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Original Research Article

Anti-Oxidant and Antimicrobial Studies of *Tinospora cordifolia* (Guduchi/Giloy) Stems and Roots under In Vitro Condition

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ABSTRACT

Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines and healing properties. The present study was aimed to evaluate the anti-oxidant and antimicrobial properties of stem and root of T. cordifolia. Total phenolic contents of different solvent extracts were determined and found that ethanol extract had the highest phenolic content of 0.3213 mg g-1. Antioxidant assays were also carried out by using different in vitro models such as total reducing power, hydrogen peroxide scavenging activity assay and hydroxyl redical scavenging activity. The Ethanol extract showed the highest total antioxidant activity. The H2O2 scavenging and hydroxyl free radical scavenging activity was maximum 87.2 % and 91.0% found in case of ethanolic steam extract respectively. The antimicrobial activity of ethanolic and methanolic extract of root and stem of *T. cordifolia* were also evaluated against some pathogenic microorganisms viz. E. coli, B. subtilis, A. niger and Candida sp. it was found that the various concentration of extract viz. 50, 100, 150 and 200 mg ml-1 were tested. It was observed that the increasing in concentration there was also increasing in antimicrobial activity reveled by increase in size of zone of inhibition. The methanolic stem extract exhibits highest antimicrobial activity against all four pathogens. The study shown that the extract of *T. cordifolia* has a wide range of anti-oxidant as well as antimicrobial activity against bacterial as well as fungal pathogens.

INTRODUCTION

The evidence of medicinal value of some plants has been observed since ancient times. These plants are still widely used as ethnomedicine around the world. Currently, one-half of the pharmaceuticals dispensed having plant origins, very few are intended for use as antimicrobials, since we have relied on bacterial and fungal sources of these activities [1]. From time immemorial, man depends on plants for medicine. *T. cordifolia* a medicinal plant found in the Asian subcontinent including India, Nepal, Bangladesh Malaysia etc. with great medicinal properties including antioxidants, antimicrobial, anti-diabetic, anti-ageing [2]. The antimicrobial properties and cytotoxic activity of *T. cordifolia* has been evaluated in its crude methanolic extract, Petroleum ether, carbon tetrachloride, and chloroform extract [3].

Antioxidant properties were also studied to treat most diseases and to prolong the storage stability of foods [4].

Tinospora cordifolia is one of the most important plants used in indigenous system of medicine. There are innumerable references of its uses in traditional medicine. *T. cordifolia* is a useful medicinal plant; it has good impacts on health, prolongs life, enhances memory, and improves the quality of voice and fertility [3]. In the present study the anti-oxidants and antimicrbial activity of the aqueous, ethanol and chloroform extracts from the stems & root of T. cordifolia was evaluated by using some in- vitro model.

MATERIALS AND METHODS

Collection of Plant material

The Roots and Stems of *T. cordifolia* were collected from district Bokaro, Jharkhand.

Preparation of extraction

The stem and roots were washed, dried in hot air oven at 500C and then finely powdered. The powder was used for extraction. Sequential extraction of the powder was done with ethanol and methanol. 30g of the powder was extracted in 100mL of the solvents in conical flasks on a shaker for 24h at room temperature. The extracts were filtered with Whatmann filter paper no. 1 and dried by evaporation. The roots and stem extracts obtained was screened for antioxidant and antimicrobial activity.

STUDY OF ANTIOXIDANT PROPERTY OF EXTRACTS

Total polyphenolic content

Total soluble phenolic content of T. cordifolia was estimated by Folin-Ciocalteu's reagent method using Gallic acid as a standard phenolic compound. Solvent extracted solutions (0.3 mL in triplicate) was mixed with 1.5 mL of 10% freshly prepared Folin-Ciocalteu's reagent and after 3 min, 1.2 mL of 7.5% (W/V) sodium carbonate was added and mixed thoroughly. The tubes were placed in boiling water for one minute, cooled and the absorbance was measured at 650 nm in a spectrophotometer against a reagent blank. The concentrations of the total phenolic compounds in the extract were obtained by extrapolating the absorbance of Gallic acid on standard Gallic acid graph. The experiment was repeated thrice and concentration of total phenols was expressed as mg/g of dry extract.

Hydrogen peroxide scavenging activity assay

Aliquot of 1.0 mL of 0.1mM H_2SO_4 and 1.0 mL of various concentration of extracts were mixed, followed by 2 drops of 3% ammonium molybdate, 10mL of 2M H_2SO_4 and 7.0 mL of 1.8M KI. Then mixed solution was titrated with 5.09 mM NA_2SO_3 until yellow color disappeared. Percentage of scavenging of hydrogen peroxide was calculated as:

$$\% \text{ inhibition} = (V_0\text{-}V_1)/V_0 \times 100$$

Where, V_0 = volume of Na_2SO_3 solution used to titrate the control sample in the presence of hydrogen peroxide (without extract) and

V_1 = volume of solution Na_2SO_3 used in the presence of the extract.

Hydroxyl radical scavenging activity

The reaction mixture contained 60µl of 1.0mM $FeCl_3$, 90µl of 1mM 1,10-phenanthroline, 2.4 mL of 0.2 M phosphate buffer (pH 7.8), 150µl of 0.17 M H_2O_2 and 1.5 mL of extracts.. Adding hydrogen peroxide started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 532nm was measured with a spectrophotometer. The hydroxyl radicals scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = (A_0 - A_1) / A_0 \times 100$$

Where, A_0 = absorbance of the control (blank, without extract) and

A_1 = absorbance in the presence of extract.

