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CLINICAL APPLICATIONS OF HEXANE EXTRACT OF *CEIBA PENTANDRA* LEAVES EXTRACTS ON ANTIOXIDANT ACTIVITY AND URINARY TRACT INFECTION PATHOGEN CONTROL

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ABSTRACT

Urinary tract infection is the major disease to human beings, particularly it cause severe problem to women. *Ceiba pentandra* is the commonly available plant may have lot of bio medical application. In this present investigation we used hexane extract of *Ceiba pentandra* for the controlling of urinary tract infection causing pathogens *Bacillus subtilis*, *E. coli*, *Klebsiella pneumonia* and *Pseudomonas* sp. the plant extracts are tested for its phytochemicals using various test of Carbohydrates/glycosides, Phenolic compounds, Alkaloids, Flavonoids, Steroids and Triterpenoids, Saponins, Fat and Oils and Tannins. The plant extracts are having very good antioxidant activity was confirmed usong non enzymatic antioxidant assay (Free radical scavenging assay) of DPPH radical assay, Nitric oxide radical inhibition assay and hydroxyl radical scavenging assay.

KEYWORDS: Antioxidant, urinary tract infection, *Ceiba pentandra*, antibacterial actvtiy.

INTRODUCTION

Scientific classification of *Ceiba pentandra*

Kingdom: Plantae
Division: Angiosperms
Class: Eudicoids
Order: Malvales
Family: Malvaceae
Genus: *Ceiba*
Species: *Ceiba pentandra* Linn

Figure: *Ceiba pentandra* leaves and tree.

C. pentandra can be found in various types of moist evergreen and deciduous forests, as wellas in dry forests andgallery forests. As a pioneer species, it mostly occurs in secondary forests (Figure 1). *Ceiba pentandra* having good anti-fungal activity against disease causing fungus namely *Epidermophyton flocosum*, *Microsporum canis*, *Trichopyton rubrum* and *Candida albicans.* The fungicidal effects while others had fungistatic effect on the organism (Nwachukwu et al., 2008). The hexane extract of extract of *Ceiba pentandra*roots on Ethanol(EtOH)-induced ulcer and Pylorus ligated (PL)-inducedulcers in rats showing good anti-ulcer activity and the result indicated a dose- dependent antiulcerogenic activity in *C. Pentandra* (Bhushan et al., 2011). The anti-inflammatoryactivity of two new isoflavoneglucosidevavain 3¢-*O*-*â*-Dglucoside and its aglycon, vavain, from the bark of *Ceiba pentandra*, together with the known flavan-3-ol, (+)-catechin was proved (Ylva Noreen et al 1998). The hypoglycaemic activity was reported in the *Ceiba pentandra* root bark extract in normal and alloxaninduced diabetic rats. (Saif-ur-Rehman *et al* 2010). The hypoglycaemic and hypolipidaemic effects of feed prepared with *Ceiba pentandra* leaves was investigated in alloxan induceddiabetic rats. The good hypolipidaemic activity was observed due to the reduction of plasma glucose, plasma lipids {totalcholesterol (TC), triglyceride (Tg), high density lipoprotein(HDL), low density lipoprotein (LDL) and very low densitylipoproteins (VLDL)}, total protein and albumin were determined in the induced rats.

(Aloke et al., 2011). The Hepatoprotective activity was reported the protective activity of ethylacetate fraction of methanol extract of stem bark of *Ceiba pentandra*against paracetamol-induced liver damage in rats. A significant reduction in serum enzymes GOT, aspartate aminotransferase, alkaline phosphatase, total bilirubin content and histopathological screening in the rats treated gave indication that ethyl acetate fraction of mhexane extract of extract of *Ceiba pentandra* (Bairwa et al., 2011).

In this present study we have collected the *Ceiba pentandra* leaves from local area. And prepared the leaf powder from dried leaves and prepared the *Ceiba pentandra* leaf extracts by using Hexane. The phytochemicals analysed using various biochemical analysis. The antimicrobial activity of hexane extract of *Ceiba pentandra* tested against *Pseudomonas sp., Bacillus subtilis, Escherichia coli* and *Klebsiella pneumonia*. Finally the we analyze the free radical scavenging activity *of Ceiba pentandra* leaves extract using DPPH radical scavenging activity, Nitric oxide radical inhibition assay and Hydroxyl radical scavenging assay.

