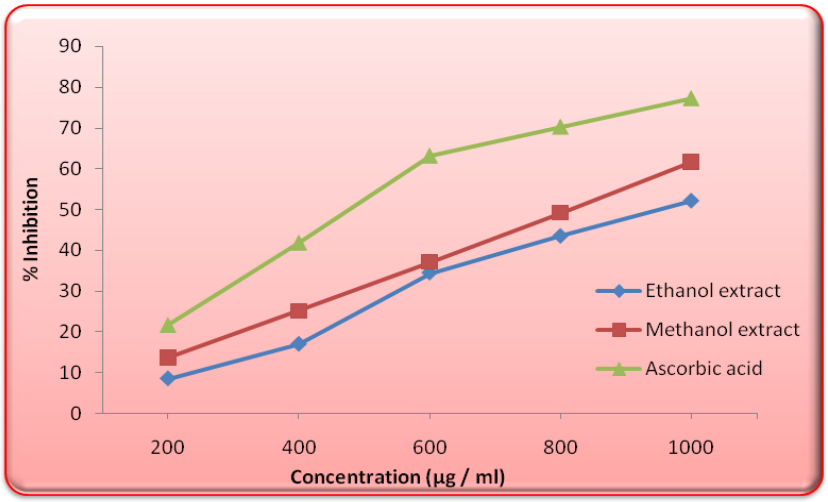

Figure 1: Antioxidant activity of *Barleria buxifolia* – DPPH assay

IC_{50} value of Ethanol extract : 950.02 µg/ml
IC_{50} value of Methanol extract : 811.73 µg/ml
IC_{50} value of Ascorbic acid (standard) : 476.55 µg/ml

Table 8: Antioxidant activity of *Barleria buxifolia* -FRAP assay

S. No.	Extracts tested	Absorbance at 593 nm				
		50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)	250 (µg/ml)
1	Ethanol extract	0.09 ± 0.03	0.16 ± 0.06	0.28 ± 0.04	0.39 ± 0.07	0.51 ± 0.02
2	Methanol extract	0.11 ± 0.07	0.19 ± 0.20	0.31 ± 0.12	0.43 ± 0.38	0.54 ± 0.02
3	Ascorbic acid	0.30 ± 0.21	0.39 ± 0.05	0.45 ± 0.17	0.57 ± 0.36	0.65 ± 0.04

The experiment was conducted in triplicates (n=3).

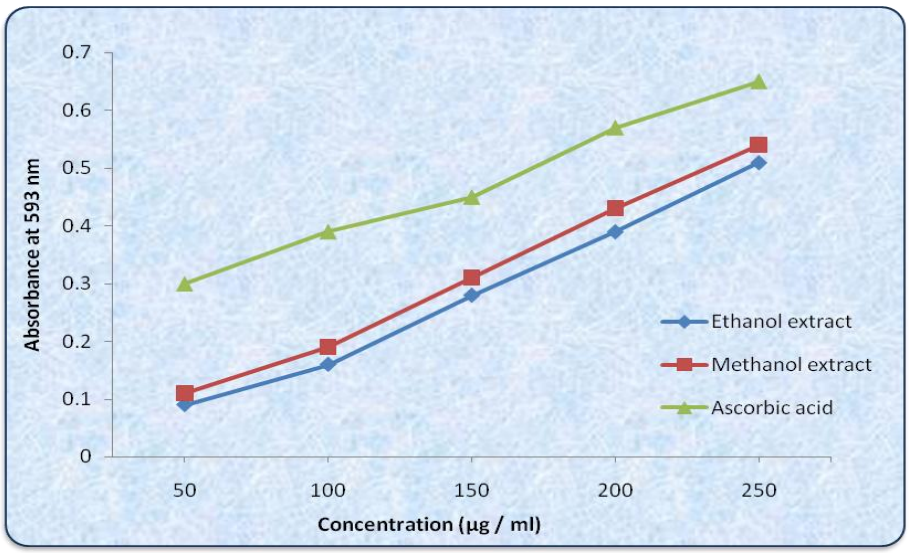

Figure 2: Antioxidant activity of *Barleria buxifolia* – FRAP assay

Table 9: Antioxidant activity of *Barleria buxifolia* -ABTS^{+} scavenging assay

S. No.	Extract tested	% inhibition				
		200 (µg/ml)	400 (µg/ml)	600 (µg/ml)	800 (µg/ml)	1000 (µg/ml)
1	Ethanol extract	18.67 ± 0.87	25.40 ± 0.36	36.72 ± 0.40	48.63 ± 0.19	56.73 ± 0.37
2	Methanol extract	23.56 ± 0.10	32.17 ± 0.56	44.90 ± 0.16	53.58 ± 0.20	60.88 ± 0.29
3	Ascorbic acid	27.93 ± 0.67	39.45 ± 0.29	51.40 ± 0.35	65.34 ± 0.79	74.40 ± 0.17

The experiment was conducted in triplicates (n=3).

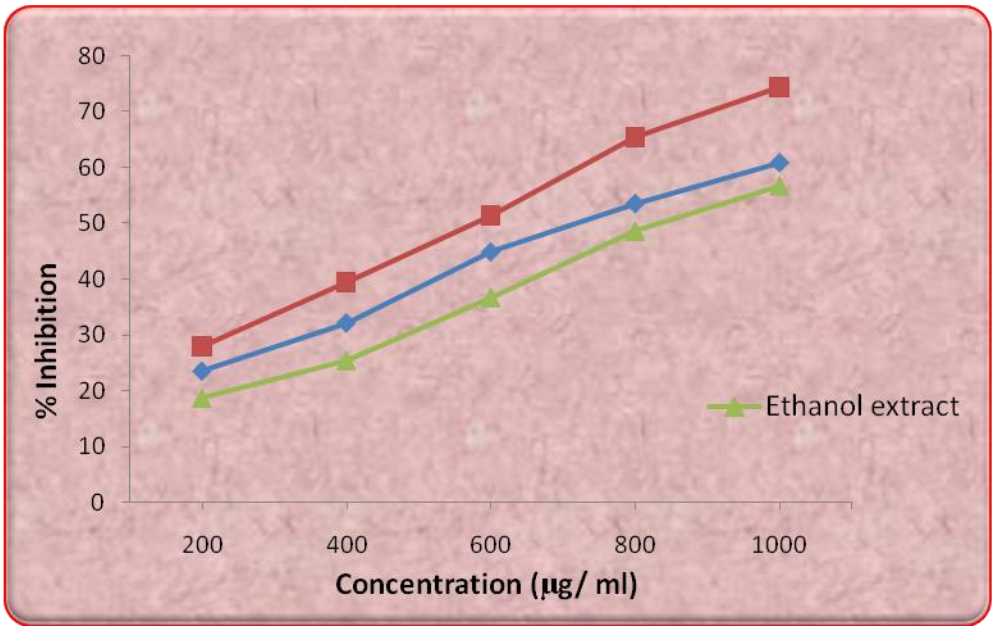

Figure 3: Antioxidant activity of *Barleria buxifolia* – ABTS assay

IC_{50} value of Ethanol extract : 833.83 µg/ml
IC_{50} value of Methanol extract : 717.51 µg/ml
IC_{50} value of Ascorbic acid (standard) : 576.56 µg/ml

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