ANTIMICROBIAL STUDY OF ROOTS AND STEM EXTRACT

Antibacterial activity

Preparation of McFarland standard

Exactly 0.5 McFarland equivalent turbidity standards was prepared by adding 50 µl of 1% barium chloride solution to 9950 µl of 1% sulphuric acid solution and mixed thoroughly. This solution was used to prepare the test and control inocula. Exactly 0.5 McFarland gives an equivalent approximate density of bacteria 1 x 10^8 cfu.

Inoculums preparation by direct colony suspension method

Small volume of sterile water was poured inside a test tube to which 50 µl of 24 hr broth culture was added until it match the 0.5 McFarland standard turbidity.

Antibacterial sensitivity assay

The sensitivity of the test organism viz. *E. coli*, *B. subtilis*, to the extract of *T. cordifolia* was carried out using the agar well diffusion method. The plates of Muller Hinton agar were prepared. 100 µl of culture was inoculated. Sterile L-shaped spreader was used to spread the organism all over the surface of the medium and allowed to dry for about

5 minutes. Four wells were bored in the agar using sterile cork borer. Different concentrations of the plant extract were prepared in the order 50 $mgml^{-1}$, 100 $mgml^{-1}$, 150 $mgml^{-1}$, 200 $mgml^{-1}$ in four different test tubes and placed in a test tube stand. Exactly 50 µl of each concentration was introduced into each well on the medium and was allowed to stand on bench for about half an hour for proper diffusion. The plates were incubated aerobically at 37 °C for 24 hours. The zone of inhibition was measured with antibiotic zone scale in mm.

Antifungal activity assay

The screening of Antifungal activity was done by using the simple well diffusion Method. The fungal isolates used in the present study were *Aspergillus niger and Candida* sp. The plates of potato dextrose agar were prepared. 100 µl of culture was inoculated. Sterile L-shaped spreader was used to spread the organism all over the surface of the medium and allowed to dry for about 5 minutes. Four wells were bored in the agar using sterile cork borer. Different concentrations of the plant extract were prepared in the order 50 mgml-1, 100$mgml^{-1}$, 150 $mgml^{-1}$, and 200 $mgml^{-1}$ in four different test tubes and placed in a test tube stand. Exactly 50 µl of each concentration was introduced into each well on the medium and was allowed to stand on bench for about half an hour for proper diffusion. Incubation period of 24-48 hours at 28^0C was maintained for observation of antifungal activity of plant extracts. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extracts. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm.

RESULTS AND DISCUSSION

Total polyphenolic contents

The total polyphenolics content in ethanol as well as in methanol extract was determined from calibration curve and expressed in Gallic acid equivalents. Calibration curve for Gallic acid was found to be linear with in experimental range. The results are depicted in figure 1. From the experiment it was found that the ethanol recovered more polyphenolic contents than the methanolic extracts both in root and stem. It has already been reported that total phenolic content in ethanolic leaves extract of *T. cordifolia* has higher value as compared to methanolic and other solvent extracts. In a similar study it was reported that the level of polyphenols in the ethanol extract was 5.1±0.25 mg/g higher as compared to methanol, chloroform, hexane and aqueous extracts of *T. cordifolia* [5]. Medicinal plants are an important source of antioxidants [6]. Natural anti-oxidants increase the anti-oxidant capacity of the plasma and reduce the risk of certain diseases [7]. Polyphenols are the major plant compounds with anti-oxidant activity. Typical phenolics that possess anti-oxidant activity are known to be mainly phenolic acids and flavonoids [8]. It is reported that the phenolics are responsible for the variation in the anti-oxidant activity of the plant [9]. They exhibit anti-oxidant activity by inactivating lipid free radicals or preventing decomposition of hydro peroxides into free radicals [10].

Total reducing power

Figure 2 depicts the reductive effect of *T. cordifolia.* The antioxidant activity, the reducing power of *T. cordifolia* in case of ethanolic extracts is higher than methanolic extracts. The increase in the absorabanc with increased concentration indicates its reducing activity. All extracts showed significantly higher activities indicating that *T. cordifolia* consist of hydrophilic polyphenolic compounds that cause the greater reducing power. In a Similar study it was reported that, the reducing power of different solvent extracts using ferricyanide method indicates that the reducing ability of the extracts increased with the concentration [5]. Reducing power is associated with its anti-oxidant activity and may serve as a significant reflection of the anti-oxidant activity [11]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid per oxidation processes, so that they can act as primary and secondary anti-oxidants [12].

Hydrogen peroxide scavenging assay

The ethanol extracts (roots and stem) showing more percentage inhibition as shown in figure 3. *T. cordifolia* extract demonstrated hydrogen peroxide scavenging activity in ethanolic extract is higher as compared to methanolic extract. In a similar study it has been also reported that the ethanolic leaves extract of *T. cordifolia* contains higher amount of scavenging activity as compared to other solvent system so it may be concluded that ethanol has higher tendency to elute active component that is

required for activity. It was also reported in the same study that quenching ability was generally low with all the solvents and aqueous extracts. As with ethanol extract, even at 0.7 mg/ml conc. the percentage radical scavenging abilities of other extracts were lower than ethanol extract [13]. Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress [14]. Among the extracts of *T. cordifolia*, ethanol extract showed mild scavenging activity and EC_{50} value could not reached even at 0.9 mg/ml concentration. The result supports the earlier study which showed that the EC_{50} value for superoxide scavenging could be as high as 6 mg/ml [15].

