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Morphological variability potential of *Cenchrus ciliaris* L. ecotypes on their phytochemical substances and antibacterial activities

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Introduction

Ecotypes are population of same species with different races, which is adapted to local environment Different ecotypes of a particular species may differ in their edaphic, biotic or microclimatic requirements (Linhart and Grant, 1996; Eppela *et al*., 2013) and such adaptation either become irreversible or genetically fixed variations. *Cenchrus ciliaris* L. (Tam: *kollukottaipul*, Eng. *buffel grass*, Sans*: anjan grass)* is a grass belonging to the family Poaceae and ephemeral in nature. In this species, habitat plays a significant role in the formation of ecotypes and several ecotypes are found in *Cenchrus ciliaris* L. including with varying inflorescence and florets colours and however all the ecotypes exhibiting similar growth characters. Arshad *et al*., (2007) studied on tiller growth and phonological studies of *C. ciliaris*. Phytochemical studies on the ecotypes is of paramount important and the potential of the plant ecotypes, especially their different components are required to be studied on their antimicrobial activities (Kannan and Bagam Priyal, 2015). The present study was attempted to investigate the important phyto-chemical substances for their presence or absence in three select morphological variants with differing inflorescence and floret colours and the work was included to study the antimicrobial activity of the plant extracts prepared from different components in *Cenchrus ciliaris* L. on the select microbial cultures, under laboratory conditions.

Materials and Methods

Sampling: Three morphological variants of similar age group of *Cenchrus ciliaris*, L. producing inflorescences with varying colour – white, green and black ecotypes were collected from three different naturally growing locations at *Kappalur* (white variant), *Perungudi* (green variant) and *Paravai* (black varient), Madurai District, Tamil Nadu. Stem, leaves and inflorescence were separated and kept for air- drying, till attaining constant weight. Then the dried samples were finely powdered samples were stored for further analysis.

Phytochemical analysis: Exactly weighed 20gms of powdered plant samples were separately subjected for sequential extraction, using different solvents, according to their increasing polarity. The extract obtained from stem, leaf and inflorescence components of *C. ciliaris* L. was prepared with a series of non-polar to polar solvents by heat extraction methods in soxhlet apparatus. Then the extracts obtained were filtered using Whatman No.1 filter paper and then concentrated at 40˚C by using evaporator. The residual extracts were stored in refrigerator at 4˚C in 5 ml. clean sterile glass bottles. Different extracts were used for phytochemical analysis. Qualitative analysis was done for the prepared extracts to find-out the absence or presence of bioactive compounds by using standard methods.

Plant extracts were prepared from different parts the ecotypes of *C. ciliaris* viz., leaves, stem and inflorescence, by dissolving in methanol and those extracts were used to test the antimicrobial activity. *In vitro* antimicrobial activity was examined for the experimental plant extracts, separately obtained from the morphological variants of *C. ciliaris* on three gram-negative bacteria viz., *Escherichia coli*, *Pseudomonas florescent* and *Klebsiella Pnemoniae*. Nutrient agar medium was prepared for culturing the plates. To detect the effect of plant extract, along with the control medium, plant extract mixed in the nutrient agar medium was used to culture the select bacterial strains, simultaneously.

Bacterial activity analysis: The antibacterial activity of the stem, Leaf, inflorescence extracts, was determined using agar well method. Nutrient agar medium was inoculated with the given microorganism by spreading the bacterial inoculums on the media. Wells with 5mm diameter were punched in the agar and filled with plant extracts. Standard antibiotics Gentamycin (10mg/disc) were used. The experiments were replicated for three times. The plates were kept at 4° C for 1 hour to effect the diffusion of extract, thereafter were incubated at 37° C for 24 hours. Antimicrobial activities were assessed in terms of the zone of inhibition developed in the cultures. The inhibition zone was compared with the standard reference antibiotics.

