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PHYTOCHEMICAL SCREENING, ANTI-OXIDANT POTENTIAL AND ANTIMICROBIAL ACTIVITIES IN THREE SPECIES OF CESTRUM PLANTS

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ABSTRACT

Plants have richest source of bioactive compounds as phytochemicals in the form of secondary metabolites. In this study three *Cestrum* species *C. aurantiacum, C. nocturnum and C. diurnum* were investigated for phytochemicals and revealed presence of Alkaloids, Anthraquinones, Cardiac Glycosides, Carbohydrates, Flavonoids, Phenolic compound, Tannins and Terpenoids. Antioxidant potential was estimated by DPPH and FRAP assay and *C. diurnum* showed higher antioxidant potential than that of the other two species in both assays. Different solvents such as ethanol, methanol, butanol, propanol and acetone were used to extract the bioactive compounds from the leaves of these plant species. The antimicrobial activity of these solvent extracts was tested against three bacterial (*S. typhi*, *P. aeruginosa* and *K. pneumoniae*) and two fungal species (*Aspergillus* and *Trichoderma*) by agar well diffusion method. *C. aurantiacum* showed maximum antibacterial activity, and maximum antifungal activity was detected for butanol extract of *C. nocturnum* against *Aspergillus*.

KEYWORDS: ***Cestrum*, Phytochemical, Antioxidant, Antimicrobial**

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INTRODUCTION

India has a rich plant flora distributed throughout the country and have been used for the treatment and cure for various diseases. Plants are the richest resource of on drugs, and extracts of various species of edible plants, herbs, and spices have long been used for food preservation because of the presence of potentially effective antimicrobial compounds[1, 2]. The medicinal plants contain not only minerals and primary metabolites but also a diverse array of potential chemical compounds which play an important role in adsorbing and neutralizing free radicals[3, 4]. Free radicals generates in pathogenesis of many diseases like atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease and in the aging process[5]. Radical scavenging antioxidants are particularly important in anti oxidative defence in protecting cells from the injury of free radical**s**. Thus plants are screened for antioxidants to enhance the properties of food for health benefits[6, 7]. The genus *Cestrum* (Solanaceae) with more than 300 species is globally distributed in tropical and subtropical regions throughout the world including India, southern China, Australia, USA and Bangladesh[8]. Several applications of *Cestrum* have been documented and the toxicity of the species to humans and livestock has been reported[9, 10]. The leaves of *Cestrum* have shown significant analgesic and bactericidal activity. Also, inhibitory effect on the central nerve system and cardiac arrhythmic effect of *Cestrum* is documented and also used for the treatment of burns and swellings[11]. The oil of these species is known to be mosquito repellent and hence *C. nocturnum* and *C. diurnum* are used to prevent malaria in African Nations[12]. The study with plant extracts of *Cestrum diurnum* showed promising activity to combat against larvae of *Culex quinquefasciatus* (Diptera: Culicidae) and *Anopheles stephensi*[13]. In this study *C. aurantiacum, C. nocturnum and C. diurnum* were investigated for phytochemicals and determination of their antioxidant and antimicrobial potential.

MATERIALS AND METHODS

Extract Preparation

The plant material *C. aurantiacum, C. nocturnum* and *C. diurnum* were obtained from and authenticated by Gandhi Krishi Vigyana Kendra (GKVK), Bangalore. The plant leaves were air dried under shade and powdered. For extraction, 5gm powder was mixed with 50ml of solvent. Extraction continued until the extraction solvents became colourless. The obtained extracts were filtered and from the collected filtrate the solvent was removed by evaporation.

Preliminary Phytochemical Analysis

The different solvent extract was subjected to preliminary phytochemical screening according to the protocols[14] to the presence of phenolic content, glycosides, anthraquinones, terpenoids, proteins, flavinoids, tannins, lignin and Saponins.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay

For determination of DPPH radical scavenging activity 1ml of methanol extracts (0.2 to 1mg/ml) was mixed with 3ml of a methanolic solution of 0.1mM DPPH. The mixture was shaken well and incubated at room temperature for 30 min and absorbance was measured at 517nm in a spectrophotometer.

