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Antibacterial, Anti-inflammatory and Antioxidant Potential of Ethanol Extract of *Ipomoea staphylina* Roem. & Schult

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

In the present study we evaluate the antibacterial, anti-inflammatory and antioxidant activity of medicinal plant *Ipomoea staphylina* Roem & Schult. Many bacteria are harmful to human beings and animals. Theses bacteria make diseases the host organisms. Many antibiotics are available in the marker for the treatment of bacterial diseases. However, antibiotics cause unwanted side effect. Thus, the study focused to evaluate ethanol extract of *Ipomoea staphylina* against bacterial pathogens. Antibacterial activity was evaluated by the method of well diffusion method. Antiinflamatory and antioxidant activity was evaluated by in vitro study method. Ethanol extract of *Ipomoea staphylina* showed antibacterial activity against different bacteria isolated from chicken at dose depended manner *ie.,* higher dose of plant extract possessed maximum inhibition zone (21.67 ± 1.45 mm) ($P<0.05$) against selected bacteria. Moreover, plant ethanol extract possessed anti-inflammatory activity and antioxidant activity at dose dependent manner ($P<0.05$). Higher dose (100 µg/ml) of ethanol extract of *Ipomoea staphylina* showed maximum anti-inflammatory activity (68.38 %) and antioxidant activity (72%). From this result it is concluded that the medicinal plant *Ipomoea staphylina* Roem. & Schult. possessed antibacterial, anti-inflammatory and antioxidant activity. Further detailed study will be conducted for the new drug candidate discovery.

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1. INTRODUCTION

Antimicrobial agents are more important to reduce the global burden of infectious diseases by killing performing against pathogenic microbes [1]. However, emergences of multidrug resistant (MDR) strain in pathogenic bacteria have become a significant public health threat against antimicrobial agents [2,3]. Multidrug resistances development has serious problem to the management of infectious diseases caused by pathogens [4]. This is due to continuous usage of antibiotics in human and veterinary field [5]. Common problematic multidrug resistant pathogens are *E. coli* such produced ES*β*L, penicillin-resistant *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, vancomycin resistant *Enterococcus*, methicillin-resistant *S. aureus*, and extensively drug-resistant *Mycobacterium* tuberculosis [6].

Thus, in the evidence of rapid spread of resistant clinical isolates, we need to find out new antimicrobial agents are importance [7,8]. Considering this problem, many researchers are focused to develop or discover new novel agents with broad-spectrum therapeutic potency.

A maximum number of medicinal plants have been accepted as valuable natural antimicrobial compounds and are the alternative medicine for the treatment of multidrug resistance bacterial infections [9]. World Health Organization (WHO) reported that medicinal plants would be the best source for getting variety of drugs used for many diseases including microbial pathogens [10]. Many plants have been used as an antimicrobial trait due to their secondary metabolites [11,12]. Plants are possessed wide range of secondary metabolites such as alkaloids, tannins, phenolic compounds, and flavonoids, possessed *in vitro* antimicrobial activities against multi drug resistance human pathogens [13,14]. Thus the present research was carried out to evaluate the antimicrobial activity, antioxidant and anti-inflammatory activity of herbal plant *Ipomoea staphylina* Roem. & Schult.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material and Preparation of Extract

Medicinal plant *Ipomoea staphylina* Roem. & Schult. (Common name: Oonan Kodi) was collected manually from local area. The plant material was identified authenticated by Dr. S. Soosairaj, (Specimen access No. SJCBT 2873). Leaf of the plant was washed with running water, then dried under shadow and then powdered. Then the extraction was prepared using this powder by the method of Soxhlet extraction method using ethanol as a solvent. After that, the crude extract was concentrated using a rotary evaporator. Then the extract was stored at 4^0C in refrigerator until further study.

