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

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Abstract: *The aim of the study was to investigate the phytochemical, the antimicrobial and the antioxidant activity of three plant species, namely C*estrum elegans, C. fasicilatum, C.parqui. *The Phytochemicals were screened qualitatively and quantitatively. The phytochemicals such as anthraquinones, terpenoids, flavonoids alkaloids were present in all the three species, whereas each species showed absence of saponins. Phenol was found in minute quantities. Six solvents were used to extract the bioactive compounds from the leaves of these plant species, namely ethanol, methanol, butanol, propanol, acetone, and petroleum ether for antibacterial activities and ethanol, methanol and butanol alone for antifungal activities by agar diffusion method. Maximum zones of inhibition was seen in propanol and acetone extracts and hence this extract can be used for antibacterial activity.Antifungal activity was seen mostly in Trichoderma and Aspergillus. Antioxidant activity was done by DPPH and FRAP assay and yielded good results.*

Keywords: *Cestrum Sp Solanaceae, Phytochemicals, Antioxidants, Antimicrobial, Medicinal Plants.*

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INTRODUCTION

Solanaceae, a family of flowering plants is commonly known as "nightshade" includes species which are of ornamental value although being toxic to mammals. Many members of the Solanaceae family are used by humans, and are important sources of food, spice and medicine. However, *Solanaceae* species are often rich in alkaloids whose toxicity to humans and animals ranges from mildly irritating to fatal in small quantities. All members of the *Solanaceae* family contain an alkaloid toxin called solanine. For the current study, three different species of the genus cestrum belonging to the family of *solanaceae* , namely ***Cestrum elegans, C. fasicilatum, C.parqui*** were analysed for phytochemical screening, anti-oxidant potential and anti-microbial activities. These medicinal plants are evergreen woody shrubs well known for their ornamental value albeit being heavily perfumed.

Green cestrum (*Cestrum parqui*) is a large poisonous shrub belonging to the Solanaceae family. The plant is also known as green poison berry or Chilean cestrum.

Green cestrum was originally introduced into Australia from South America as an ornamental shrub for gardens. Since that time, it has become naturalised in areas of south-eastern Queensland, eastern New South Wales (NSW) and parts of Victoria and South Australia.

Cestrum parqui L´Hér. (Solanaceae) is a shrub native of South America where is knowledge by their toxic properties for animals, attributed to alkaloid presence. It is characterized by its fetid fragrance crushing leaves (Ragonese and Milano, 1984). However it is used as ornamental plant in Tunisia (Chaieb et al., 2007), and it has also saponins. These are heterosidic substances formed by a steroid or triterpenic aglycon and a sugar chain. These molecules are well known for their toxic activities against several micro-organisms (Oleszek and Bialy, 2006). The majority of research studies dealing with the biological activities of saponins are mainly concentrated on the antifungal activity. Some authors presume that this activity constitutes their principle function in plant physiology (Oleszek et al., 1999; Mert- Türk, 2006). This activity is probably due to the interference of saponins with fungal membrane sterols (ergosterol) by the formation of a saponin/sterol complex 002 Int. Res. J. Plant Sci. generating pores formation and consequently, loss of cell integrity. Furthermore, these saponins with alcaloidic aglycone can not only bind to sterol but also straightforwardly extract it from the membrane (Morrissey and Osbourn, 1999). The mechanism of action of

saponins has been elucidated against *Fusarium solani* where mutant strains defective out of membrane sterols are able to infect the green fruits of tomato rich in α- omatine (a steroidic saponin) (Morrissey and Osbourn, 1999; Papadopoulou et al., 1999). Many research works have showed that the activity of saponins is more related to their sugar chain and thus, any modification of this chain can provoke modification of the biological activity of the entire molecule. Consequently, fungi can resist to plant saponins by the partial or the total degradation of sugar chains thanks to their enzymatic arsenal (Bourab et al., 2002; Barile et al., 2007; Tsuzuki et al. 2007; Stuardo and San Martyn, 2008; Yadava and Chakravarti, 2009).

The antifungal activity constitutes an interesting property for the assessment of saponin's activity and quantity in plants. Moreover, the antifungal tests are relatively precise and simpler than chromatographic and spectroscopic techniques. In fact, some *in vitro* tests are conducted by using fungal species belonging to *Trichoderma* genus because they are particularly sensitive to saponins (Jurzysta, 1986). Cells of the prokaryotes such as bacteria do not contain membrane sterols suggesting that these organisms are not sensitive to this type of molecules as reported by several authors (Morrissey and Osbourn,1999). Sprag et. al., (2004) pointed that saponins did not inhibit microbial growth of dense populations. In this work, the antibacterial and antifungal activity of the crude saponic extract (CSE) of a local ecotype of Cestrum *parqui* was evaluated against several bacterial and fungal agents and the interaction of saponins and membrane sterols was studied in relation with their eventual biological activities.