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of its toxic effects. So Scavenging of hydrogen peroxide can help in elimination of toxic effects [16]. Hydrogen peroxide scavenging activity of the sample extract is directly related to the antioxidant activity of the extract, the extract having good hydrogen peroxide scavenging capability showing good antioxidant activity.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activities were evaluated and are depicted in figure 4. It was found that the ethanolic extract has better hydroxyl radical scavenging activity than methanolic extract. Hydroxyl radical (OH^-) is very reactive and can be generated in biological cells through Fenton reaction. The potential scavenging abilities of extract might be due to the active hydrogen donor ability of the hydroxyl substitution. Similarly high molecular weight and proximity of many aromatic rings and hydroxyl groups are more important for free radical scavenging by specific functional group [13]. A study carried out has shown the hydroxyl radical scavenging activity of *T. cordifolia* aerial parts with an EC_{50} value of 0.02 mg/ml, the difference in the EC_{50} value can be attributed to the distribution of secondary metabolites that may fluctuate between different plant organs [17].

Antimicrobial activity of stem and root extract of *T. cordifolia* in different micro-organisms

The antimicrobial activity of methanolic and ethanolic extract of *T. cordifolia* is tested against different bacterial and fungal strain. The results had shown that different extracts shows different inhibitory action against different microbial strains. To study the antimicrobial activity of *Tinospora* plant against different microbes, we used powdered stem and roots sample. As we used roots and stem samples against bacterial and fungal strains we came out with the result that methanol extract of roots and stem showing more antimicrobial activity in comparison to ethanolic extract of roots and stem extracts of *T. cordifolia*. The screening of antibacterial activity was done by using the simple well diffusion method. A previous study shows that methanol has more zone of inhibition than other solvents. Methanol is showing value of 9mm and other solvents showing 0.08, 0.15, and 0.25mm respectively in case of *E. coli.* In case of other bacteria and fungi same thing was noticed, that methanol is showing more antimicrobial activity than ethanol and other solvents [18].

Here we can see that methanol extract of stem and roots are showing more zone of inhibition than ethanol extracts. Control used for bacteria is Ciprofloxacin and for fungi it's Amphoterecin. Methanolic extract have shown higher zone of inhibition as compared to ethanolic extract. Methanolic extract have higher potency for bacterial and fungal infections. Different extract have shown different activity for bacterial and fungal strains but ethanolic root extract have shown minimum zone of inhibition and so it may be concluded that ethanolic extract of roots doesn't have good antifungal activity but it possess good anti bacterial activity. It is clear from the above figures that methanoloic extract of roots and stem showed maximum inhibitory action against bacterial as well as fungal strains. The results are depicted in figure 5 to 8.

The results of the present study are encouraging as all the investigated plants showed antimicrobial potential, although the method of extraction and the plant part used affected their antimicrobial activity. Despite the tremendous progress in human medicines, infectious diseases are still a major threat to public health. Their impact is intense in the developing countries particularly due to relative unavailability of the medicines and the emergence

of drug resistance [19]. In case of immunocompromised, AIDS and cancer patients, drug resistant bacteria, virus and fungal pathogens have further complicated the treatment of infectious diseases [20]. Thus, in the present scenario of multiple drug resistance to infectious pathogens, it is important that the search for new antimicrobial substance from alternative sources including plants be initiated [21]. Contrary to synthetic drugs, antimicrobials from plants are safe and possess effective therapeutic potential to treat several infectious diseases [22].

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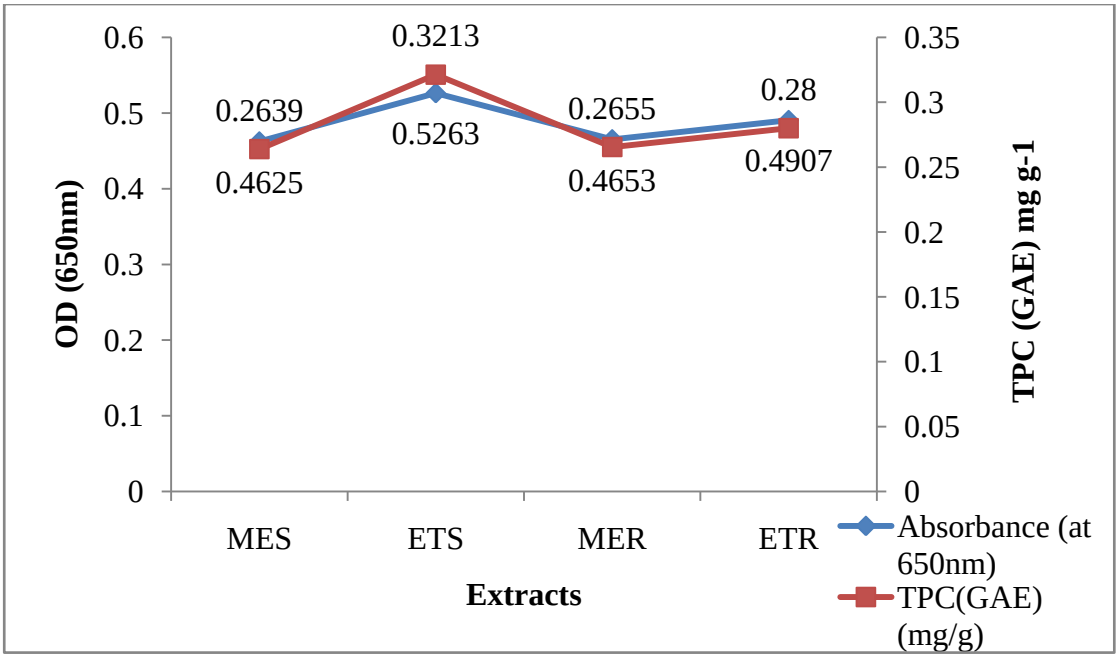