2.2 Antimicrobial Activity

2.2.1 Agar well diffusion method

Antimicrobial assay of different concentration of ethanol extract of *Ipomoea staphylina* Roem. & Schult. was performed by agar well diffusion method against bacteria isolated from chicken. Different bacterial colonies such as *Escherichia fergusonii, Escherichia coil, Shigella sonnei, Escherichia sps* and *Escherichia coli –I* were isolated from chicken and used for the evaluation of antibacterial activity. The test bacteria were inoculated in Nutrient broth medium and incubated at 37^0C for overnight. Then the different concentration of ethanol extract of and *Ipomoea staphylina* Roem. & Schult. was prepared using Dimethyl Sulfoxide (DMSO). Six wells of 6 mm were bored in agar plate with the help of sterile cork-borer (6 mm). Each well was filled with 50μl of different concentrations of ethanol extract and positive control (Amikacin 30 mcg) was used for comparison study. Then this allowed for the diffusion about 30 minutes at room temperature and incubated for 18-24 hours at 37^0C. After incubation, the formation of inhibition zone around the well which corresponds to the antimicrobial activity of tested compounds was observed. The zone of inhibition (ZOI) was observed and measured in mm.

2.3 *In Vitro* Anti-inflammatory Activity

2.3.1 Inhibition of albumin denaturation

The anti-inflammatory activity of *Ipomoea staphylina* was studied by using slightly modified method of inhibition of albumin denaturation according to Mizushima et al [15] and Sakat et al [16]. Plant extract and 1% aqueous solution of bovine albumin fraction was mixed and pH of reaction mixture was adjusted using small amount of 1N HCl. Then the extract was

incubated at 37 ºC for 20 min and then heated to 51^{o}C for 20 min, after cooling the samples the turbidity was measured at 660nm. (UV-Visible Spectrophotometer Model 371, Elico India Ltd) The experiment was performed in triplicate. The inhibition of protein denaturation was calculated and given in percentage.

Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control

2.4 Antiproteinase Action

The test was performed according to the modified method of Sakat et al [16]. 0.06 mg trypsin, 1 ml 20mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations were added and mixed well. Then the mixture was incubated at 37^{o}C for 5 min. To this 1ml of 0.8% casein was added. Then the mixture was incubated for 20 min. After this, 2ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

2.5 Membrane Stabilization

2.5.1 Preparation of Red Blood cells (RBCs) suspension [17,18]

The Blood was collected from healthy chicken for 2 weeks prior to the experiment and transferred to centrifuge tubes. Then the sample tubes were centrifuged at 3000 rpm for 10min. Then these tubes were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline.

2.5.2 Heat induced haemolysis [17,18]

1ml test sample of different concentrations and 1ml of 10% RBCs suspension was added and mixed well, saline and Aspirin were used and control and standard test tube. All tubes were incubated in water bath at 56 ºC for 30min. At the end of incubation tubes were cooled under running tap water. Then the mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. The Percentage inhibition of Haemolysis was calculated as follows:

Percentage inhibition = (Abs control –Abs sample) X 100/Abs control.

2.6 Antioxidant Activity

2.6.1 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) assay

The scavenging potency of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical of ethanol extract of *Ipomoea staphylina* was determined [19]. The absorbance at 517 nm was measured to assess the remaining amount of DPPH. Butylated hydroxytoluene (BHT) was applied as a standard. The ability to scavenge DPPH radicals was calculated using the following equation:

Inhibition (%) = ($A_{control} - A_{test}$) / $A_{control}$ X 100.

Where;

$A_{control}$ = The absorbance of the control reaction.

The results were expressed as the half maximal inhibitory concentration (IC_{50}) and compared with standard. All measurements were fulfilled in triplicate and mean values were calculated.

2.7 Ferric Reducing Antioxidant Power Assay

The reducing antioxidant power of plant material was determined by using the method of Oyaizu [20] with some modifications. Various concentrations of ethanol extract and standard drug L-ascorbic acid were mixed with phosphate buffer (2 ml) and to this 2 ml of 1% potassium ferricyanide were added. This mixture was then incubated at 50°C for 20 minutes. Next to, 2ml of 10% trichloroacetic acid was added and the mixture was centrifuged at 1000 rpm for 10 minutes. Then the supernatant was mixed with 2ml of distilled water and added 1ml of 0.1% ferric chloride solution. Then the absorbance's were measured at UV-Vis spectrum at 700nm and recorded.

