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Phytochemical and Antimicrobial screening of ethanol and hexane extracts of *Urochloa ramose*

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ABSTRACT

Urochloa ramosa is well-known for its environmental benefits, which include stabilizing and reclaiming polluted soils, eliminating root-knot nematodes that damage crops, and treating cardiovascular problems, duodenal ulcers, and hyperglycemia, nephritis, and snake bites. Using recognized procedures, phytochemicals in ethanol and hexane extracts were qualitatively evaluated. Using the disc diffusion method, an in vitro antimicrobial experiment was conducted against the fungus Candida albicans, the gram-negative bacteria Pseudomonas aeruginosa and Escherichia coli, and the gram-positive bacteria Bacillus subtilis and Staphylococcus aureus. It was discovered that Urochloa ramosa hexane extract had a 70% efficacy rate against Candida albicans, demonstrating strong antifungal properties.

Keywords -: Antimicrobial activity, Phytochemicals, *Urochloa ramose*

INTRODUCTION

Urochloa ramosa commonly known as 'Brown top millet' is an annual weedy grass in Poaceae. In India, it is often cultivated for grains and as a forage crop. It is also grown as a nurse crop especially on slopes, which can assist in the establishment of a perennial crop. The ash of the leaves is to treat cardiovascular diseases, duodenal ulcer, hyperglycemia, nephritis and snake bites by traditional healers. It is used in environmental remediation projects, as it accumulates significant amounts of zinc and lead from the soil and hence it can be a potential source for easy removal of toxic minerals. The plant also possesses antimicrobial, antioxidant, anti-inflammatory and antiangiogenic properties. Plants are rich source of bioactive phytochemicals .Phytochemicals acts as components of defense sys-tem that not only protect plants from stress conditions but also these phytometabolites (primary and secondary metabolites) can also be used as herbal drugs by humans, as they act as natural antioxidants thereby preventing the risk of Cancer development (2). Invasiveness and toxigenic qualities of pathogenic microorganisms has led to the spread of many infectious diseases. In order to control the spread of pathogens, modern medicines rely upon the use of synthetic antibiotics (3). Unscientific usage of broad spectrum of antibiotic not only causes severe oxidative stress in

human beings but also it has lead to the development of multidrug resistant pathogens for which the need of the hour is to de-pend on herbal medicines that will enable preventing infectious microbial diseases. In the present work, both anti-bacterial and antifungal activities of ethanol and hexane extracts were assessed by disc diffusion method (4).

Materials and Methods

All reagents and solvents were of analytical grade used in the present study. The plant was collected from its natural habitat and authenticated by a Botanist from R.G.P.G.College, CCS University Meerut. The leaves were thoroughly washed, dried under shade, fine powdered, sieved and stored till further use in air tight container. 100 g of leaves were weighed and subjected to sequential extraction using solvents like hexane, chloroform, ethyl acetate, acetone ethanol and methanol (non polar to polar) taken in Soxhlet apparatus. Hexane extract and ethanol extracts were filtered and used for screening of phytochemicals and assessment of biological activities like anti-microbial, activities.

PHYTOCHEMICAL ANALYSIS

The hexane and ethanol extracts of the plant were subjected to preliminary phytochemical analysis to trace the presence of phytochemicals like Carbohydrates, Tannins, Saponins, Alkaloids, Flavonoids, Glycosides, Quinones, Phenols, Terpenoids, Cardiac glycosides, Amino acids, Coumarins, Anthraquinones, Steroids, Lignin, Phlobatannins, Anthocyanin, Balsams, Volatile oils and Fatty acids (Table 1) as per the standard methods (11-13).

ANTIMICROBIAL ACTIVITY

Gram-positive bacteria *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538) and Gram-negative bacteria - *Pseudomonas aeruginosa* (ATCC 9027), *E. coli* (ATCC 25922) and fungi *Candida albicans* (ATCC 3147) were used for the determination of antimicrobial potential of ethanol and hexane extract of *U. ramosa* by disc diffusion method. 0.1 g of extract was transferred to 100 ml of ethanol and hexane and dissolved to form a final concentration of 1 mg/ml. This forms stock solution of the extract. Extract of 20 µl of various concentrations - 50, 100, 200, 400 µg/mL dissolved in Dimethyl formamide were impregnated onto 6 mm diameter sterile discs. Dimethyl formamide loaded disc was considered as negative control. Sterile discs containing various concentrations of extracts were layer onto the lawn of microbial colonies in the petri plate; it was followed by incubation at 37 ˚C for 24 hrs to determine the zone of inhibition in mm. Gentamycin and Flucanazole were used as positive control (14-16).