Phytochemical screening: it includes screening the plants for phytochemicals , which are chemicals naturally occurring in plants (secondary metabolites) like alkaloids,terpenoids,quinones , flavanoids ,saponins, tannins etc. The compounds were assessed qualitatively and quantitatively.

Anti-oxidant potential: it is the capability of an organism to scavenge free radicals which are harmful to the organism. The plants were subjected to the scavenging activity assays in order to fathom their anti-oxidant potential.

Anti-microbial activity: with increasing number of diseases being discovered there is a constant need to evaluate different plants for their anti-microbial activity. These plants

were analysed for both anti bacterial as well as anti fungal properties against common disease causing organisms.

In order to carry out the investigation, the plants were subjected to solvent fractionations with different organic solvents.

MATERIALS AND EXTRACTION

1. **Plant Material**: The three plant species namely ***Cestrum elegans, C. fasicilatum, C.parqui*** were obtained from Kanakapura, Taluk, Bangalore district, Karnataka. They are not indigenous varieties but were grown and cultivated there. The leaves were taken as the plant material for the study. The leaves were dried (shade drying) for a week and ground to fine powder.

2. Plant Extraction:The The plants were extracted in three different solvents; Water, Methanol and Ethanol. The extraction was carried out by leaving the plant samples overnight in their respective solvents. For phytochemical screening, the concentration was maintained at 0.1g/ml of aqueous extract, while for Anti-oxidant potential the concentration was maintained at 1g/ml of Methanolic and Ethanolic extracts. As for Anti-microbial activity, the concentration was 0.1mg/ml in seven different organic solvents – Ethanol (EtOH), Methanol (MeOH), Butanol (BuOH), Propanol (PrOH), Acetone(Ac),Petroleum ether(PE) and Water.

3. Microbial Cultures: For Anti-microbial assays, three bacterial species and two fungal species were used for the study. Bacterial species *included Klebsiella pnemoniae, Pseudomonas* and *Salmonella typhi*. The two fungal species included *Aspergillus niger* and *Trichoderma* .

METHODS

1. **Phytochemical Screening:** Qualitiative tests were performed for different phytochemicals like: Carbohydrates, Proteins, Cardiac glycosides, Anthraquinones, Terpenoids, Flavonoids, Saponins, Tannins, Lignins, Phenols, Alkaloids and Diterpenes.

   Quantitative tests were perfomed to quantify the amount of Carbohydrates, Proteins, Alkaloids and Flavonoids which were expected to be present in higher amounts in the plants taken for study.

2. **Anti-oxidant Potential**: Two assays were performed to assess the scavenging activities of the three plants. The Two assays are:

(i) **DPPH (Dipheny pyruvic hydroxide) Assay**: DPPH is characterised as a free radical which has maximum absorbance at 517nm. When the anti-oxidant compound reacts with the free radical, it will donate one proton to the free radical, and hence the free radical will get scavenged. Hence the colour changes from violet to pale yellow. Different aliquots of the methanolic plant extracts were taken ranging from 0.2g/ml to 1g/ml.The scavenging activity percentage was calculated from the following formula:

$$\text{Scavenging activity \%} = (A_{Control} - A_{sample})/ (A_{control})$$

Where $A_{control}$ = Absorbance of Control

And A_{sample} = Absorbance of Sample

(ii) **FRAP (Ferric reducing ability of Plasma) assay**: Reducing power was determined by the method of Yildrim et al 2000 with slight modification. The ethanolic extract (1g/ml) was taken in aliquots of 0.2-1g/ml. The samples were then 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. 2.5ml of 10% Trichloroacetic acid was added to the mixture, followed by centrifugation at 3000rpm for 10mins.The upper layer of solution (2.5ml) was mixed with 2.5ml of distilled water and 2.5ml of 0.1% of Ferric Chloride. Absorbance was read at 700nm. Increased absorbance of the reaction mixture indicates increase in reducing power.

3. **Antimicrobial Acitivity**: The Antibacterial and Antifungal activities were assessed by agar diffusion method. The plants in six different solvents were subjected to this method and incubated for 72hrs at 37°C for the bacterial cultures and 5days at room temperature for the fungal cultures. The diameters of the zones of inhibition were measured and noted.