Fig.1. Total polyphenolic contents (TPC) of *T. cordifolia* of Stem and Roots extract in terms of Gallic acid.

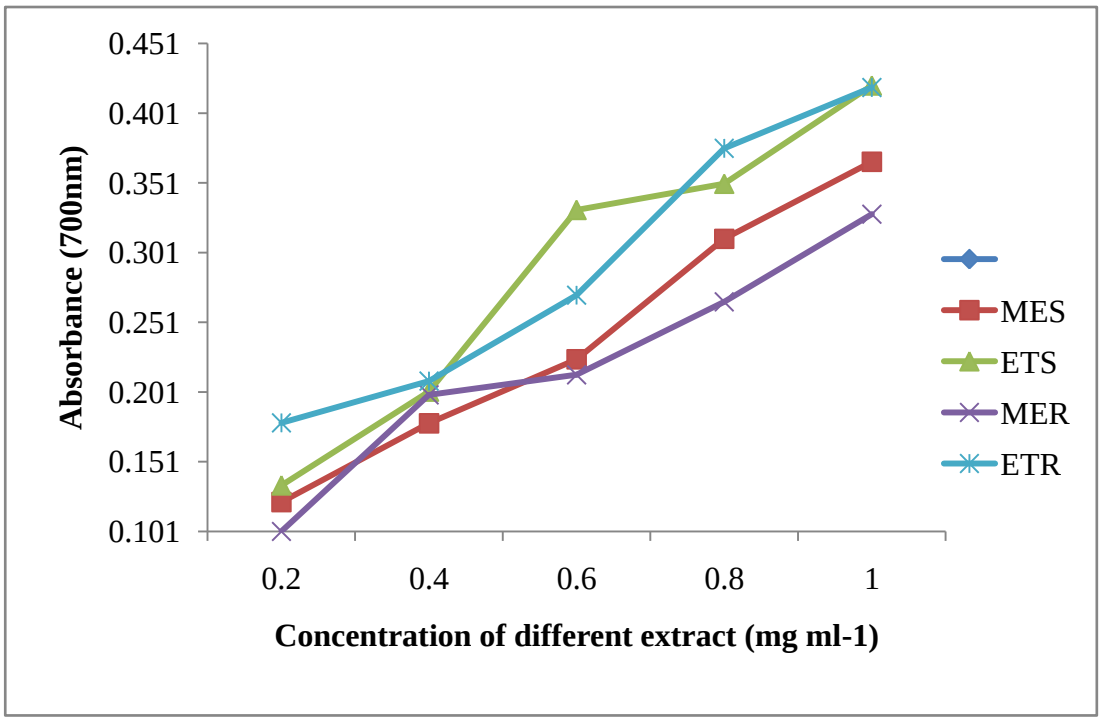

Fig.2. Reducing Power Activity of *T. Cordifolia* of Methanolic and Ethanolic Stem and Roots Extract

