

Anti-inflammatory, Antioxidant, Antibacterial effect and Phytochemical Analysis of *Mucuna pruriens* seed extract

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Abstract : The ethanol extracts of seeds of the medicinal plant *Mucunapruriens* was screened their phytochemical characters for their antimicrobial, antioxidant and anti-inflammatory activities. GC-MS analysis revealed that the presence of 6 compounds at the retention time with minor peaks. These peaks were corresponds to Ethane, 1, 2, 2- trichloro-1,1-difluoro-, Hexadecanoic acid, Ethyl ester, N,N-Dimethyl-2-Cyclohexyloxyethylamine, CyclotrisiloxanE, Hexamethyl, Trimethyl[4-(2-Methyl-4-oxo-2-Pentyl)Phenoxy]Silane, Silicic acid, Diethyl bis(trimethylsilyl) ester. The ethanol extract was found to possess the highest antimicrobial activity against harmful pathogenic microorganisms. The scavenging activity of the extract against DPPH, Nitric oxide and hydroxyl free radicals revealed excellent potential comparable to that standard. Ethanolic seed extract showed significant anti-inflammatory activity and the inhibition percentage against protein denaturation and proteinase action is 73% and 64% respectively and the concentration is 300 µg/ml.

Keywords : *Mucuna pruriens*; Antibacterial; antioxidant; free radical scavenging; GC-MS analysis.

Introduction

Medicinal plants have high amount and variety of phytochemicals like flavonoids, alkaloids, polyphenolsetc^{1, 2}. This served as a rich source of therapeutic agents. Medicinal plants are the basis for production of synthetic drug³. The natural herbal products highly consumed to treat variable harmful diseases are attracting great interest to discover and develop a new drug^{4, 5, 6}. In recent years, plants products are used in alternative therapies and a small kind of research has been properly examined phytochemically in terms of their pharmacological properties.

The plant *Mucuna pruriens* is tropical legume, belongs to the genus *Mucuna* of which consists of 100 species of climbing vines and shrubs mostly distributed in the tropical areas especially in tropical Africa, India and the Caribbean. The genus *Mucuna* belongs to the family Leguminosae⁷ and is commonly known as velvet bean or cowitch. Seeds are 12 mm long, ovoid and its colour is shiny black or brown^{8, 9, 10}. This plant is enormously consumed by pharmaceutical industries due the presence of high content of L-3,4- dihydroxy phenyl alanine (L-DOPA) in the *Mucuna* seeds¹¹. It is an unusual non protein amino acid. This protein is used to treatment of Parkinsonism disease. The other chemical compounds such as, mucunain⁹, glutathione lecithin, gallic acid,β- sitosterol and serotonin. Commonly, the mature seed contains about 3.1 to 6.1% L-DOPA, with trace amounts of 5-hydroxy tryptamine (serotonin), nicotine, dimethyl tryptamine (DMT), bufotenine, 5-MeO-DMT and beta-carboline¹². It is used against a wide range of disorders, such as urinary tract, neurological and menstruation disorders, constipation, edema, fever, tuberculosis, ulcers, PD¹³ and helminthiasis like elephantiasis¹⁴. The seeds of *M. pruriens* have many pharmacological activity including astringent, laxative,

astringent, laxative, anthelmintic, aphrodisiac, alexipharmic and tonic^{14, 15}. Several studies also showed that seed possesses powerful antimicrobial activities¹⁶.

Ethyl acetate and methanol extract of *M. pruriens* exhibited high antioxidant and free radical scavenging activity due to the presence of large amount of phenolics compounds^{17, 18}. The aqueous extract of the seeds of *M. pruriens* exhibited hypoglycemic activity¹⁹ and anti-venom activity^{20, 21, 22}. The aim of this investigation is to prepare and identify the phytochemicals present in the Ethanolic extract of *M. pruriens* seeds using GC- MS analysis. Finally analyse the therapeutic applications are antimicrobial, anti-inflammatory and in-vitro antioxidant activities of *M. pruriens* seeds.

Materials And Methods

Collection and Drying of plant materials

The dried seeds of *Mucuna pruriens* were collected from Chennai in Tamil Nadu. The seeds were washed thoroughly three times with water and once with distilled water. The plant materials were air dried and powdered. The powdered samples were hermetically sealed in separate polythene bags until the time of extraction.

Preparation of plant extract

About 5 g of powdered seeds were extracted successively with 100 ml of ethanol at 40-50°C in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use. Compound identification was examined by using GC-MS analysis by comparing the retention times with those of authentic compounds and the spectral data obtained from library data of the corresponding compounds.

Antibacterial activity of Plant Extract

The antibacterial activity of ethanol extracts of *Mucuna pruriens* seeds were performed by agar well diffusion method against pathogenic bacteria, *Streptococcus sp*, *Klebsiella planticola*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The inoculums were spread on the Muller Hinton agar medium and 5 wells with 6 mm diameter were made. Then different concentration of (20, 40, 60, 80, 100 µL) purified seed extracts of *Mucuna pruriens* leaf extract were added and commercial antibiotic discs are maintained as control. Plates were incubated for 24 hours at 37°C. After incubation the results of plates were examined and recorded, as the presence or absence of zone around the well. This experiment was repeated for three times.

