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EVALUATION OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF METHANOLIC LEAF EXTRACT OF *CALLISTEMON VIMINALIS*

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Abstract

Callistemon viminalis (bottle brush) belongs to family Myrtaceae that has a great medicinal importance.*C.viminalis* leaf shows various types of activities such as free radical scavenging activity, calcium channel blocking activity, antifungal, antibacterial, antidiabetic, antithrombin and herbicidal activity. The current work describes the antibacterial, phytochemical analysis as well as antioxidant activities of methanolic leaf extract of *C.viminalis*

The phytochemical analysis of methanolic leaf extract of *C.viminalis* showed the presence of alkaloids, flavonoids, tannin & phenol, saponins, carbohydrates, amino acids and proteins. In the present study the total phenolic content in the leaf extract was found 1201.06 ± 27.13 g equivalent of gallic acid per100g of extract and antioxidant activity which was measured as reducing power assay (FRAP) was found 12.98 ± 0.82%. Antibacterial activity of leaf extract of *C.viminalis* was screened by disc diffusion method against isolated bacterial strain of *E. coli*, and *S.aureus.* The methanolic leaf extract was found active against both Gram-positive and Gram-negative bacteria, which showed maximum diameter of inhibition zone 15 mm in *E. coli* while 12mm in *S.aureus.*

Based on these results, we may conclude that the methanolic leaf extract of *C.viminalis* showed good antioxidant free radicals due to their hydroxyl groups of phenolic content. These data support the use of such plant based medicines in treatment of infectious diseases where access to commercial antibiotics is restricted. The leaf extract is active against human bacterial pathogens thus emerging as potential sources of new antibacterial compounds.

Key words: *Callistemon* viminalis, antibacterial, antioxidant activities, phytochemical screening.

Introduction

Herbal plants are being used as traditional health remedies by 80% of the world population in Asia, Latin America and Africa. Herbal medicines are reported to have minimal side effect (Doughari, 2006).*Callistemon* is a genus of 34 species of shrubs in the family *Myrtaceae*. *Callistemon* species are commonly referred to as bottlebrushes because of their cylindrical, brush like flowers resembling traditional bottle brush. It is also used as weed control (Wheeler, 2005) and as bioindicators for environmental management (Burchett et al., 2002). It is expected that it might also be

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a storehouse of many chemicals of medicinal and pharmacological interest. Several research works on the various parts of the plant have been reported for their anti-thrombin, anti tuberculosis properties (Krishna Panda et,al 2013).The phytochemical studies revealed the presence of C – methyl flavonoids, lipid and betulinic acid. Betulinic acid (3β-hydroxylup-20-(29)-en-28-oic acid) is a pentacyclic triterpenoids which has property to selectively kill human melanoma cells without affecting healthy cells (Bhatia et,al 2015). Due to its apparent specificity for melanoma cells, betulinic acid seems to be a more promising anti-cancer substance. It is also found to retard the progression of HIV -1 infection, by preventing the formation of cellular aggregates *(*Cassels & Asencio 2010). Moreover; it has antibacterial properties and inhibits the growth of both *Staphylococcus aureus* and *Escherichia coli* (Pisha et al, 1995).

Keeping all these points in view the present investigation was carried out to see phytochemical screening of methanolic leaf extract of *C. viminalis*, evaluation of antibacterial activity against the selected bacterial species *S. aureus and E.coli* as well as estimation of total phenolic content (TPC) and antioxidant activity by ferric reducing power assay.

Materials and methods

Collection of plant materials

C. viminalis leaves were procured from Botanical garden of Department of Botany SLS, Dr .B.R Ambedkar University Agra, and the identification of the plant was confirmed by the taxonomist of the same Department.

Preparation of callistemon leaf extract

Crude leaf extract was prepared by Soxhlet extraction method described by Okeke et al (2001*)*. About 45 gm of leaves powder material were uniformly packed into a thimble and run in Soxhlet extractor separately. It was exhaustible extracted with 250 ml methanol for the period of about 48 hours and 22 cycles or till the solvent in the siphon tube of an extractor become colourless. After that extracts were filtered with the help of filter paper and solvent evaporate from extract in rotary evaporator to get the syrupy consistency. The residue was dried over

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anhydrous sodium sulphate to remove trace of alcohol. Then extract was kept in refrigerator at 4°C for detect antibacterial activity and analyzed their physical and chemical property.

Phytochemical screening of crude extract

Extracts were tested for the presence of active principle such as steroid, tannins, phenols, flavonoid, alkaloids, glycoside, triterpinoids, carbohydrates and proteins. By standard procedures followed by (Debela, 2002).

Determination of total phenolic content (TPC)

Total phenol content was estimated using Folin-Ciocelteau reagent based assay as previously described with little modification by Singleton and Rossi (1965). One ml of leave extract in methanol, 5ml of Folin -Ciocalteau reagent (diluted tenfold) and 4 ml (75 g/l) of Na_2CO_3 were added. The mixture was allowed to stand at 20^{o}C for 30 min and the absorbance of the developed colour was recorded at 765 nm using UV-VIS spectrophotometer. 1 ml aliquots of 20, 40, 60, 80, 100µg/ml methanolic gallic acid solutions were used as standard. Total phenolic content was expressed in g GAE/100g dry weight of sample. All determination were performed in triplicates and values were expressed in mean±SD.