RESULTS:

The phytochemical screening of the three plant species ***Cestrum elegans, C. fasicilatum, C.parqui***, revealed the following phytochemicals :

Test	C.*elegans*	*C. fasicilatum*	*C.parqui*
Carbohydrates	Positive	Positive	Positive
Cardiac Glycosides	Positive	Positive	Positive
Anthraquinones	Positive	Positive	Positive
Terpenoids	Positive	Positive	Positive
Proteins	Positive	Positive	Positive
Flavonoids	Positive	Positive	Negative
Saponins	Negative	Negative	Negative
Tannins	Positive	Negative	Positive
Lignins	Positive	Positive	Positive
Phenol	Negative	Negative	Negative
Alkaloids	Positive	Positive	Positive
Diterpenes	Positive	Positive	Positive

The quantitative results for the phytochemicals are given below

Carbohydrates

	Concentration (mg/ml)	**Absorbance at 630nm**
	0	0
	0.5	0.034
	0.75	0.05
	1	0.07
	1.25	0.09
	1.5	0.12
C.*elegans*	18.55	1.441
C. fasicilatum	31.88	2.441
C.parqui	18.28	1.42

Proteins

	Concentration (µg/ml)	**Absorbance at 765nm**
	0	0
	200	0.34
	400	0.68
	600	0.95
	800	1.19
	1000	1.47
C.*elegans*	1489.86	2.28
C. fasicilatum	809.86	1.26
C.parqui	663.2	1.04

The Carbohydrates content in **C.*elegans*** (30.88mg/ml) was higher than that obtained for ***C. fasicilatum*** (17.55mg/ml) and ***C.parqui*** (17.28mg/ml), whereas the protein content in **C.*elegans*** (1389.76µg/ml) was higher than ***C. fasicilatum*** (709.76 µg/ml) and ***C.parqui*** (763.2 µg/ml). This indicates variations in the carbohydrates and protein content between different species.

Phenol

Concentration (µg/ml)	**Absorbance at 765nm**
0	0
5	0.054
10	0.12
15	0.26
25	0.56
50	1.83
100	2.84
200	5.005
300	6.77
400	8.69
500	9.57
C.*elegans* 7.12	0.14
C. fasicilatum 3.67	0.21
C.parqui 3.18	0.22

The Phenol content was found to be very minute quantities within the test. **C.elegans** (6.12 µg/ml) showed double the quantity of Phenol as compared to **C. Fasicilatum** (4.67 µg/ml) and **C.parqui** (4.18 µg/ml). The graphs showing Carbohydrates content, Protein content and Phenol content are displayed. The Value of the phytochemical content has been obtained from the respective formulas using X and Ý coordinates and R2.

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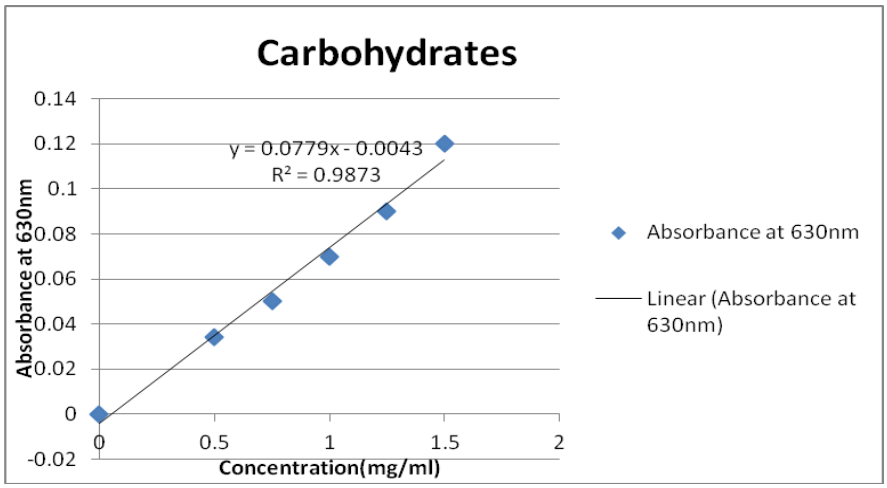