MATERIALS AND METHODS

Collection of plant material

The medicinal plant *Ceiba pentandra* were collected from Wandiwash, Tiruvanamalai district Tamilnadu, India.

PREPARATION OF PLANT EXTRACT

After the collection of *Ceiba pentandra* medicinal leafs plant were placed in clean tray and allowed for shade drying. The plant were subjected to surface sterilization using tween 20 and then dried in shade. The dried plant leafs were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve.

The *Ceiba pentandra* leafs plants powdered (250g) was defatted by treating with pet-ether and then extracted with hexane solvent by using Soxlet apparatus. The solvent was removed under vacuum to get the solid mass. The residue was weighted and stored in air and water proof containers, kept in refrigerator at 4^0C. From this stock, fresh preparation was made whenever required.

Preliminary phytochemical analysis

Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test

Filtrates were treated with 2 drops of alcoholic α-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 dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides

Extracts were hydrolysed with dil. HCl and then subjected to test for glycosides.

Modified Borntrager's Test

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test

Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test

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

Wagner's Test

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's 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 Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

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 phytosterols
Salkowski's Test
Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Libermann Burchard's test
Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of saponins
Froth Test
Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

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

Test for Fixed oils and Fats
a. Spot test
A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

b. Saponification test
A few drops of 0.5 N alcoholic potassium hydroxide solution is added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated on a water bath for 2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

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.

Antibacterial activity of Plant Extract
The bacterial cultures are isolated from urinary tract infected sample. The urinary tract infected pathogens are identified as *Bacillus subtilis*, *E. coli*, *Klebsiella pneumonia* and *Pseudomonas* sp. The antibacterial activity of hexane extracts of *Ceiba pentandra leaves* were performed by agar well diffusion method against pathogenic bacteria, *Bacillus subtilis*, *E. coli*, *Klebsiella pneumonia*, and *Pseudomonas*. Fresh overnight culture of each strain was swabbed uniformly onto the individuals plates containing sterile Luria Bertani agar and 5 wells were made with the diameter of 6 mm. Then 20 µL, 40 µL, 60 µL, 80 µL and 100 µL of purified plant extracts of *Ceiba pentandra leaves* were poured into each well and commercial antibiotic discs are placed as control and incubate for 24 h at 37^{o} C. After incubation the different levels of zonation formed around the well and it was measured. This experiment was repeated for three times.

Non enzymatic antioxidant assay (Free radical scavenging assay)
DPPH radical assay
The DPPH free radical scavenging activity of hexane extract of extract of *Ceiba pentandra leaves* was determined by according to the method of Gyamfi (1999). Typically, different concentration (2-10 µg/ml) of plant extract was mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 µl of 50 mM Tris-HCl buffer (pH 7.4) and incubated for 30 min. After incubation, the reduction in the number of DPPH free radicals was measured based on the absorbance at 517 nm. BHT was used as controls. The percent inhibition was calculated from the following equation:
% Inhibition = [Absorbance of control – Absorbance of test sample/Absorbance of control] ×100

Nitric oxide radical inhibition assay
Nitric oxide radical inhibition activity of plant extract can be estimated by the use of Griess Illosvoy reaction (Ebrahimzadeh et al 2010) with slight modifications i.e. using naphthyl ethylene diamine dihydrochloride (0.1% w/v). The reaction mixture prepared by mixing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and *Ceiba pentandra leaves* extract (10-100 µg/ml) or standard solution (rutin, 0.5 ml). This reaction mixture was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture was mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. The absorbance of these pink colour solutions was measured at 540 nm against the corresponding blank solutions. Rutin used as a standard.
% Inhibition = [Absorbance of control – Absorbance of test sample/Absorbance of control] ×100

Hydroxyl radical scavenging assay
Hydroxyl radical scavenging assay using plant extract was performed by Halliwell et al method (1987) with slight changes. To this assay, 1.0 ml of the reaction mixture contained 100 µl of 28 mM 2-deoxy-2-ribose (dissolved in phosphate buffer, pH 7.4), 500 µl solution of various concentrations of the *Ceiba pentandra leaves* (10 to 100 µg/ml), 200 µl of 200 µM $FeCl_3$ and 1.04 mM EDTA (1:1 v/v), 100 µl H_2O_2 (1.0 mM) and 100 µl ascorbic acid (1.0 mM) and incubated at 37°C for 1

hour. The amount of deoxyribose degradation was measured by the TBA reaction (Bouchet 1998). Measure the absorbance at about 532 nm against the blank solution. Vitamin E was used as a positive control. % hydroxyl inhibition = (Abs of the control- Abs of the extract sample/Abs of the control × 100.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of Hexane extract of *Ceiba pentandra* leaves