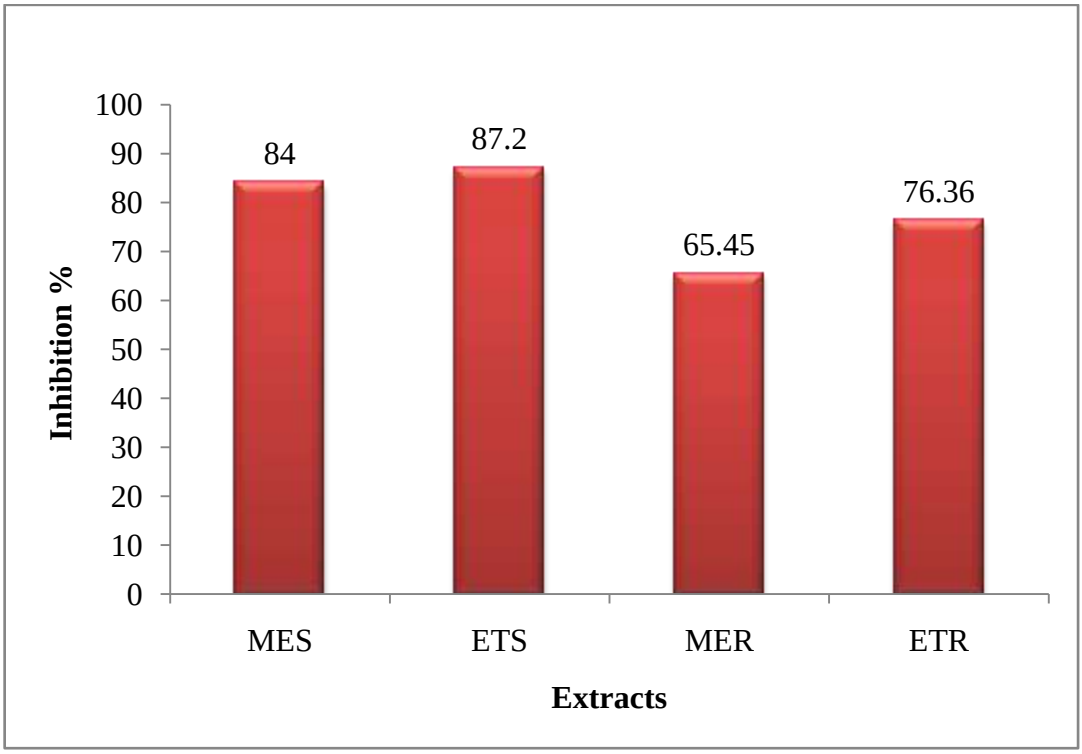

Fig.3. Hydrogen peroxide scavenging assay *T. cordifolia* of Stem and root extract

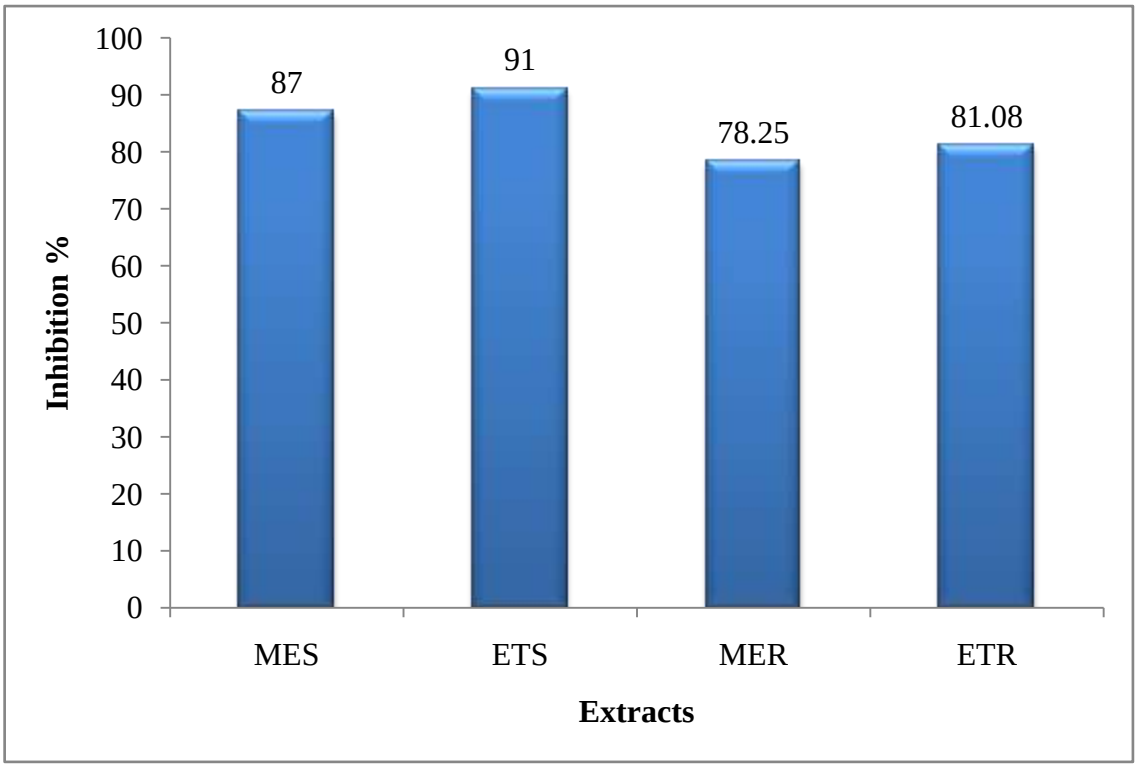

Fig.4. Hydroxyl radical scavenging activity of *T. Cordifolia* of Stem and root extracts