FRAP (Ferric reducing ability of Plasma) assay

For determination of reducing power, 1ml of the ethanol extract (0.2-1mg/ml) was mixed with 2.5ml of 0.2M Phosphate buffer (pH 6.6) and 2.5ml of 1% Potassium Ferricyanide. The mixture was incubated at 50°C for 20mins and then 2.5ml of 10% Trichloroacetic acid was added and centrifuged at 3000rpm for 10mins. The upper layer (2.5ml) was mixed with 2.5ml of 0.1% of Ferric Chloride and Absorbance was taken at 700nm.

Collection of Microorganisms for Antibacterial Activity

The pathogenic microorganism were isolated from clinical samples collected from diagnostic testing labs and indentified on the basis of morphological, biochemical and physiological characteristics. The isolated bacterial species were found to be *S. typhi*, *P. aeruginosa* and *K. pneumoniae,* and fungal species was found to be *Aspergillus* and *Trichoderma.*

Determination of Antibacterial Activity

The antibacterial activity was determined by the agar well-diffusion method. Overnight grown bacterial culture was transferred to a sterile Petri plate with Mueller Hinton agar medium (Hi Media Laboratories Limited, Mumbai, India) and was spread with a sterile spreader to create a lawn. Wells of 6mm were punched into the previously seeded MH agar plates using sterile cork borer. About 50µl of the different solvent extract was placed in the wells and allowed to diffuse for 2 hrs at 4ºC and the plates were incubated at 37°C for 24 hrs. The antibacterial activity was determined by measuring the diameter of the zone of inhibition for each well and expressed in mm.

Determination of Antifungal Activity

The antifungal activity was determined by the agar well-diffusion method. Fungal cultures grown for 72 hrs were transferred and spread on a sterile Petri plate with potato dextrose agar (PDA) (Hi Media Laboratories Limited, Mumbai, India). Wells of 6mm were punched using sterile cork borer and about 50µl of the different solvent extract were placed in the wells. The plates were kept at 4ºC for 2 hrs and then incubated at 28°C for 72 hrs. The antifungal activity was determined by measuring the diameter of the zone of inhibition for each well and expressed in mm.

RESULTS AND DISCUSSION

Phytochemical screening of *C. aurantiacum, C. nocturnum* and *C. diurnum* revealed the presence of Alkaloids, Anthraquinones, Cardiac Glycosides, Carbohydrates, Flavonoids, Phenolic compound, Tannins and Terpenoids (Table1).

Table1

Phytochemical screening of C. aurantiacum, C. nocturnum and C. diurnum.

Test	*C. aurantiacum*	*C. nocturnum*	*C. diurnum*
Alkaloids	+	+	+
Anthraquinones	+	+	+
Cardiac Glycosides	+	+	+
Carbohydrates	+	+	+
Flavonoids	+	+	+
Phenol	+	+	+
Saponins	-	-	-
Tannins	+	+	+
Terpenoids	+	+	+

Antioxidant activity was determined by DPPH and FRAP assay and all species showed Antioxidant potential in both assay (Figure 1&2). The obtained results from the anti-oxidant assays indicated that they are highly active against free radicals and hence can prevent cellular damage which is resultant of high free radicals. The scavenging activity of *C. diurnum* was found to be higher than that of the other two species, indicating that it has a higher antioxidant potential. FRAP assay also showed increased absorbance with increase in concentration which indicated reducing power potential.