Activity index for each extracts was calculated, using the following formula:

$$\text{Activity index} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

Results and Discussion

The work was started with the characterization of the experimental plant ecotypes on their biochemical compounds present in the different components. Protein, carbohydrate, phenol, flovonoids, saponins, and alkaloids were found to be presented in all the three morphological variants of the experimental plant, irrespective of the components (Table 1). Extracts prepared from different parts of white and black types of *C. ciliaris* were found that the absence of glycosides; whereas in green ecotypes, glycosides were found from the extracts, prepared separately from stem leaves and inflorescence. White and green variant were shown positive results for the presence of steroids in all their plant parts. In black variant this biochemical substance was present only in their leaves. Coumarin was present both in white and green variants of *Cenchrus ciliaris* whereas, their compound was not found in the extracts prepared from the different parts of black variant.

E. coli and *B. subtilis* were found susceptible organism grown in the medium augmented with the methanol extract of *Cenchrus ciliaris*. Antimicrobial activity was assessed in terms of inhibition zone and activity index. All the extracts exhibited better antibacterial activity against *Proteus mirabilis,* K*lebsiella pnemoniae* and *Pseudomonas florescent* (Table 2). Black variant leaves and inflorescence extract prepared using methanol had the controlling activity against *K. pnemoniae,* more efficiently, when compared with *P. mirabilis* and *P. florescent*. The activity index results showed the same trend (Table 2). Bio activity of crude extracts of leaves of *Cenchrus* grass extracted in different polar solvents act against some pathogenic microbes (Premlata *et al*., 2011). Results of the present study clearly showed that extracts obtained from the experimental plants and their morphological variants inhibited the bacterial growth by which this phenomenon was observed through the formation of inhibition zone developed.

Table 1: Phytochemical screening of extracts, prepared from different ecotypes of *Cenchrus ciliaris*, L. (+ indicates the presence and – represents the absence of the substances)

Bioactive groups	White Ecotype			Green Ecotype			Black Ecotype		
	Stem	Leaf	Florets	Stem	Leaf	Florets	Stem	Leaf	Florets
Phenol	+	+	+	+	+	+	+	+	+
Flavanoids	+	+	+	+	+	+	+	+	+
Saponins	+	-	+	+	+	+	+	+	+
Glycosides	-	-	-	+	+	+	-	-	-
Steroids	+	+	+	+	+	-	-	+	-
Terpenoids	+	-	+	+	+	+	+	-	+
Alkaloids	+	+	+	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+	-	-	-

Table 2: Antimicrobial Activity of extracts of three ecotypes of *Cenchrus ciliaris* prepared from Methanol solvents on *Proteus mirabilis, Pseudomonas florescent, Klebseilla pnemoniae;* IZ- Inhibition zone. AI – Activity index

Ecotypes of *Cenchrus ciliaris*	**Plant component**	**Control on the microbial population**					
		P. mirabilis		***P. florescent***		***K. pnemontae***	
		IZ	AI	IZ	AI	IZ	AI
White	Stem	0.0	0.0	1.5	0.6	1.5	0.6
	Leaves	1.7	0.62	1.2	0.48	1.6	0.64
	Inflorescence	1.3	0.52	1.4	0.56	1.5	0.6
Green	Stem	1.3	0.52	1.0	0.4	2.0	0.4
	Leaves	1.5	0.6	1.4	0.32	1.5	0.6
	Inflorescence	1.0	0.40	1.0	0.4	1.0	0.4
Black	Stem	1.4	0.56	1.5	06	1.8	0.72
	Leaves	1.3	0.56	1.0	0.4	2.4	0.96
	Inflorescence	0.6	0.24	1.0	0.4	1.4	0.56

Conclusion

The morphological variations detected on the basis of phytochemistry is one of the valuable methods to establish proper selection procedures on populations to identify the optimum yield of phytochemical substances, leads to the production of effective drugs to sustain the health of human beings. Further, this present study has further prospects to establish studies on the genomic studies, leading to genetic similarities as well as dissimilarities. Further studies are required to test the efficacy of plant and their ecotypes on the various biological activities and cattle feed value and further implications of the *C. ciliaris* ecotypes on live stock development.

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