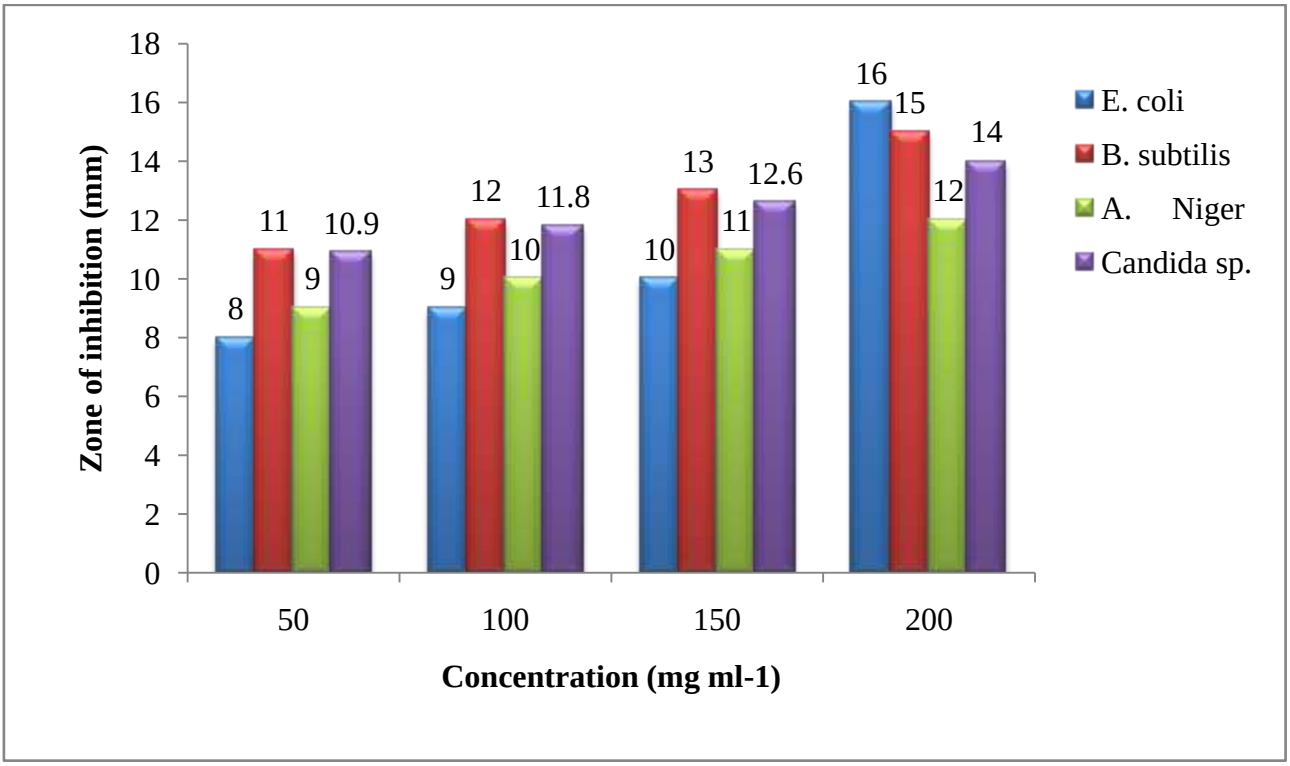

Fig.5. Antimicrobial activity of *T. cordifolia* steam extract (Methanolic)

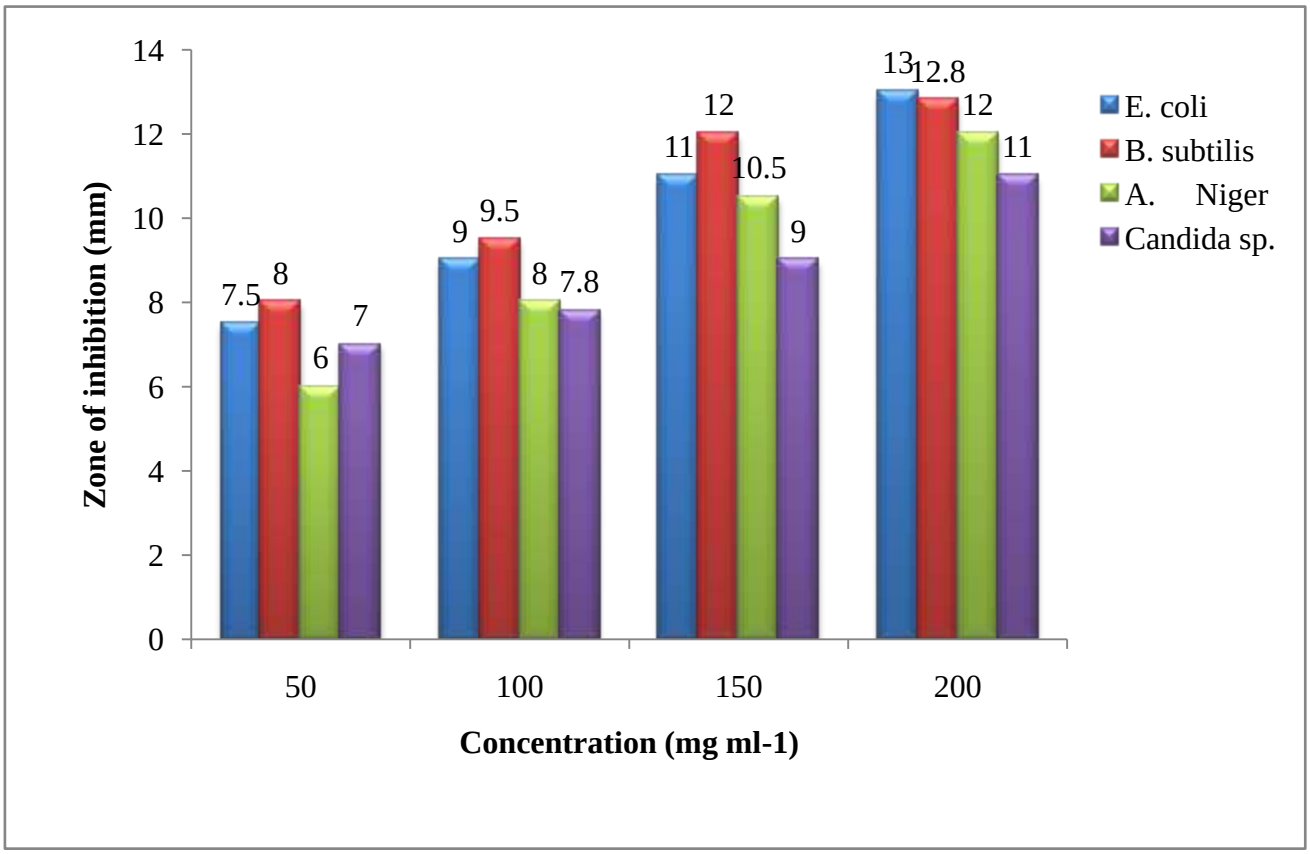

Fig.6. Antimicrobial activity of *T. cordifolia* root extract (Methanolic)