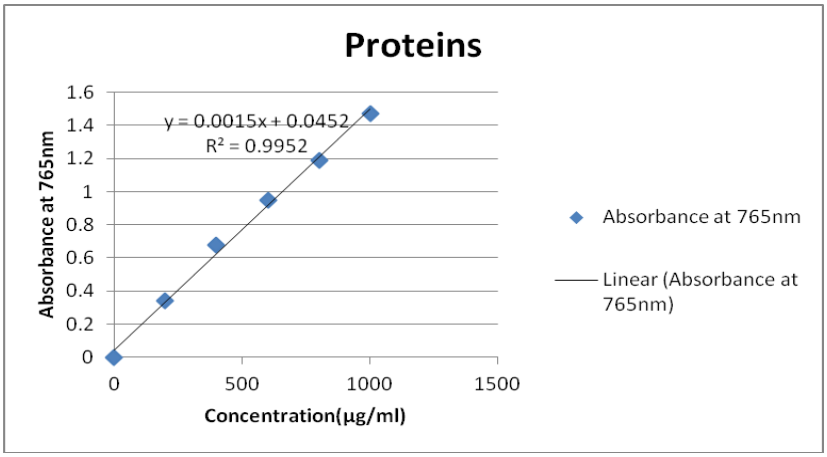

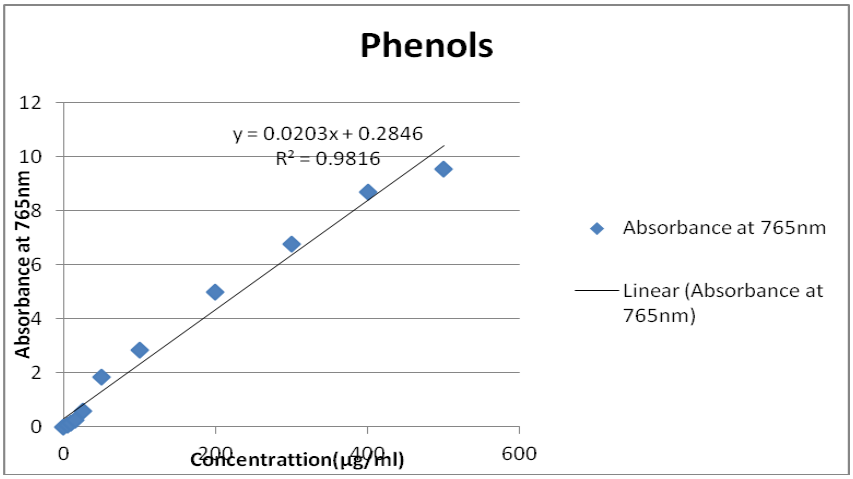

ANTIBACTERIAL ACTIVITY

The results of diameters measured of the zones of inhibition for antimicrobial activity is tabulated:

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Antibacterial activity									
	S typhi			*Pseudomonas*			*K.Pneumoniae*		
	CA(cm)	CN(cm)	CD(cm)	CA(cm)	CN(cm)	CD(cm)	CA(cm)	CN(cm)	CD(cm)
ETHANOL	1.4	1.2	1	1.3	1	1.1	1.2	0.7	0.8
METHANOL	1.1	1.4	1.2	0.9	1	1.1	1.4	1.2	1.1
BUTANOL	1.2	1.4	1	1.3	1.5	1	1.3	1	1.0
PROPANOL	1.5	1.4	1.2	1.1	1.1	1.3	1.2	1.1	1
ACETONE	1.2	1.1	1.3	1.3	1.2	1	1.8	1	0.8
PETROLEUM ETHER	1.1	0.9	0.9	1.1	0.9	0.8	0.9	0.9	1
Antifungal activity									
	Aspergillus			*Trichoderma*					
	CA(cm)	CN(cm)	CD(cm)	CA(cm)	CN(cm)	CD(cm)			
ETHANOL	1.2	0.8	1.0	1.2	1.0	1.3			
METHANOL	1.2	1.2	1.3	1.2	1.1	1.1			
BUTANOL	1.4	1.6	1.2	0.7	0.8	1.0			

RESULTS AND DISCUSSION:

The three plants of our study, ***Cestrum elegans, C. fasicilatum, C.parqui***,are known to have hallucinogenic properties besides being toxic in high quantities causing nausea,headache,vomiting and irritation to humans. These plants being under the solanaceae family are known to contain alkaloids such as solanin .In the current study, the plants were found to be negative for the presence of saponins, ***Cestrum elegans*** 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 ***Cestrum elegans*** 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.

The solvent extracts of ***Cestrum elegans, C. fasicilatum, C.parqui***, in EtOH, MeOH, BuOH, PrOH, Ac, PE showed moderate antimicrobial activities. The zones of inhibition in three bacterial species Pseudomonas, Klebsiella pneumoniae and Salmonella typhi were higher in the case of PrOH followed by BuOH, MeOH PE and Acetone. In the case of Fungi, the zone of inhibition was higher in BuOH for Aspergillus and MeOH for Trichoderma.

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