2.8 Determination of Metal Chelating Activity

Metal chelating activity of selected medicinal plant was measured by adding 0.1 mM $FeSO_4$ and 0.25 mM and then 0.2 mL of plant extract [21]. Then incubated at room temperature for 10 min and then absorbance of the mixture

was recorded at 562 nm. Chelating activity was calculated using the following formula:

Metal chelating activity = $(A_{control} - A_{sample})/A_{control} \times 100$

Where

$A_{control}$ is the absorbance of control reaction (without plant extract), and A_{sample} is the absorbance in the presence of a plant extract.

3. RESULTS AND DISCUSSION

3.1 Antibacterial Activity

Recently there has been considerable interest in the use of plant material as an alternative method to control pathogenic microorganism [22] and many components of plants products have been shown to be specially targeted against resistant pathogenic bacteria [23]. The emergence of multidrug resistant strain of many pathogens is a serious threat and makes chemotherapy more difficult. Antibiotic resistance is the major problem that continues challenge to the healthcare sector in both developing and developed countries. The emerging spread of multidrug resistant pathogens has substantially threatened the current antibacterial therapy. Moreover, the current cost of most of the chemotherapeutic agents is unbearable to the public especially in developing countries like India [24]. Therefore attempts must be directed towards the development of effective natural, non-toxic drug for treatment. Many reports are available on the antiviral, antibacterial antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [25]. The methanol leaf extract of *L. Indica* and *A. reticulata* showed the activity against all the five tested microorganisms but the activity was very significant against *P. vulgaris* and *P. aeruginosa.*

This has necessitated searching new source of antimicrobial substances from plants. They produce many bioactive compounds that known for therapeutic properties. In the present study we evaluate the antimicrobial activity of ethanol extract *Ipomoea staphylina* against different strain of *E. coli* isolated from chicken. Evaluation of the antimicrobial activity of ethanol plant extract of *Ipomoea staphylina* was determined initially by the well diffusion method against different bacterial colonies was isolated from chicken. These organisms were frequently encountered in infectious diseases to the poultry industries. In the present study different concentrations of ethanol extract of *Ipomoea staphylina* used and exhibited a varying degree of antibacterial activity against different bacterial colonies (Fig. 1). It was observed that higher concentration of ethanol extract showed maximum zone of inhibition (ZOI) against all different straining of *E. coli* bacteria especially *E. coli* (21.67 ± 1.45 mm) followed by *Escherichia fergusonii* (19.67 ± 1.20 mm). Minimum inhibition zone was observed in *Escherichia coli -I* strain (17.33 ± 0.8 mm) Moreover, the antibiotic drug showed more or less similar inhibition zone to the plant extract. Although, some plant extracts showed good antibacterial activity against different tested bacterial isolates as judged by their MIC values [26,27,2]. This result was similar to those of other studies that reported antibacterial activity of methanolic extract of *O. corniculata* [28]. Many researchers has been widely observed and accepted that herbal plants possessed medicinal value due to the presences of bioactive compounds that dissolve in different solvent systems [29]. *C. tamala* extract showed antimicrobial activity against only one tested bacterium, *S. aureus* (ATCC 25293). Hassan Waseem et al. [30] detected the antimicrobial activity of *C. tamala* against a number of organisms. They possessed different degrees of antimicrobial activity against all tested gram positive and gram negative bacteria contrary to our result where only *S. aureus* was found to be effective. Phytochemicals flavonoids, terpenoids, tannins, and alkaloids present in extracts of *C. tamala* show antihelminthic, antidiarrhoeal, and antimicrobial activities. MIC value shows that these plant extracts have the least antimicrobial activity. In the present study the plant *Ipomoea staphylina* many bioactive compounds like flavonoids, alkaloid, terpinoids etc. are involve in the pharmacological activity.