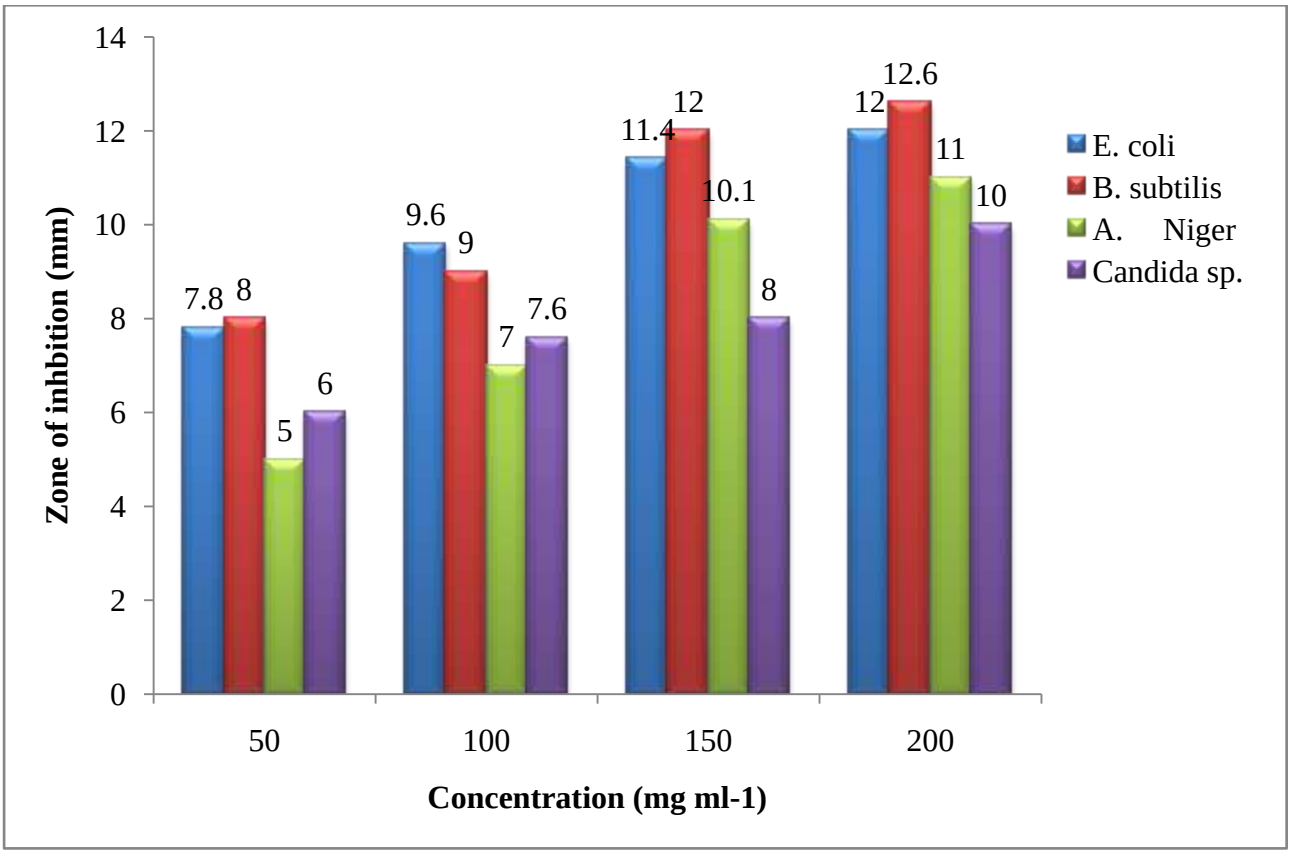

Fig.7. Antimicrobial activity of *T. cordifolia* stem extract (Ethanolic)