Table 1. Qualitative Phytochemical tests

Phytochemicals	Test	Observation
Carbohydrates	1ml extract + 1ml Molish's reagent+ 2 drops of sulphuric acid	Purple colour
Tannins	1ml extract + 2ml 5% ferric chloride	Dark blue colour
Saponins	1ml extract + 1ml distilled water+ shaken well	Foam formation
Alkaloids	1ml extract + 1ml HCl + 2 drops of Mayer's reagent	Green colour
Flavanoids	1ml extract + 1ml 2N sodium hydroxide	Yellow colour
Glycosides	1ml extract + 2ml chloroform + 1ml 10% ammonia solution	Pink colour
Quinones	1ml extract + 1 ml sulphuric acid	Red colour
Phenols	1ml extract + 1 ml distilled water + 4 drops 10% ferric chloride	Blue/green colour
Terpenoids	1ml extract + 1 ml chloroform + 1ml sulphuric acid	Reddish brown colour
Cardiac glycosides	1ml extract + 1 ml glacial acetic acid + 4 drops of 10% ferric chloride + 1 ml sulphuric acid	Brown colour
Amino acids	1ml extract + 2 drops of 0.2% ninhydrin+ heat	Pink/purple colour
Coumarins	1ml extract + 1 ml 10% sodium hydroxide	Yellow colour
Anthraquinones	1ml extract + 4 drops 10% ammonia solution	Pink colour
Steroids	1ml extract + 1ml chloroform+ 4 drops sulphuric acid	Brown ring formation
Lignin	1ml extract + 1ml Phloroglucinol-HCl	Red/violet colour
Phlobatanins	1ml extract + 4 drops 2% HCl	Red colour precipitate
Anthocyanins	1ml extract + 1ml 2N sodium hydroxide + heat	Bluish green colour
Balsams	1ml extract + 1ml 90% ethanol + alcoholic ferric chloride	Dark green colour
Volatile oils	1ml extract + 4 drops dilute HCl	White precipitate
Fatty acids	1ml extract + 2ml ether + mixed, evaporated on filter paper	Transparency on filter paper

STATISTICAL ANALYSIS

Values are means of three independent replicates (n = 3) and ± indicates standard deviation. Means followed by the same letter(s) within the same column are not sig-nificantly ($p \leq 0.05$) different according to Tukey's HSD.

Results and Discussion

Qualitative analysis of phytochemicals

The phytochemicals found in ethanol and hexane extracts are presented in Table 2.

Table 2. Details of phytochemicals found in ethanol and hexane extracts of *U. ramosa*

Phytocompounds	Ethanol extract	Hexane extract
Carbohydrates	+	-
Tannins	+	-
Saponins	+	+
Alkaloids	-	-
Flavonoids	+	-
Glycosides	-	+
Quinones	-	+
Phenols	+	-
Terpenoids	+	+

Cardiac glycosides	-	+
Amino acids	+	-
Coumarins	-	+
Anthraquinones	-	-
Steroids	-	+
Lignin	+	-
Phlobatannins	-	+
Anthocyanin	+	-
Balsams	+	-
Volatile oils	-	+
Fatty acids	-	+

Note: + indicates presence and - indicates absence of phytoconstituents

Antimicrobial activity

Antimicrobial activity of two extracts of *U. ramosa* was determined by evaluation of zone of inhibition formed around the discs. Among five different concentrations, 400 µg/ml showed maximum activity against all the microbes viz.; *Bacillus subtilis* (8.0 mm and 9.0 mm), *Staphylococcus aureus* (5.0 mm and 7.0 mm), *Pseudomonas aeruginosa* (6.0 mm and 8.0 mm), *E. coli* (8.0 mm and 11.0 mm) *and Candida albicans* (12 mm and 13 mm) respectively. For-mation of zone of inhibition was recorded in *Bacillus subtilis* and *Candida albicans* at all the concentrations in both ethanol and hexane extracts. There was no formation of zone of inhibition at 50 µg/ml concentration in *Staphylococcus aureus, Pseudomonas aeruginosa* and *E. coli* in both the extracts. It was observed that ethanol and hexane extracts were more effective against *Bacillus subtilis* and *Candida albicans* and in particular there was more growth retardation and arrest in *Candida albicans* indicating potent antifungal property than antibacterial property of hexane extract (Table 3; Fig. 1).

Table 3. Antimicrobial activity of ethanol and hexane extract of *U. ramosa* - Zone of inhibition (mm) induced by plant extract by disc diffusion method.