S. No	Phytochemical constituents	Hexane extract of *Ceiba pentandra* leaves
1	**Carbohydrates/glycosides**	Absent
2	**Phenolic compounds**	Present
3	**Alkaloids**	Absent
4	**Flavonoids**	Present
5	**Steroids and Triterpenoids**	Present
6	**Saponins**	Absent
7	**Fat and Oils**	Present
8	**Tannins**	Present

Antimicrobial activity of Plant leaves

The antibacterial activity of hexane extract of *Ceiba pentandra* leaves extract shown in the figures 2-5. It clearly indicates the antibacterial capability of the medicinal plant *Ceiba pentandra.* There are three different plant extracts concentrations 25 µl, 50 µl and 75 µl were tested against the bacterium like *Bacillus subtilis*, *E. coli*, *Klebsiella pneumonia* and *Pseudomonas.* These bacteria are Pathogenic and opportune bacterial isolates. In this the 75 µl concentration shows the maximum zone of inhibition indicates the concentration of plant extract having role in the killing of bacteria.

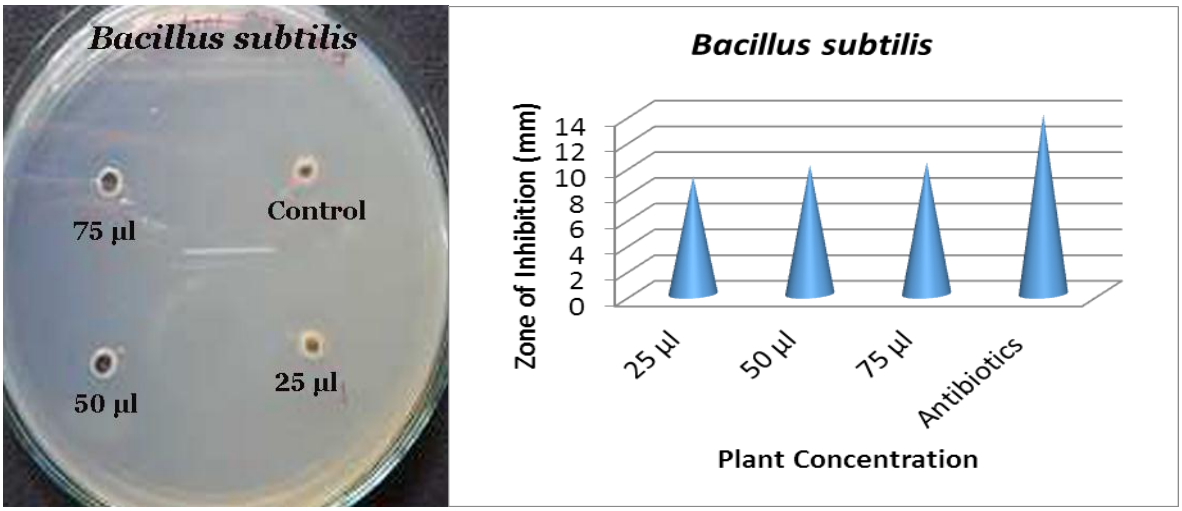

Figure 2: Antibacterial activity of hexane extract of *Ceiba pentandra* leaves extract against *Bacillus subtilis.*