Non enzymatic antioxidant assay (Free radical scavenging assay)

DPPH radical assay

Typically, different concentration (10-50µg/ml) of seed extract was mixed with 1ml of 0.1mM DPPH in methanol solution and 450µl of 50mM Tris-HCl buffer (pH 7.4) and incubated for 30 min and inhibition was measured absorbance at 517nm. BHT was used as control²³. The percentage of inhibition was calculated from the following equation:

$$\% \text{Inhibition} = \left[\frac{C_{\text{abs}} - T_{\text{abs}}}{C_{\text{abs}}} \right] \times 100$$

Nitric oxide radical inhibition assay

Nitric oxide radical inhibition activity of plant extract can be estimated by the use of Griess-Ilosvay reaction. The reaction mixture prepared by mixing sodium nitroprusside (10mM, 2ml), phosphate buffer saline (0.5ml) and *Mucuna pruriens* seed extract (10-50µg/ml) or standard solution (rutin, 0.5 ml). This reaction mixture was incubated at 25°C for 150 min and 0.5 ml of this reaction mixture was mixed with 1ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid), then stand up for 5 min for completing diazotization. Then, 1ml of naphthylethylenediamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. The absorbance of these pink color solutions was measured at 540nm against the corresponding blank solutions.

$$\% \text{Inhibition} = [C_{\text{abs}} - T_{\text{abs}} / C_{\text{abs}}] \times 100$$

Hydroxylradical scavenging assay

To this typical assay, 100 μ l of 28 m M2-deoxy-2-ribose, 500 μ l solution of various concentrations of the *Mucuna pruriens* (10 to 50 μ g/ml), 200 μ l of 200 μ M FeCl₃ and 1.04 mM EDTA (1:1 v/v), 100 μ l H₂O₂ (1.0 mM) and 100 μ l ascorbic acid (1.0 mM) and incubated for 1 hour at 37°C. After incubation, the absorbance of reaction mixture was measured at about 532 nm against the blank solution. Vitamin E was used as a positive control.

$$\% \text{Inhibition} = [C_{\text{abs}} - T_{\text{abs}} / C_{\text{abs}}] \times 100$$

Anti-inflammatory activity of Amazing bean

Inhibition of albumin denaturation

The inhibitory activity of ethanolic seed extract was determined according to Mizushima et al and Sakat et al^{24, 25}. Typically, different concentrations of extract were mixed with 1% bovine albumin fraction and were incubated at 37 °C for 20 min. After incubation the reaction mixture was heated at 51 °C for 20 min then allow cooling. After cooling, the absorbance of the reaction mixture was measured at 660 nm. The experiment was performed in triplicate. The inhibition percentage of protein denaturation was calculated as follows:

$$\% \text{ inhibition} = (\text{Control}_{\text{Abs}} - \text{Sample}_{\text{Abs}}) \times 100 / \text{control}_{\text{Abs}}$$

Inhibition of haemolysis

The inhibition of haemolysis was evaluated by following the method²⁶. To this analysis, 1 ml of different concentration of seed extract (50-300 μ g/ml) was mixed with 1 ml of 10% RBCs suspension, saline solution instead of extract was added to the control. Aspirin was used as a standard drug. The reaction mixtures were centrifuged and incubated at 56 °C for 30 min in a water bath, then cool the solution. After cooling the reaction mixtures were centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was measured at 560 nm. The Percentage inhibition of Haemolysis was calculated as follows:

$$\% \text{ inhibition} = (\text{Control}_{\text{Abs}} - \text{Sample}_{\text{Abs}}) \times 100 / \text{control}_{\text{Abs}}$$

Results and Discussion

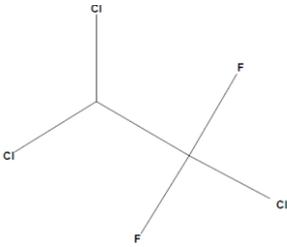
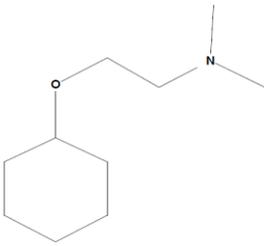
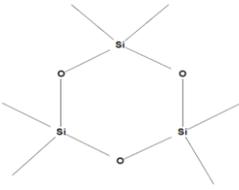
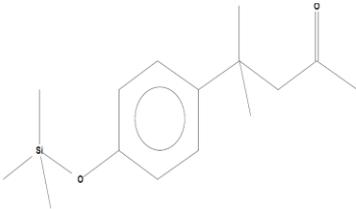
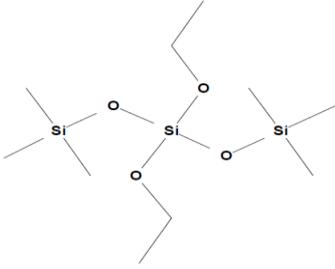
GC-MS Analysis of ethanolic *Mucuna pruriens* seeds extract

The presence of phytochemical constituents in the *M. pruriens* seed extracts which is responsible for the antimicrobial, antioxidant and anti-inflammatory activity was analyzed by using GC-MS. Figure 1 shows the phytochemical screening results showed 6 retention peaks. The retention time and molecular weight of the active compounds present in the *M. pruriens* seed extract was tabulated (Table 1). The prevailing compounds are Ethane, 1, 2, 2-trichloro-1,1-difluoro-, Hexadecanoic acid, Ethyl ester, N,N-Dimethyl-2-Cyclohexyloxyethylamine, Cyclotrisiloxan E, Hexamethyl, Trimethyl[4-(2-Methyl-4-oxo-2-Pentyl)Phenoxy] Silane, Silicic acid, Diethyl bis(trimethylsilyl) ester. Hexadecanoic acid and ethyl ester are well known antioxidant compound. However, these compounds may be responsible for biological activities.