Ferric reducing antioxidant power assay (FRAP)

The Ferric reducing antioxidant power assay of methanolic extracts was determined by the method of Oyaizu (1986). 1ml of methanolic leaf extract was mixed with phosphate buffer (3.0 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K_3Fe (CN) $_6$] (2.5 ml, 1%). The mixture was incubated at 50^{o}C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer. Increased absorbance of the reaction mixture indicates increase in reducing power. Ascorbic acid was used as standard 50µg/ml. Percent inhibition was calculated using the following expression:

$$\% \text{ inhibition} = A_{blank}\text{-}A_{sample} / A_{blank} \times 100$$

Where, A_{blank} and A_{sample} stand for absorption of blank sample and absorption of tested extract solution respectively. All determination were performed in triplicates and values were expressed in mean±SD

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Microbiological assay

The agar disc diffusion method was employed for the determination of antibacterial activities of the methanolic leaves extract of C. *viminalis* (Mukherjee et al., 1995). The MIC of the extract was also determined using a two-fold dilution method. The bacteria were first grown in nutrient agar for 18 hour before use. The inoculum suspensions were standardized. It was performed using an 18 h culture at 37°C in 10 ml of Mueller Hinton Broth. The cultures were adjusted to approximately 10^5CFU/ml with sterile saline solution. Five hundred micro liters of the suspensions were spread over the plates containing Mueller-Hinton agar using a sterile cotton swab in order to get a uniform microbial growth on test plates and then tested against the effect of the plant extracts at the concentration of 50 mg/ml, 250 mg/ml, 125mg/ml, 62.5 mg/ml, and 31.25. mg/ml. All petridishes were sealed with sterile laboratory parafilms to avoid eventual evaporation of the test samples. These plates were incubate for 24 hour at 37°C and measured the zone of inhibition in millimeter the plates later incubated at 37°C± 0.5°C for 24 hours after which they were observed for zones of inhibition. The effects were compared with that of the standard antibiotic Gentamycin at a concentration of 1mg/ml *(*Khan and Omotoso, 2003*).* This was used as positive control, while methanol was used as negative control. The inhibitory zone around test paper discs indicated as positive (growth inhibition observed) and absence of zone as negative

Statistical analysis

All measurements of were carried out in triplicates. The results are expressed as mean values ±standard deviation (SD).

Result and discussion

Preliminary phytochemical screening of C. viminalis

Table-1 shows that the phytochemical analysis of *C. viminalis* leaf showed the presence of glycosides flavonoids, alkaloids, proteins, carbohydrate, Saponins, tannin & phenol.The presence of bioactive compounds in leaf has been reported to confer resistance against microbial pathogens and thus explains the manifestation of antibacterial activity by the leaf extract used in the study (Anibijuwon and Udeze 2009)**.** Phytochemicals acts as a potential source for

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biological antibacterial activity against selective bacteria, without any adverse effects on human beings (Doughari, 2009).

Table 1: Table showing phytochemicals present in C.*viminalis*

Phytochemicals	**Methanolic leaf extract of *Callistemon viminalis***
Alkaloids	+
Glycosides	+
Flavonoids	+
Proteins and amino acids	+
Steroids	-
Carbohydrates	+
Tannin & phenol	+
Saponins	+

Table - 2 shows the effect of concentration of methanol leaf extract with their % inhibition revealed that the antioxidant activity of the extract. The reducing power of the methanolic leaf extract and ascorbic acid were 12.98% and 87.37%, respectively. The result obtained in the study for antioxidant activity of methanol extract of leaf of C. viminalis had revealed the reducing power activity which is compared to standard antioxidant ascorbic acid. The ability of reducing power of methanolic leaf extract of leaf of C. viminalis observed almost same as synthetic antioxidant, ascorbic acid. This result is consistent with investigations done by **o**ther researchers (Kumar et al., 2008; Das et al., 2008). The reducing capacity of a compound Fe^{+3} /Ferricyanide complex to the ferrous form may serve as indicatior of its antioxidant capacity(Yildirim et al., 2000) . The existence of reductones are the key of the reducing power,which show their antioxidant activities through the action of breaking the free radical chain by

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donating a hydrogen atom..The reduction of the Fe^{+3}/ferricyanide complex to the ferrous form occurs due to the presence of reductants in the solution (Xing et al., 2005).