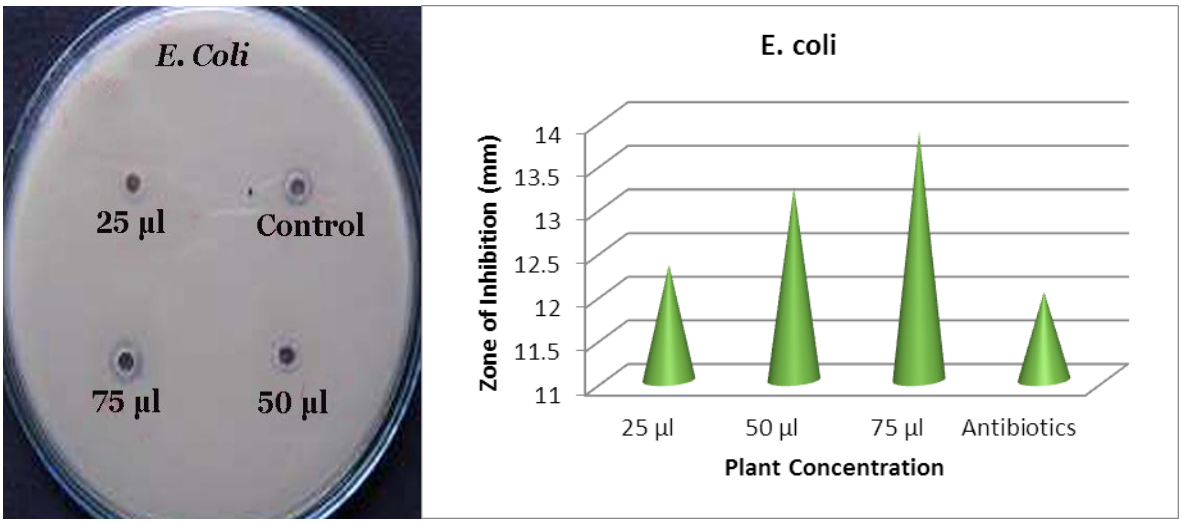

Figure 3: Antibacterial activity of hexane extract of *Ceiba pentandra* leaves extract against *E. coli.*

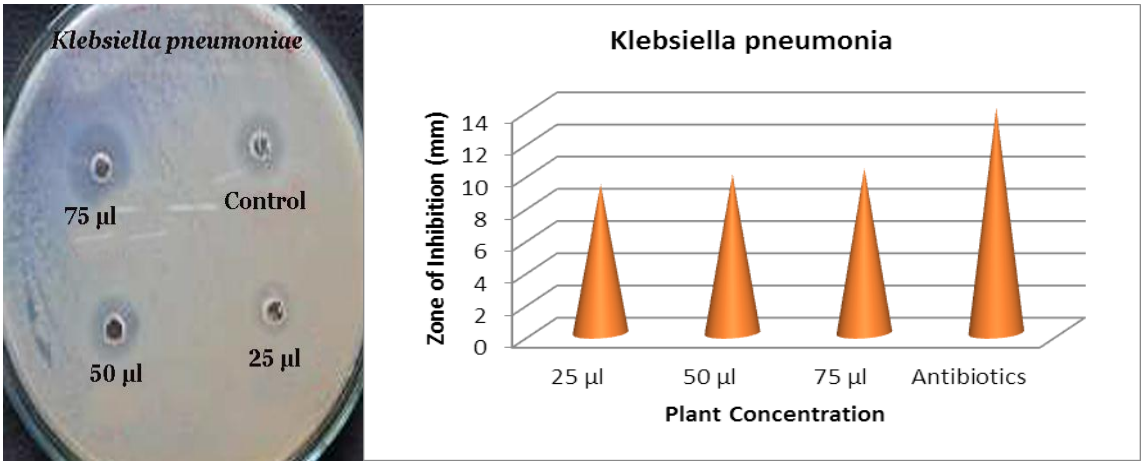

Figure 4: Antibacterial activity of hexane extract of *Ceiba pentandra* leaves extract against *Klebsiella pneumoniae*.