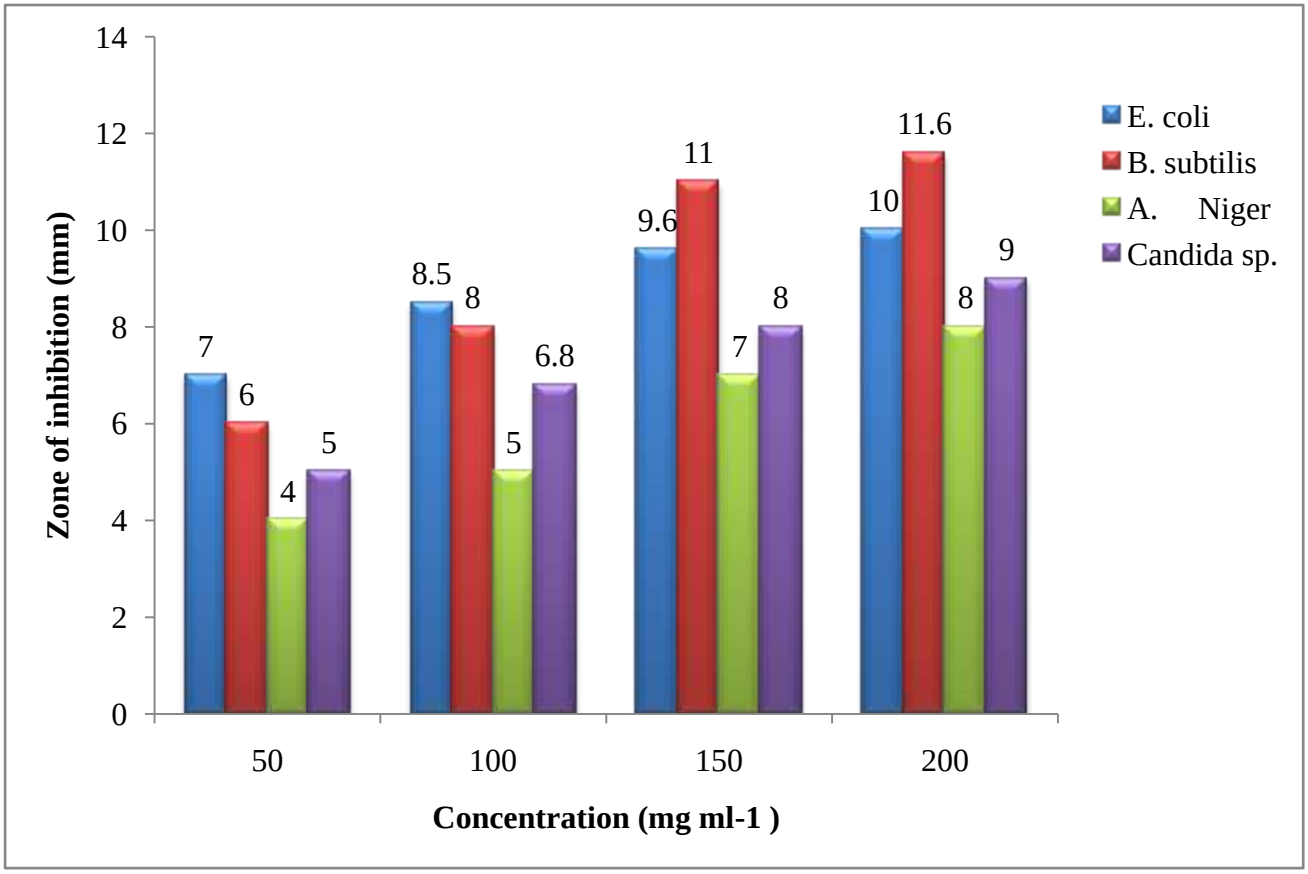

Fig.8. Antimicrobial activity of *T. cordifolia* root extract (Ethanolic)

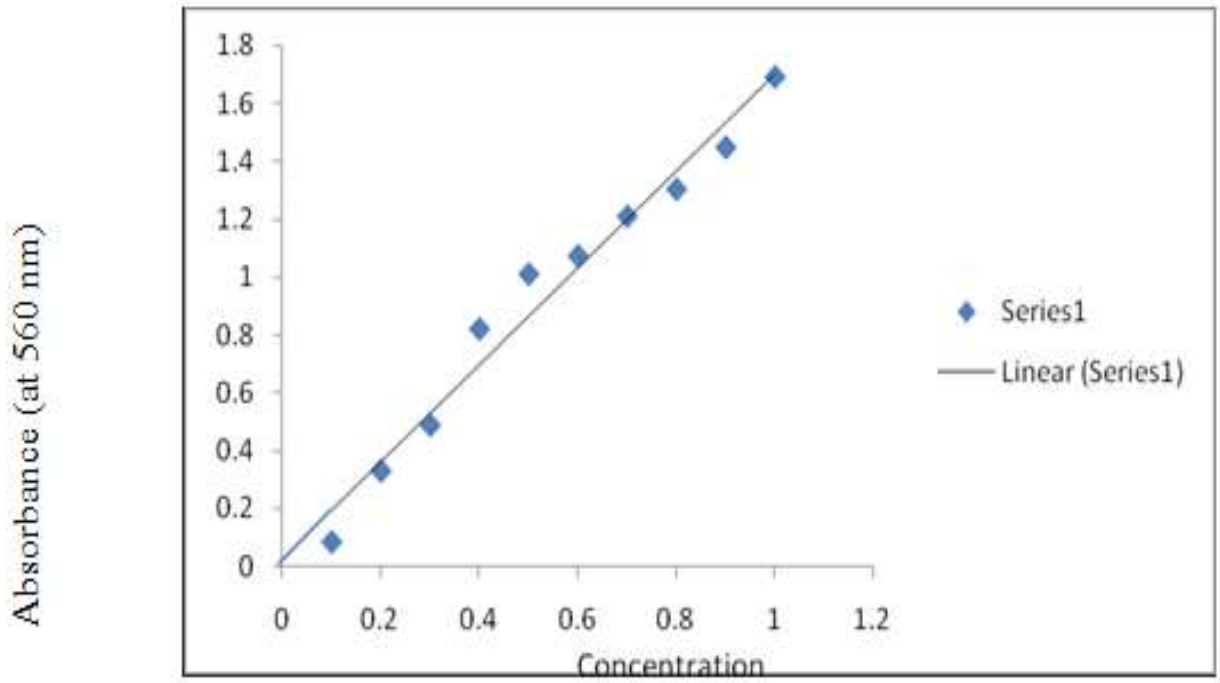

Fig.9. Standard calibration curve for Gallic acid.

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