Antimicrobial activity of Plant leaves

The antibacterial activity of ethanolic *Mucuna pruriens* seeds extract was evaluated against pathogenic bacteria. The inhibitory zone around the well indicated the absence of bacterial growth and the diameters of the zones were measured and recorded (Figure 2-5). The activity of ethanolic seed extract was compared with that of standard antibiotic tetracycline. The result shows the minimum inhibitory concentration (MIC) of seed extract against *Streptococcus sp*, *Klebsiella planticola*, *Klebsiella pneumoniae* and *Staphylococcus aureus* are 23.3 μ g, 16.5 μ g, 20.7 μ g, and 25 μ g, respectively. The higher antibacterial activity of the ethanolic extract of *M. pruriens* seeds due to the presence of active metabolites like flavonoids, alkaloids and steroids^{27, 28, 34}.

Table 1: GC- MS Analysis of Ethanolic extract of *Mucuna pruriens* seeds

Rt value	Compound	Molecular weight	Structure
2.58	Ethane, 1, 2, 2- trichloro-1,1- difluoro-	168	
18.56	Hexadecanoic acid, Ethyl ester	284	
21.11	N,N-Dimethyl-2-Cyclohexyloxyethylamine	171	
26.92	Cyclotrisiloxane, Hexamethyl	222	
28.95	Trimethyl[4-(2-Methyl-4-oxo-2-Pentyl)Phenoxy]Silane	264	
29.66	Silicic acid, Diethyl bis(trimethylsilyl) ester	296	