Figure1
Antioxidant activity by DPPH Assay

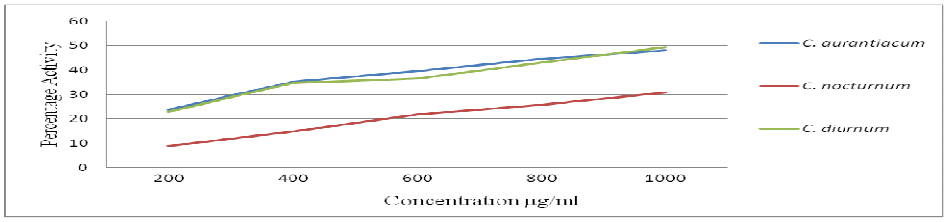
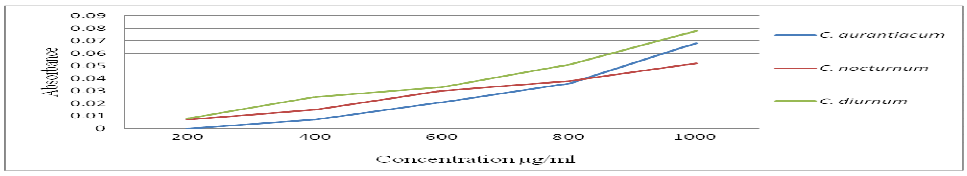


Figure2
Antioxidant activity by FRAP Assay



Antimicrobial activity was observed for five solvent extracts against three bacterial species and for three solvent extracts against two fungal species (Figure 3, 4 and 5). The different solvent extracts of these plants showed moderate antimicrobial activities against all tested microorganisms. *C. aurantiacum* showed stronger antibacterial activity compared to other two species and acetone extract of *C. aurantiacum* produced maximum zone of inhibition (18mm) against *K. pneumoniae.* In case of fungi, the zones of inhibition were highest (16mm) for butanol extract of *C. nocturnum* against *Aspergillus*. Whereas, ethanol extract of *C. diurnum* showed maximum zone of inhibition (14mm) against *Trichoderma.*

Figure3
Antimicrobial activity of C. Aurantiacum

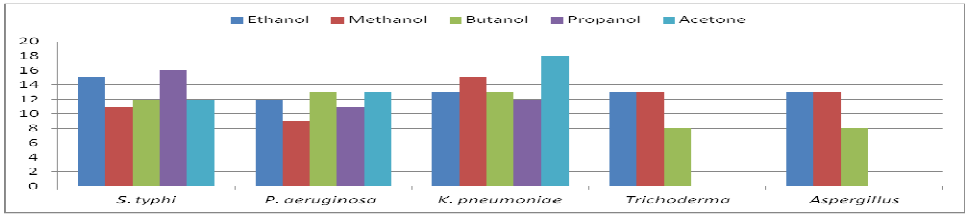


Figure4
Antimicrobial activity of C. Nocturnum

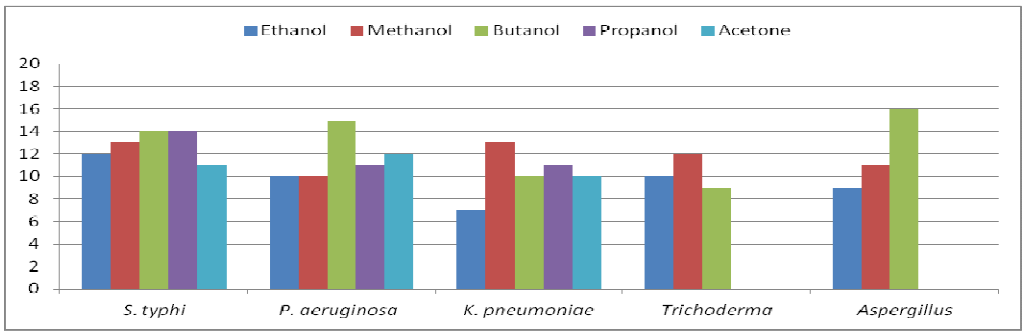
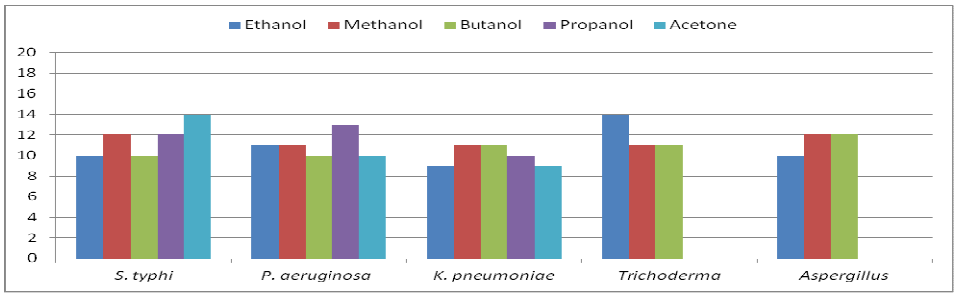