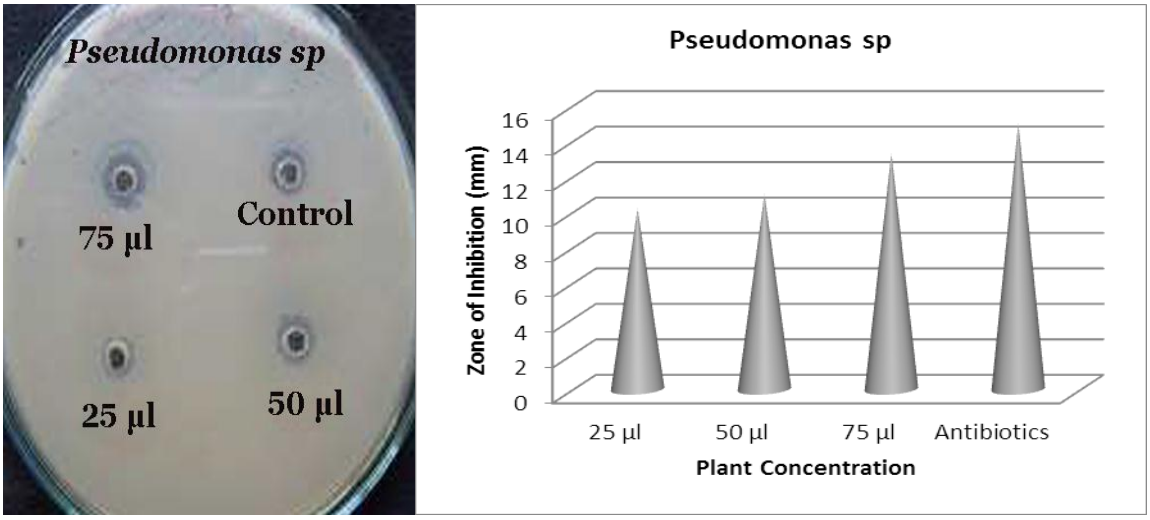

Figure 5: Antibacterial activity of hexane extract of *Ceiba pentandra* leaves extract against *Pseudomonas sp*.

DPPH radical scavenging activity

The DPPH radical scavenging activity of hexane extract of *Ceiba pentandra* leaves exhibited a significant dose dependent i.e. concentration of plant extract between 0.5 to 2.5 µg/ml. IC50 values calculated as 50% of inhibition by plant extract concentration. The results of DPPH inhibition by hexane extract of *Ceiba pentandra* leaves extract are shown in Figure 6. The 50% radical scavenging activity was observed at 2.0 µg/ml concentration of hexane extract of leaves extract. The IC 50 value of the extract was highly significant than the standard (5.65µg/ml).

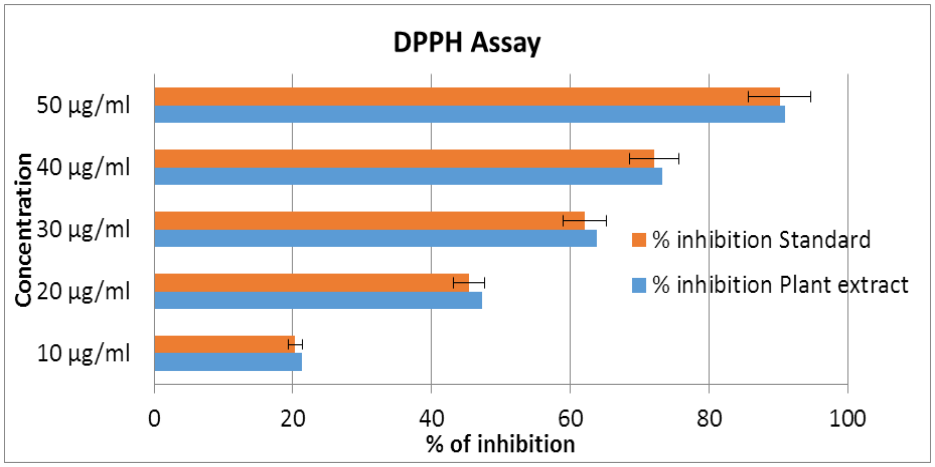

Figure 6: Effect of hexane extract of *Ceiba pentandra* leaves extract and Standard Vitamin C on scavenging of DPPH radical Results.

Nitric oxide radical inhibition assay

The nitric oxide radical scavenging activity of hexane extract of *Ceiba pentandra* leaves extract was increased while increasing the concentration of plant extract in a dose dependent manner (Figure 6). The IC50 value of the hexane extract of *Ceiba pentandra* leaves extract was 92.45 µg/ml which was similar to that of standard (91.65 µg/ml). These results showed that hexane extract of *C. pentandra* leaves is known to be an excellent antioxidant agent.