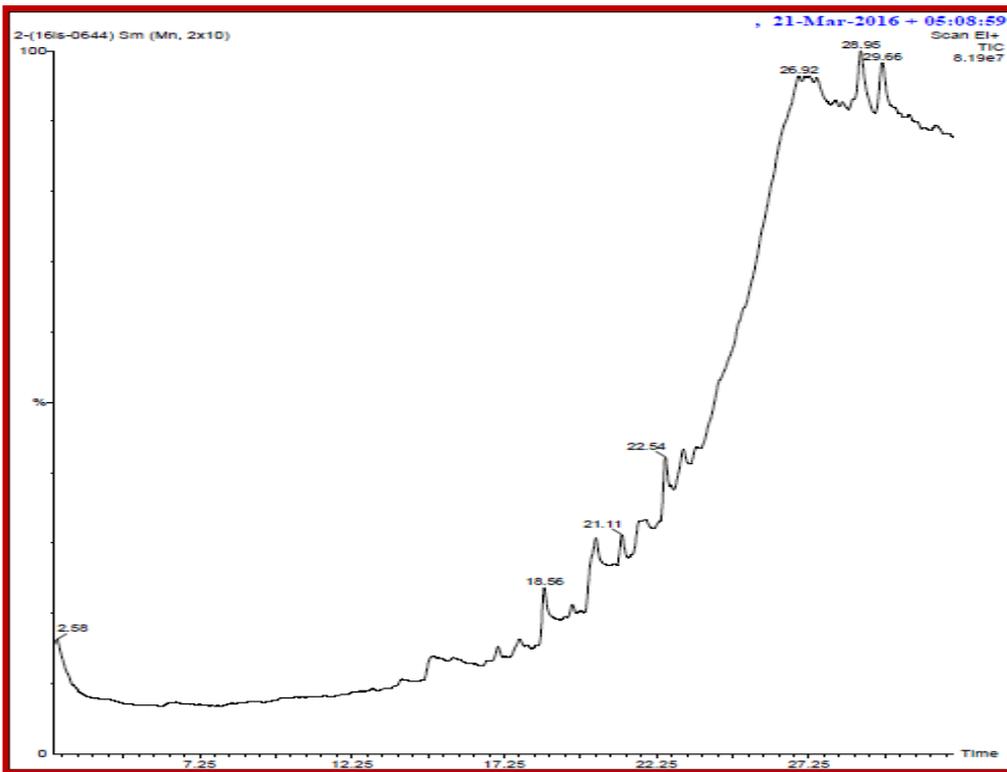


Figure 1: GC-MS of ethanolic *Mucuna pruriens* seed extract

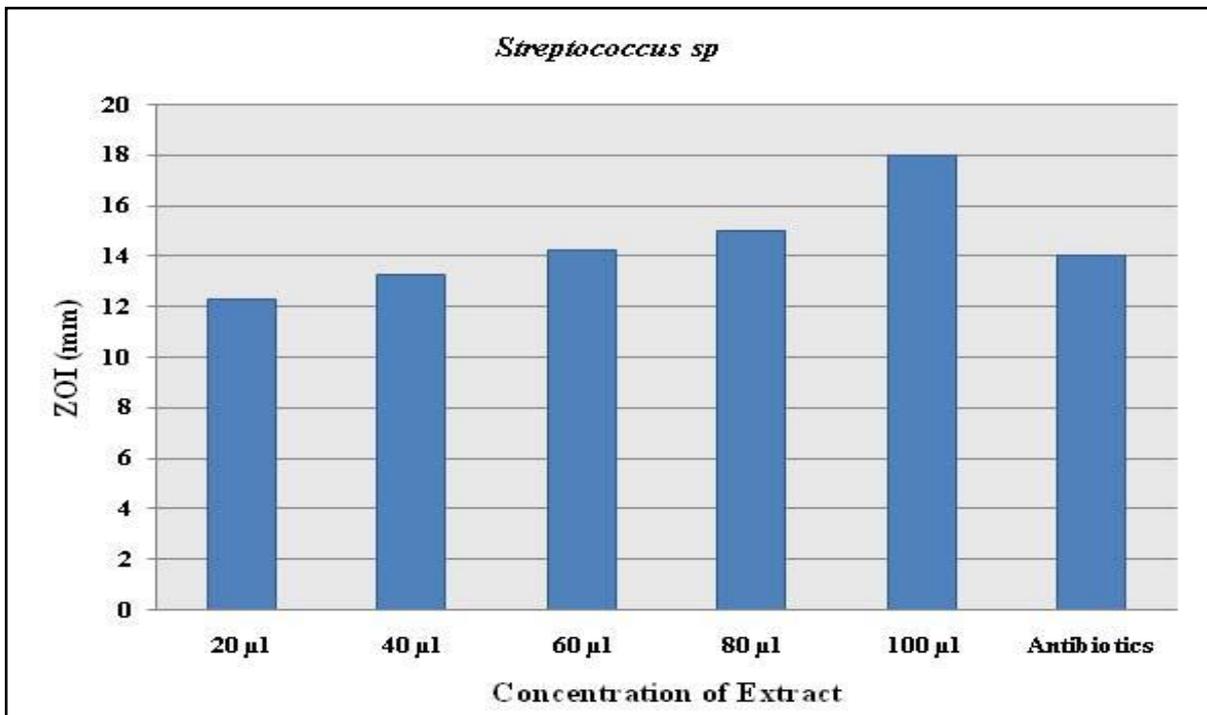


Figure 2: Antibacterial activity of ethanolic *Mucuna pruriens* seeds extract against *Streptococcus sp.*

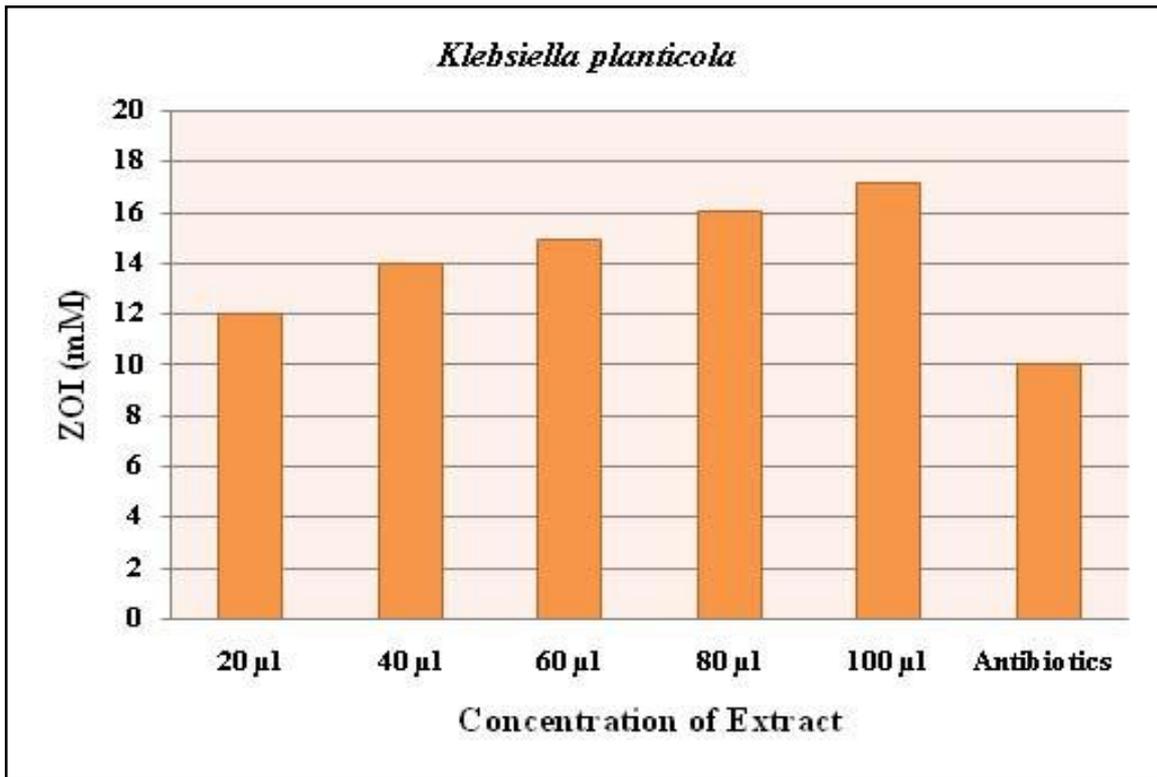


Figure 3: Antibacterial activity of ethanolic *Mucuna pruriens* seeds extract against *Klebsiella planticola*