3.2 Anti-Inflammatory Activity

3.2.1 Inhibition of albumin denaturation

Protein Denaturation is a process in which proteins lose their secondary and tertiary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. In the present investigation plant extract was inhibited protein denaturation and it was effectively inhibiting albumin denaturation

induced by continuous heating. Maximum inhibition was (68.38 %) observed at100 µg/ml and Standard anti-inflammation drug aspirin showed maximum inhibition (80.5%) compared with control (Fig. 3) and also showed statistical significant (P<0.05) between the plant extract and standard drug.

3.3 Proteinase Inhibitory Action

Neutrophils are the rich source of serine proteinase and localized in the lysosomes. Leukocytes proteinase is play a vital role in development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors. Different concentration of ethanol extract exhibited significant antiproteinase activity as shown in Fig. 3. It showed that the inhibition of inflammation was gradually increased at dose dependent manner. However, standard drug Aspirin (78.87%) showed maximum inhibition of inflammation compared to the plant extract (Fig. 3).

3.4 Heat Induced Haemolysis

Different concentration of ethanol extract of *Ipomoea staphylina* was effective in inhibiting the heat induced haemolysis at different concentrations. The results showed that inhibition of heat induced haemolysis were higher (71.63%) in maximum concentration (100µl) and that were more or less similar to the standard drug Aspirin (84.54%). Herbal plant *Ventilago maderaspatana* possessed anti-inflammatory activity, antidiabetic and anticancer activity was previously reported [31].

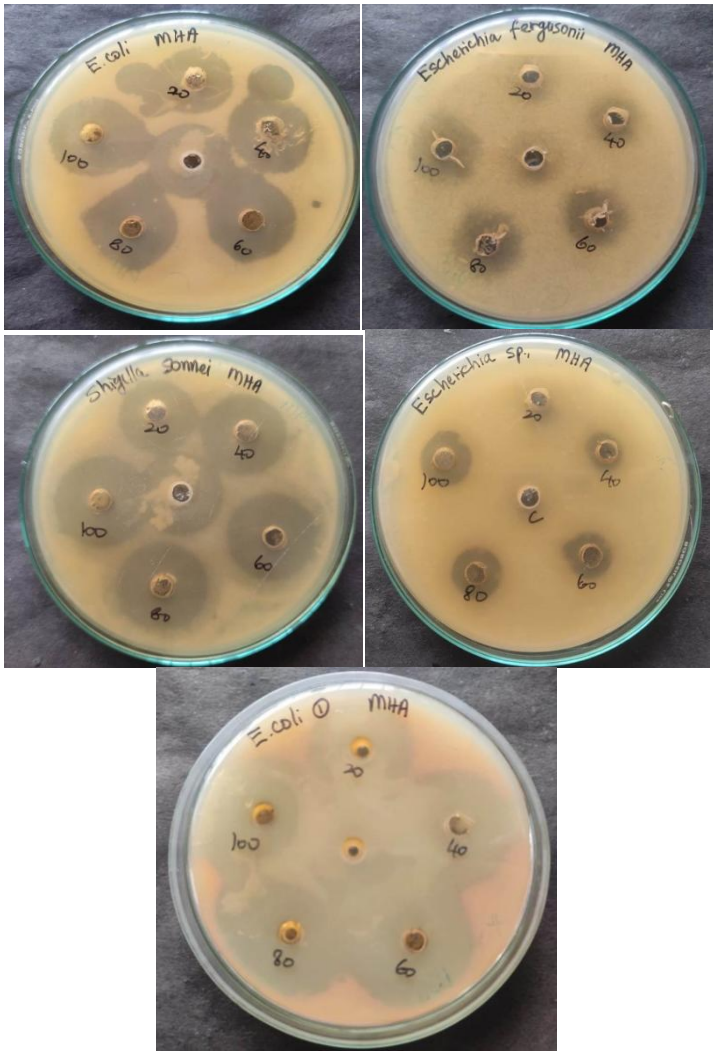


Fig. 1. Antibacterial activity of *Ipomoea staphylina* against different *E. coli* strain isolated from chicken