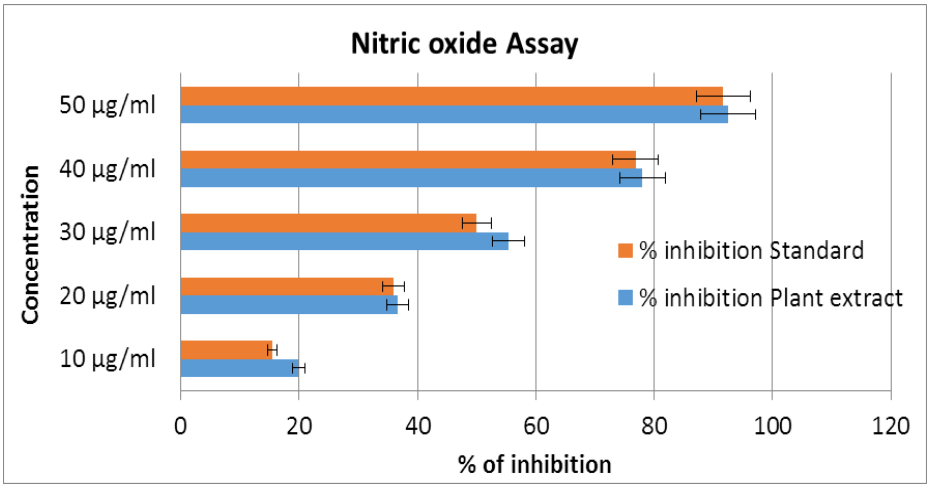

Figure 7: Effect of hexane extract of *Ceiba pentandra* leaves extract on Nitric oxide radical inhibition assay.

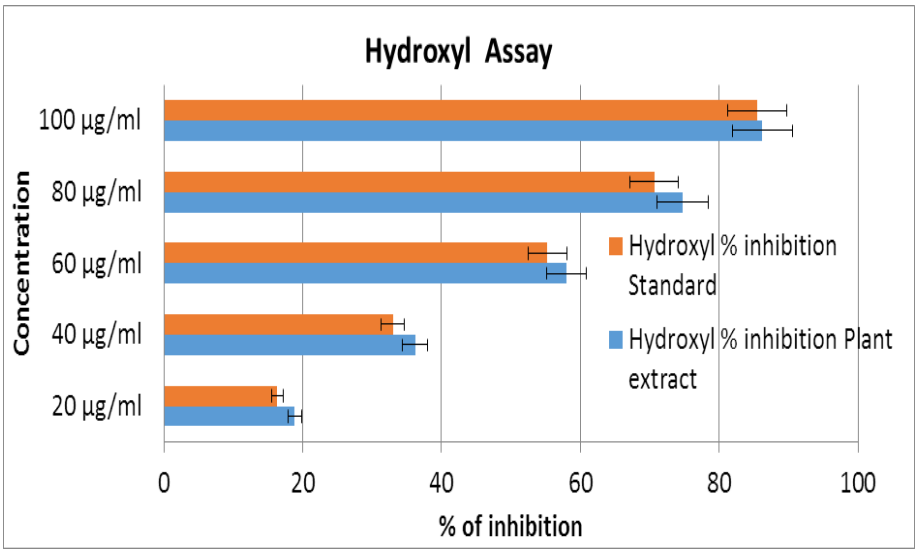

Figure 7: Effect of hexane extract of *Ceiba pentandra* leaves extract on Nitric oxide radical inhibition assay.

Hydroxyl radical scavenging assay

Figure 8 showed the hexane extract of *Ceiba pentandra* leaves extract have high inhibition activity against hydroxyl radical. The scavenging activity of plant extract against hydroxyl radical was observed by deoxyribose assay in a concentration dependent manner. It showed hydroxyl radical scavenging activity with about 50% at concentration of 30.0 µg/ml. The results are shown in Figure 8, the concentrations of 50% inhibition were found to be 30.0 µg/ml and 35.5 µg/ml for the extract and standard of vitamin E, respectively. The extract inhibition value was found to be lesser than the standard.

CONCLUSION

Herbal drugs are traditional method of treating the diseases in worldwide, the plant having ability to treat the diseases also known as medicinal plant. Several types of medicinal plants are breathing in the nature and effective in different type of diseases. In traditional systems of medicine, different parts (leaves, stems, roots and even whole plant) of *Ceiba pentandra* have been recommended for the treatment of bronchitis, diabetics, diarrhoea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, insect bite etc. All parts of this plant have numerous therapeutic activities for the treatment of a variety of diseases. It is known as a rich source of tannins, flavonoids and glycosides. Our present research investigation revealed that *Ceiba pentandra* is important medicinal plant with diverse pharmacological spectrum. The plant shows the presence of many chemical constituents which are responsible for varied pharmacological and medicinal property. The evaluation needs to be carried out on *Ceiba pentandra* in order to

uses and formulation of the plant in their practical clinical applications, which can be used for the welfare of the mankind.

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