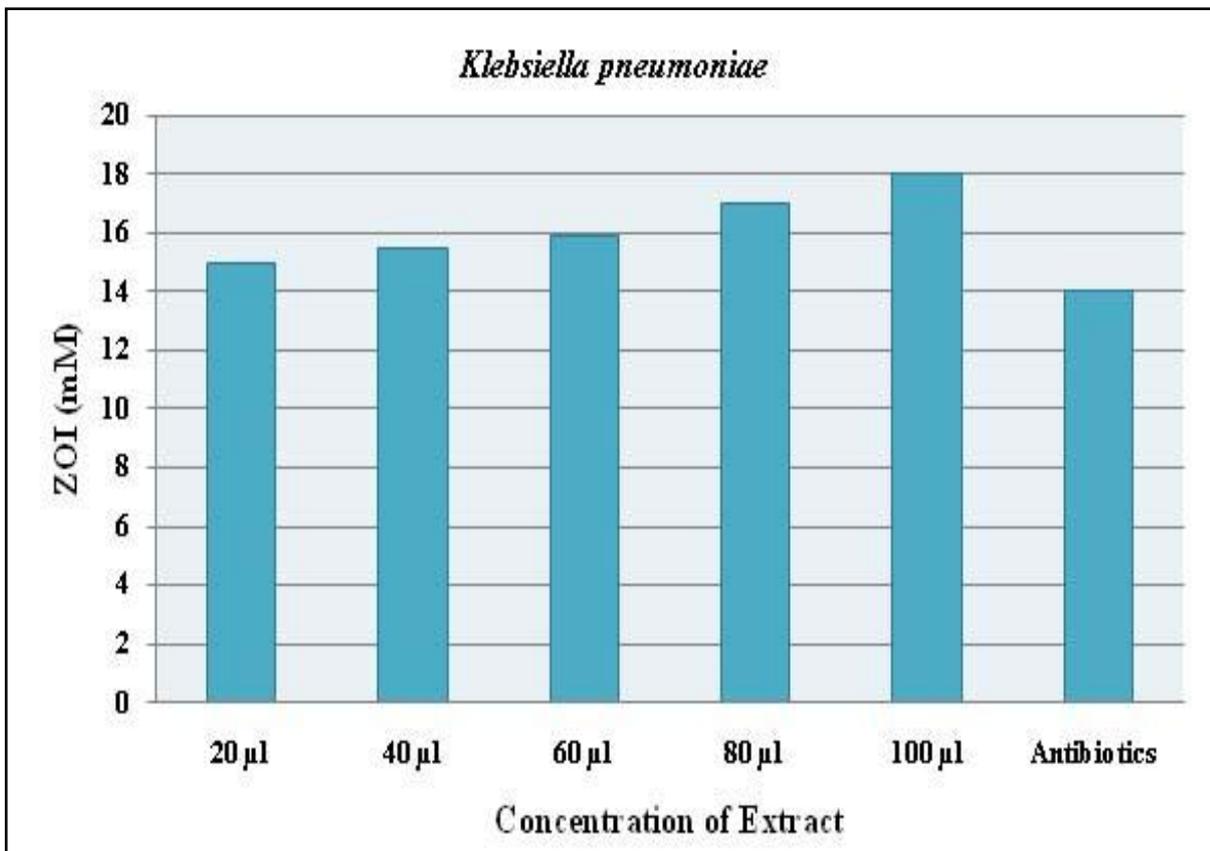


Figure 4: Antibacterial activity of ethanolic *Mucuna pruriens* seeds extract against *Klebsiella pneumoniae*.