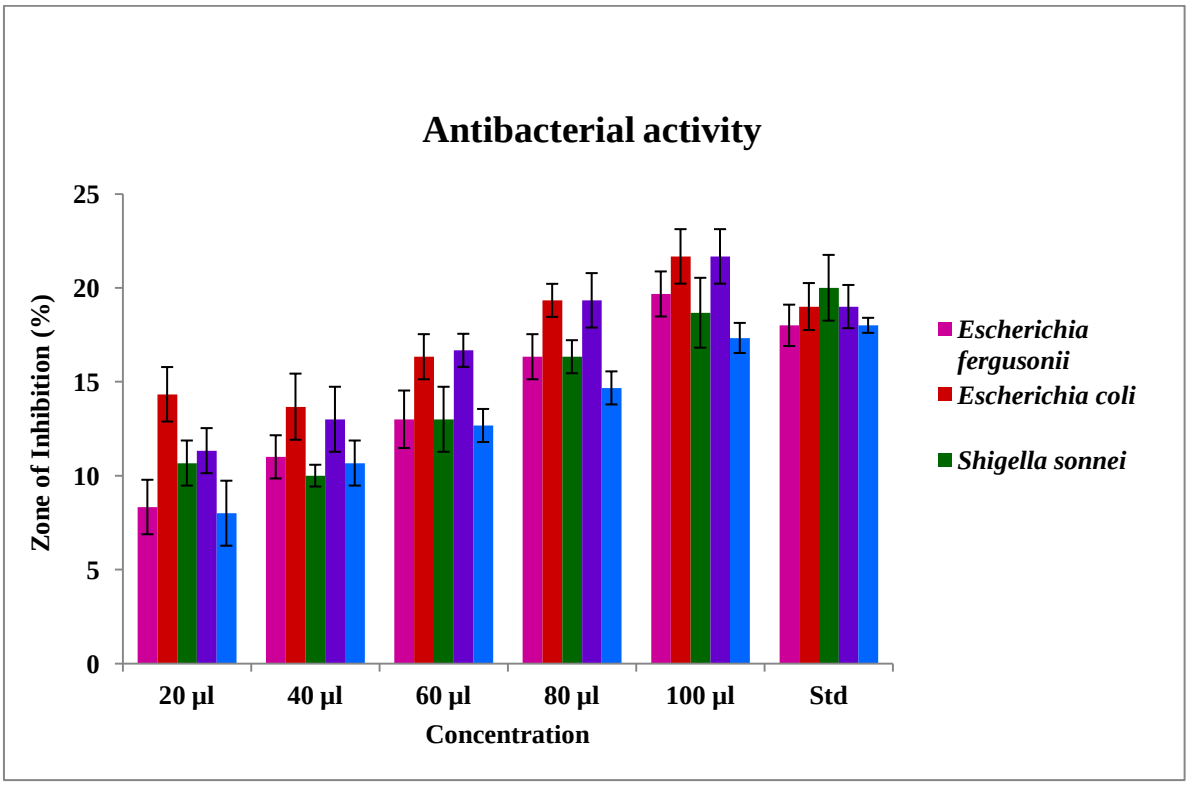
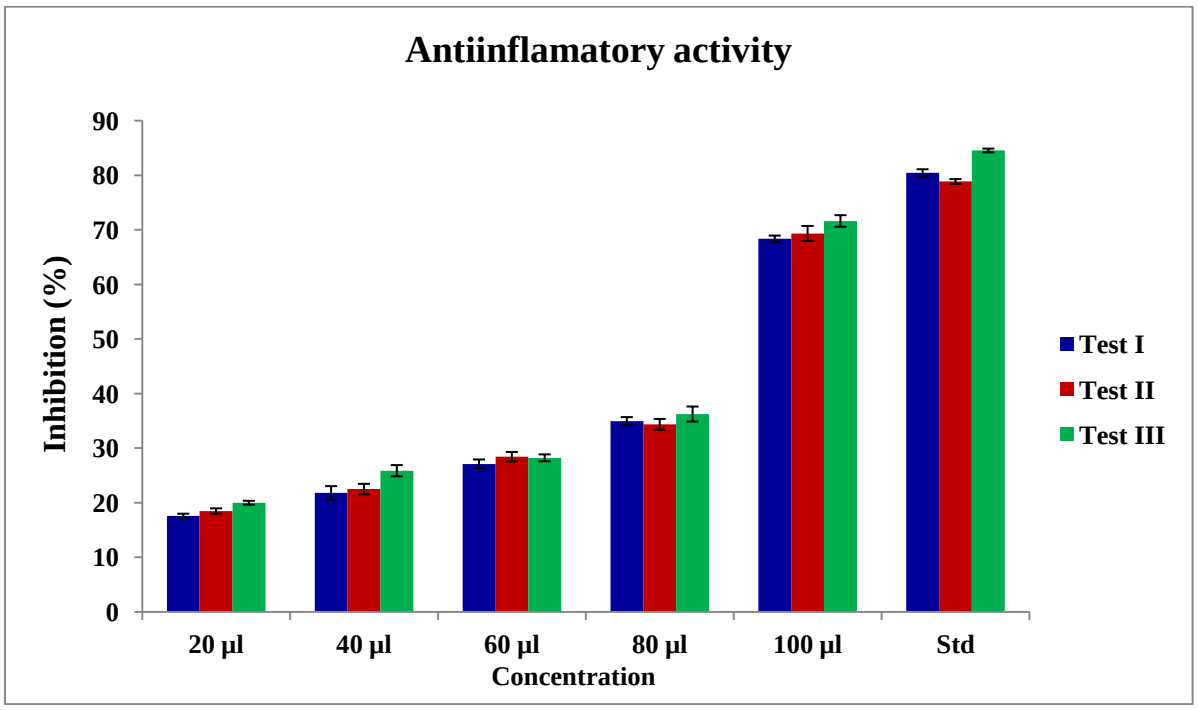