Table-2: Table showing total phenol content and ferric reducing antioxidant power assay present in *C.viminalis.*

Parameters Samples	**Total phenol content (g GAE/100g of dry weight sample)**	**Reducing power assay (% inhibition)**
Ascorbic Acid(50 µg/ml)	-	87.37±3.21
Methanolic leaves extract of *C.viminalis*(9000µg/ml conc.)	1201.06 ± 27.13	12.98± 0.82

Table-2 also shows the total phenol contents were 1201.06±27.13 g gallic acid equivalent/100g dry weight of sample in the methanolic leaf extract. The antioxidant activity has a positive correlation with phenolic contents of methanolic leaf extract of the plant. This confirms the assertion that phenolic content of plants play direct role in antioxidant properties. Furthermore, they serve as flower pigments,that act as constitutive protecting agents against invading organisms, function as signal molecules, act as allelopathic compounds (Ndakidemi, 2006). A regular intake of phenolic compounds is assumed to decrease the incidence of certain forms of cancer, and for that reason they are normally regarded as chemo-preventive agents (Sak 2014).The antioxidant properties of phenols are determined by their radical scavenging ability and consequent inhibitory action on lipid peroxidation under oxidative stress situations,(Bozin et.al,2008).

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Table 3: Antibiogram patterns for methanolic leaf extract of *C. viminalis*

Inhibitory concentration of methanolic leaf extract of *C viminalis (mg/ml)*	**Diameter of zone of inhibition for *S.aureus*(mm)**	**Diameter of zone of inhibition *For E.coli*(mm)**
500 mg/ml(D1)	10±0.75	14±0.95
250 mg/ml(D2)	9±0.51	12.33±0.67
125 mg/ml(D3)	8.33±0.73	11.66±0.47
62.5 mg/ml(D4)	8.0 ±0.47	10±0.88
31.25mg/ml(D5)	-	-
Gentamicin(PC)	35±1.73	36±1.09

In the present investigation table-3 & figure 1show antibiogram patterns for methanolic leaf extract of *C. viminalis* for different concentrations. Results showed maximum inhibition zone as 15 mm in case of *E. coli* and 12 mm in case of *S. aureus* as compared with the standard positive standard Gentamycin. As the plant produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plant secondary metabolites such flavonoids, alkaloids and triterpenoid are producing a better opportunity for testing wide range of microorganism(Mansour,2010).Our results are consistent with findings of others researchers which observed good to moderate antimicrobial activity of *Callistemon viminalis* of methanolic leaf extract *(*Dewanjee et al 2008. Abdullah 2011).In general, the Gram-negative bacteria show less sensitivity to plant extract may be due to their extra lipopolysaccharide and protein cell wall that provides a permeability barrier to the antibacterial

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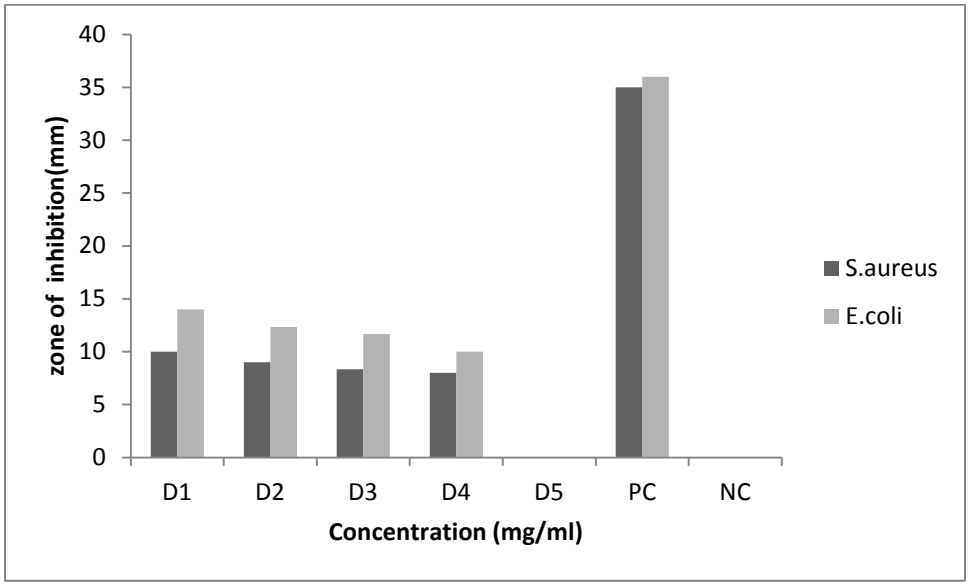

Figure 1: Graphical representation of antibacterial potential of different concentration of methanolic extract of *Callistemon* viminalis **against S. aureus and E. coli. D_1, D_2, D_3 & D_4 are dilutions of** *Callistemon* viminalis **extract, PC is positive control & NC is negative control.**

agent (Adwan and Abu- Hasan 1998). Furthermore, the Gram-positive bacteria are more sensitive to the extract because of the single layer of their cell wall, wheeas the double membrane of Gram-negative bacteria make them less sensitive (Kaur and Arora, 2009)

Conclusion

The present investigation expresses that *C. viminalis* leaves have great potential as antibacterial compound against microorganisms. These findings provide scientific evidence to support the traditional medicinal uses of the extract and indicate a promising potential of these plants for medicinal purposes. Thus it can be used in the treatment of infectious diseases caused by pathogenic bacteria. Further *in vivo* studies are required to substantiate our findings.

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