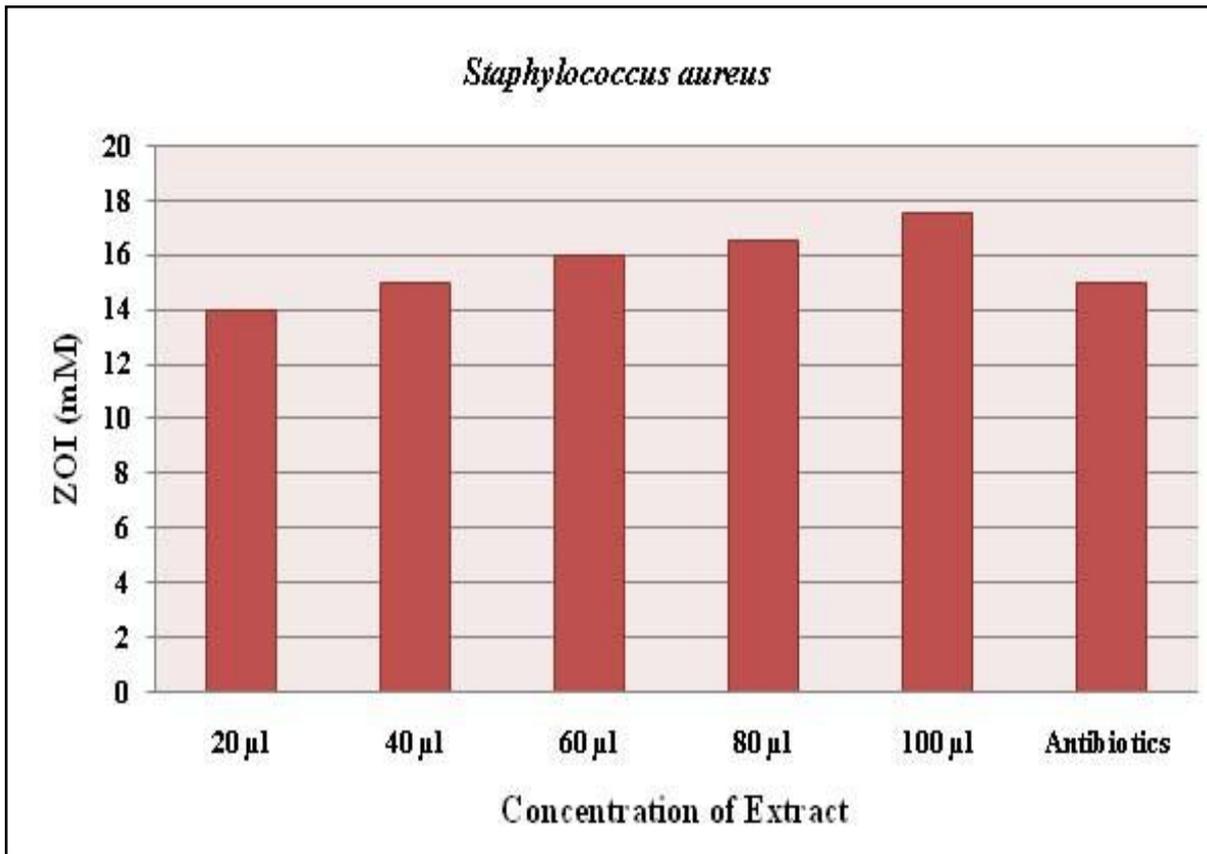


Figure 5: Antibacterial activity of ethanolic *Mucuna pruriens* seeds extract against *Staphylococcus aureus*.

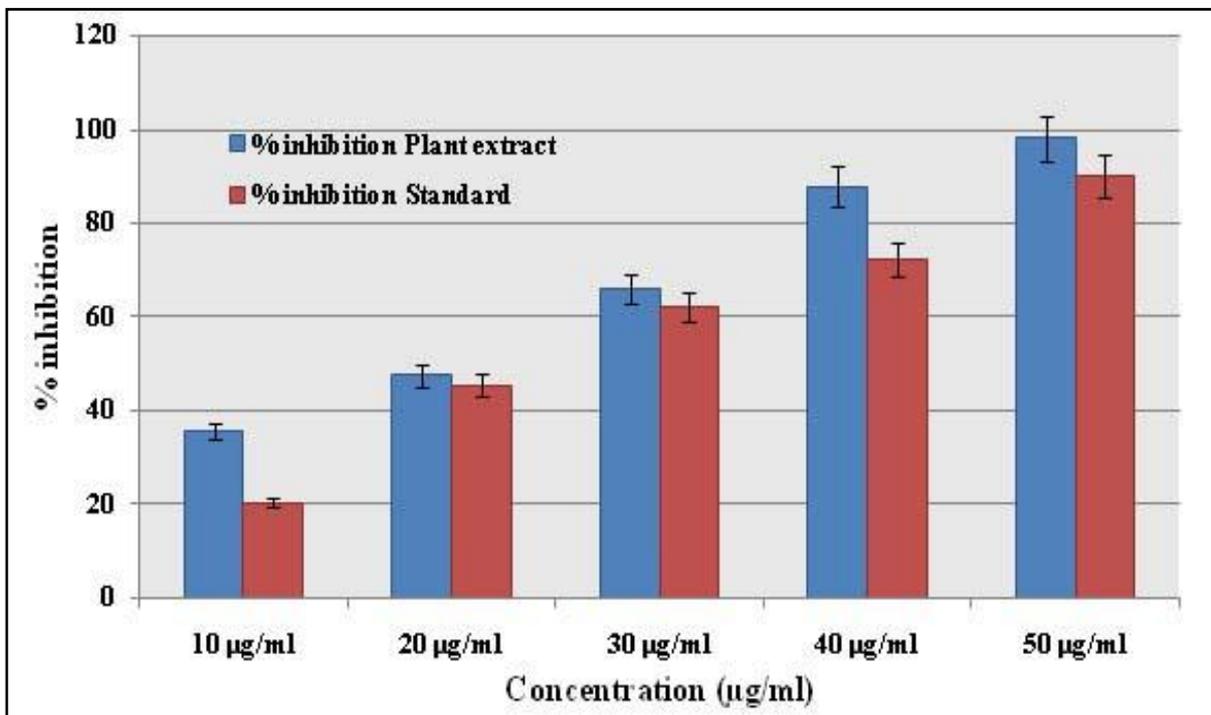


Figure 6: Effect of *Mucuna pruriens* seed extract and Standard Vitamin C on scavenging of DPPH radical Results are mean ± S.D of five parallel measurements