Fig. 2. Antimicrobial activity of ethanol extract of *Ipomoea staphylina* against bacterial strains isolated from Chicken



Test I - Inhibition of albumin denaturation; Test II- Antiproteinase action; Test III-RBC haemolysis

Fig. 3. *In vitro* anti-inflammatory activity of ethanol extract of *Ipomoea staphylina*

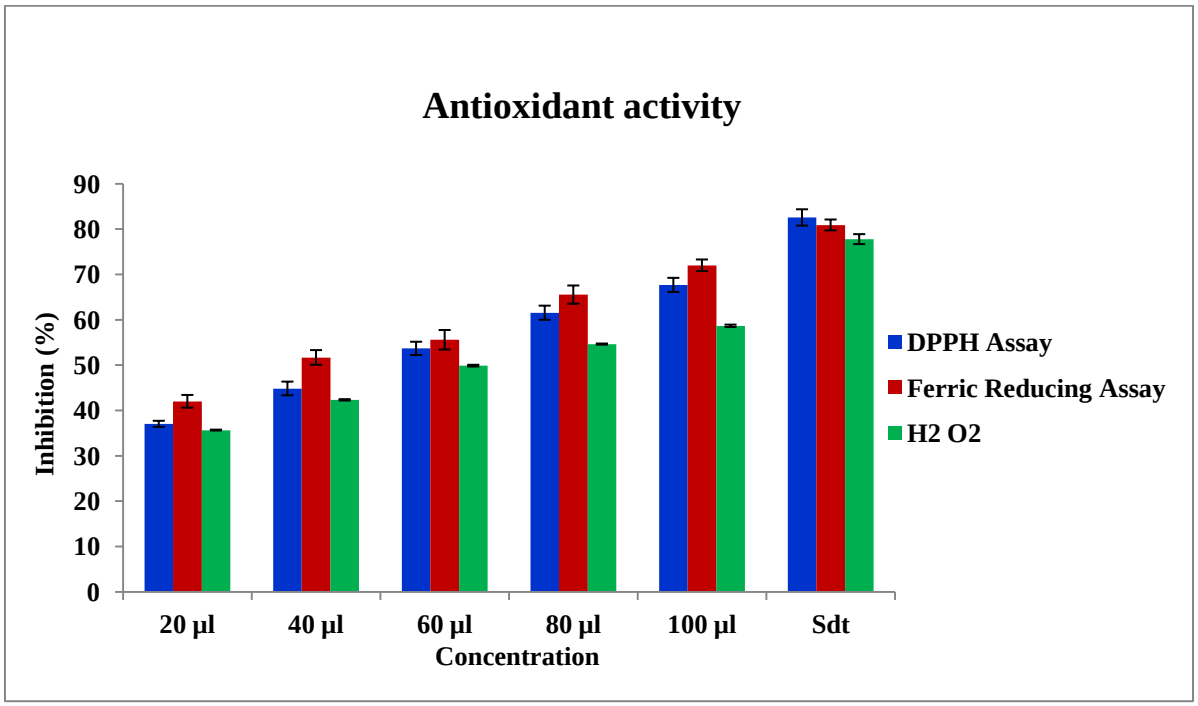


Fig. 4. ***In vitro antioxidant activity of ethanol extract of Ipomoea staphylina***

3.5 Antioxidant Activity

Increased production of reactive oxygen/nitrogen species and decreased capacity of antioxidant defences in the body leads to oxidative stress [32,33]. Generation of reactive oxygen/nitrogen species (ROS/RNS) is inevitable for aerobic organisms and in healthy cells, and it occurs at a controlled rate [34]. Under conditions of oxidative stress, ROS/RNS production is dramatically increased, resulting in subsequent alteration of membrane lipids, proteins, and nucleic acids. Oxidative damage of these biomolecules is associated with aging and a variety of pathological events, including atherosclerosis, carcinogenesis, ischemia reperfusion injury, and neurodegenerative disorders [35]. To maintain homeostasis in the redox system and protect the body against ROS and RNS, humans have evolved complex antioxidant systems, which work to avert deleterious effects of oxidative stress [36]. The body's antioxidant defense systems are of endogenous and exogenous origin [37]. Exogenous sources of antioxidants include *β*-carotene, L-ascorbic acid (vitamin C), *α*-tocopherol, and tocotrienols (vitamin E), which are derived from dietary foods we consume [38]. In the present study we evaluated the antioxidant activity of ethanol extract of *Ipomoea staphylina.