Figure5
Antimicrobial activity of C. Diurnum



CONCLUSION

In the current study, the plants were found to be negative for the presence of saponins, C.nocturnum was found to be negative for tannins. Phenols failed to answer in the qualitative test but were found to be in minute traces in quantitative test. The quantities of flavanoids and alkaloids were found to be high indicating that they are good scavengers of free radicals. The results obtained from the anti-oxidant assays indicated that they are highly active against free radicals and hence can prevent cellular damage which is resultant of high free radicals in the plants. The scavenging activity of C.nocturnum was found to be higher than that of the other two species, indicating that it has a higher antioxidant potential and hence the plants are less toxic. FRAP assay also showed increased absorbance with increase in concentration which indicates good reducing power. *C. aurantiacum* showed strong antibacterial activity against *K. pneumoniae* and *C. nocturnum* showed antifungal activity against *Aspergillus* which may be considered as a prolific approach in the search of new drugs.

REFERENCES

1. Hammer KA, Carson CF and Riley TV, Antimicrobial activity of essential oils and other plant extracts, J Appl Microbiol, 86: 985-990 (1999).
2. Nychas GJE, Tassou CC and Skandamis P, Antimicrobials from herbs and spices. In: Natural Antimicrobials for the Minimal Processing of Foods (edited by S.M. Roller), CRC Press, Woodhead Publishers, New York, 2003, pp. 176–200.
3. Akinmoladun AC, Ibukun EO, Afor E, Akinrinlola BL, Onibon TR, Akinboboye AO, Obuotor EM and Faromb EO, Chemical constituents and antioxidant activity of *Alstonia boonei*, Afr. J. Biotechnol., 6: 1197-1201 (2007).
4. Ashawat MS, Shailendra S and Swarnlata S, *In vitro* antioxidant activity of ethanolic extracts of *Centella asiatica*, *Punica granatum*, *Glycyrrhiza glabra* and *Areca catechu*, Res. J. Med. Plant, 1: 13-16 (2007).
5. Aruoma OI, Methodological considerations for characterizing potential antioxidant actions of bioactive components in plants food, Mutation Res. Fundam. Mol. Mech. Mutagen, 9(20): 523-524 (2003).
6. Ou B, Huang D, Hampsch-Woodill M, Flanagan JA, Deemer EK, Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study, J. Agric. Food Chem., 50: 3122-3128 (2002).
7. VanderJagt TJ, Ghattas R, VanderJagt DJ, Crossey M, Glew RH, Comparison of the total antioxidant content of 30 widely used medicinal plants of New Mexico, Life Sci., 70: 1035-1040 (2002).

8. Sharif M, Rahman A, Kang SC, Chemical composition and inhibitory effect of essential oil and organic extracts of Cestrum nocturnum L. on food-borne pathogens, Int J Food Sci Tech, 44: 1176–1182 (2009).
9. Mello JRB, Calcinosis-calcinogenic plants (Review), Toxicon, 41(1): 1-12 (2003).
10. Stone B, The flora of Guam, Micronesica, 6: 516 (1970).
11. Huang LG, Zhang XC, Xiao H, Ye HY, Zeng J, Analgesic effect of Cestrum *nocturnum* L. extract on mice, Chin. J. Clin. Rehab, 10: 172-174 (2006).
12. Ntonifor NN, Ngufor CA, Kimbi HK and Oben BO, Traditional use of mosquito repellent to protect human against mosquito and other insect bites in rural community of Cameroon, East Afr Med J, 83: 553–558 (2006).
13. Ghosh A, Chandra G, Biocontrol efficacy of *Cestrum diurnum* L. (Solanaceae: Solanales) against the larval forms of *Anopheles stephensi*, Nat Prod Res, 20: 371-79 (2006).
14. Harbone JB, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall London 21-26(1998).