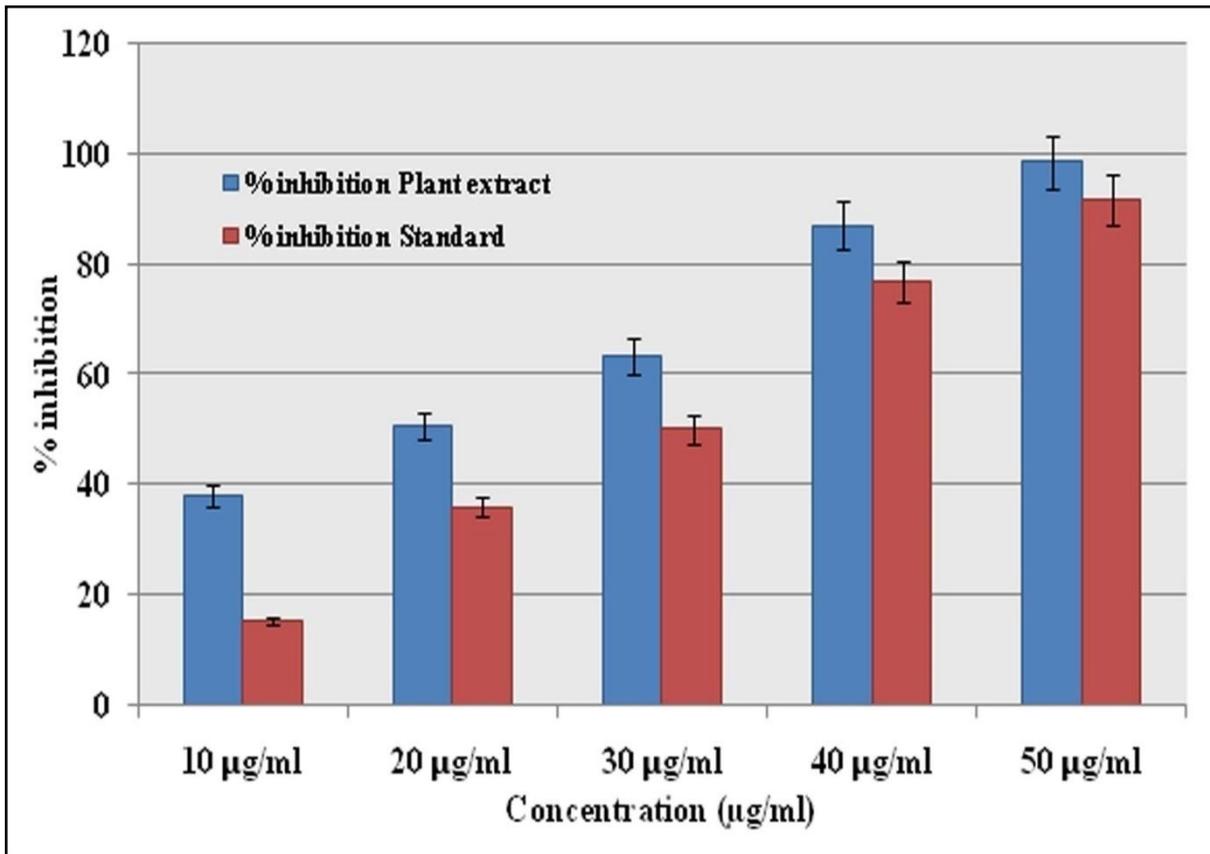


Figure 7: Effect of *Mucuna pruriens* seed extract on nitric oxide radical inhibition assay