* Ethanol extract *Ipomoea staphylina* exhibited remarkable concentration-dependent increases in absorbance against Ferric Reducing Antioxidant Power (FRAP) at a wavelength of 700nm (Fig. 4). At all the tested concentrations, high concentration of plant extracts showed maximum inhibition (72%) activity against oxidative stress. However, the standard (L-ascorbic acid) demonstrated significantly higher (80.89%) absorbance's than different concentration of ethanol extract. However there was no statistical ($P<0.05$) variation between the plant extract and standard drug. In DPPH assay, ethanol extract of *Ipomoea staphylina* demonstrated remarkable *in vitro* DPPH radical scavenging activities at dose-dependent manner (Fig. 4) and standard (L-ascorbic acid) exhibited (82.54%) significantly more or less similar ($P<0.05$) DPPH radical scavenging activities to the DPPH radical scavenging activities of different concentration of plant extract (Fig. 4). Moreover, *Ipomoea staphylina* exhibited remarkable *in vitro* hydroxyl radical scavenging activities. Different concentrations of ethanol extract *Ipomoea staphylina* demonstrated significantly higher hydroxyl radical scavenging activities. Moreover the *in vitro* hydroxyl radical scavenging activities of L-ascorbic acid standard were higher than the plant extract, however there was no significant ($P<0.05$) different between the extract and L-ascorbic acid.

4. CONCLUSION

From this study we concluded that the ethanol extract of *Ipomoea staphylina* possessed antimicrobial activity against different bacterial colonies such as *Escherichia fergusonii, Escherichia coil, Shigella sonnei, Escherichia sps* and *Escherichia coli –I* isolated from chicken and also possessed anti-inflammatory and antioxidant activity. Thus the plant *Ipomoea staphylina* was might be useful drug candidate for inflammation and oxidative stress after the detailed evaluation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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