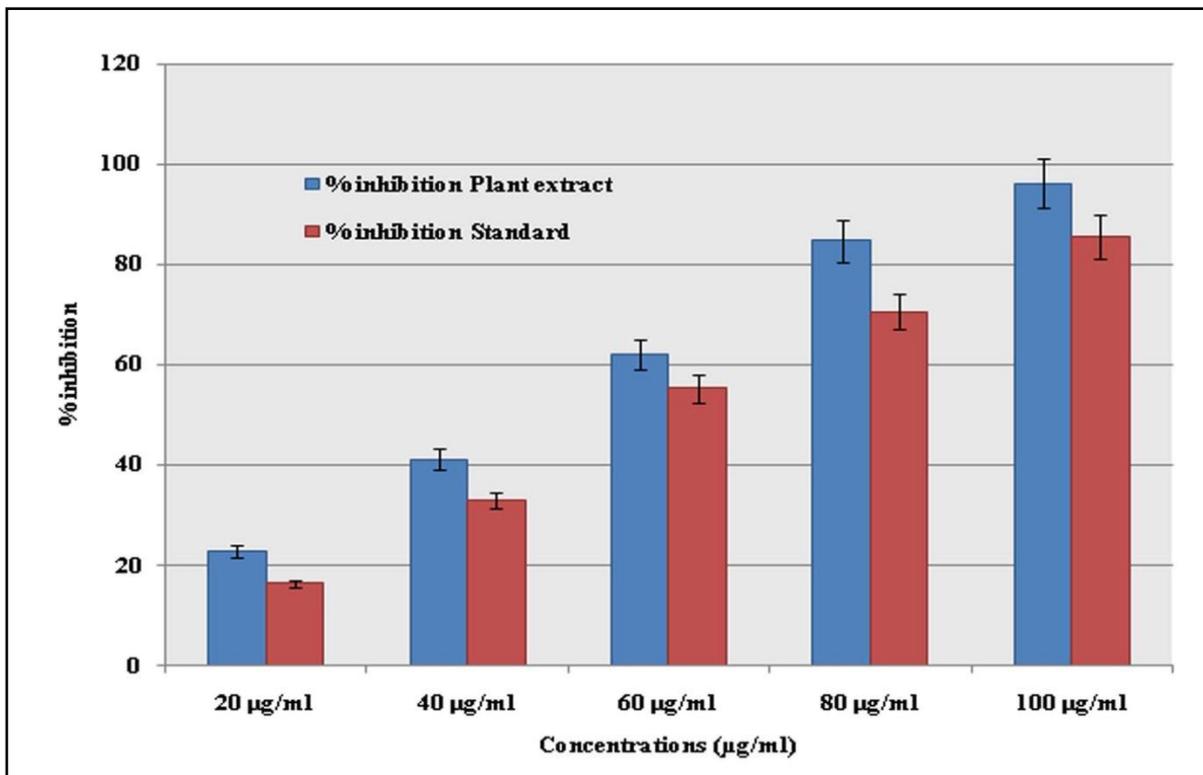


Figure 8: Hydroxyl radical scavenging assay of ethanolic seed extract of *Mucuna pruriens*

Table 2: DPPH, nitric oxide and hydroxyl scavenging activity using ethanolic extract of *Mucuna pruriens* seed

Conc. (µg/ml)	% inhibition activity					
	DPPH scavenging		Nitric oxide scavenging		Hydroxyl scavenging	
	Extract	Standard	Extract	Standard	Extract	Standard
10	26.33 ±0.12	20.35±1.25	27.93±1.13	15.48±0.63	19.87±0.86	16.32±0.22
20	50.25±0.24	45.45±0.85	49.68±1.16	35.95±0.78	39.21±0.42	32.98±0.84
30	70.85±0.65	62.12±0.98	60.36±1.23	50.02±0.69	58.02±0.37	55.28±0.96
40	80.35±0.84	72.13±1.65	81.02±1.31	76.87±2.25	81.77±0.29	70.63±0.64
50	95.05±1.05	90.25±1.24	96.45±1.46	91.65±1.34	91.25±0.57	85.45±1.21

± Standard deviation

Antioxidant activity

The antioxidant activity of ethanolic seed extract of *M. pruriens* as well as standard was examined for their scavenging DPPH, nitric oxide and hydroxyl (Figure 6, 7 and 8). The antioxidant scavenging activity against DPPH, nitric oxide and Hydroxyl is concentration dependent manner. The antioxidant activity of ethanolic seed extract is directly proportional to the concentration of the extract^{29, 30, 35, 36}. The 50% of DPPH, nitric oxide and Hydroxyl radical scavenging activity was observed at 19.85, 22.45 and 56.75 µg/ml concentration of ethanolic seed extract. The free radical scavenging activity of ethanolic *Mucuna pruriens* seeds extract was increased while increasing the concentration of extract (Table 2). The IC₅₀ value of the extract was highly significant than the standard BHT (23.95 µg/ml), rutin (32.25 µg/ml), and vitamin E (59.02 µg/ml). The minimum inhibition concentration with high activity of seed extract was found to be less than the standard.

Anti-inflammatory activity of ethanolic extract of *M. pruriens* seed

The inhibitory activity of ethanolic extract of *M. pruriens* seed, as well as standard, well known inhibitors of inflammation was determined (Table 3) by protein denaturation process. Inflammation is caused due to the denaturation of proteins^{31, 32, 33}. In this investigation, the ability of seed extract on the anti-inflammatory activity, extract inhibit the protein denaturation was evaluated. The inhibitory potential of the extract showed a concentration dependent. Maximum inhibition of 73% was observed at 300 µg/ml.

Table 3: Effect of ethanolic seed extract on inhibition of protein denaturation and proteinase action

Treatment	Conc. (µg/ml)	Absorbance at 660 nm	Protein denaturation Inhibition (%)	Absorbance at 660 nm	Inhibition of proteinase (%)
Control	-	0.45±1.05	-	0.48±1.07	-
Ethanolic seed extract	50	0.43±0.25	34	0.42±0.05	37
Ethanolic seed extract	100	0.37±1.63	46	0.35±1.03	44
Ethanolic seed extract	150	0.32±0.02	58	0.30±1.09	53
Ethanolic seed extract	200	0.26±0.23	64	0.25±0.53	60
Ethanolic seed extract	300	0.20±0.97	73	0.22±0.77	64
Standard	300	0.22±0.11	65	0.25±0.21	62

Proteinase enzyme plays an important role in the development of tissue damage during inflammations. Ethanolic seed extract was well known as proteinase inhibitor which exhibited significant proteinase inhibition activity at different concentrations (Table 3). It showed maximum inhibition of 64 % at 300 µg/ml. Furthermore, ethanol extract of seed demonstrated a markedly higher anti-inflammatory activity compared to the standard due to the presence of metabolites like phenol and esters compound.

Conclusion

The results of this present study demonstrated that the ethanol extracts of *M. pruriens* seeds possess antimicrobial, antioxidant and anti-inflammatory activities. GC-MS analysis revealed the presence of different active compounds involving in the microbial growth inhibition, antioxidant and anti-inflammatory properties. The extract serves as free radical scavenger by inhibiting the DPPH, nitric oxide and hydroxyl. The inhibition of protein denaturation causing inflammation and proteinase activity was also studied. This study gives an inspiration to identify the compound present in the *M. pruriens* seed responsible for antioxidant and anti-inflammation that could be lead compound for designing a drug in the pharmaceutical applications.

Conflict of interest

There is no Conflict of interest

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