		Concentration (µg)					
Test Pathogens	**Extract**	**50**	100	200	400	**Gentamycin**	**Flucanazole**
Bacillus subtilis	EUR	2.26 ± 0 .30b	5.00 ± 0.20a	7.20 ± 0.20a	8.06 ± 0.20c	18.00 ± 0.00a	-
	HUR	3.86 ± 0.11a	5.10 ± 0.10a	6.90 ± 0.10b	9.00 ± 0.20b		-
Staphylococcus aureus	EUR	0.00 ± 0.00c	0.00 ± 0.00d	2.06 ± 0.05f	4.96 ± 0.05f	17.86 ± 0.11a	-
	HUR	0.00 ± 0.00c	3.03 ± 0.15b	4.96 ± 0.05d	7.00 ± 0.00d		-
Pseudomonas aeru ginosa	EUR	0.00 ± 0.00c	0.00 ± 0.00d	2.03 ± 0.05f	6.00 ± 0.00e	18.00 ± 0.00a	-
	HUR	0.00 ± 0.00c	2.96 ± 0.05b	5.96 ± 0.05c	8.06 ± 0.05c		-
E. coli	EUR	0.00 ± 0.00c	1.86 ± 0.11c	3.96 ± 0.05e	7.96 ± 0.15c	13.96 ± 0.05b	-
	HUR	0.00 ± 0.00c	1.93 ± 0.11c	4.93 ± 0.11d	10.63 ± 0.55a		-
Candida albicans	EUR	3.30 ± 0.20b	6.00 ± 0.30b	8.43 ± 0.45b	12.03 ± 0.25a	-	18.83 ± 0.28a

	HUR	4.96 ± 0.25a	8.03 ± 0.15a	10.06 ± 0.20a	12.93 ± 0.11a	-	

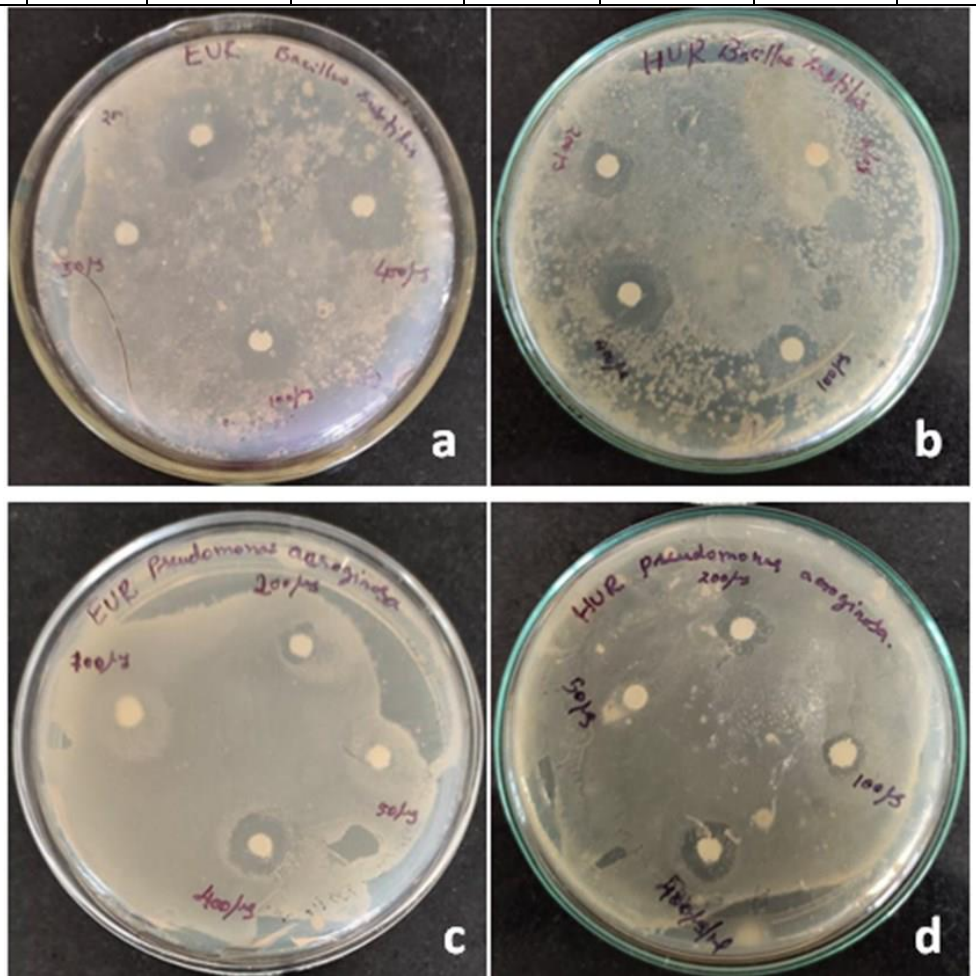

Fig. 1. The inhibition zone (mm) of ethanol (a & c) and hexane (b &d) extracts of *U. ramosa* on *Bacillus subtilis* and *Pseudomonas aeruginosa*

CONCLUSION

Presently use of antibiotics and other synthetic compounds is very much spread in medicine and agriculture that has led to the emergence of antibiotic resistant strains of microbes that eventually cause very serious problem in controlling infectious diseases as a result re-searchers are exporting new antimicrobial compounds mainly from plant source. On the basis of this antimicrobial study, it is clear that the selected plant shows a considerable activity against selected microbes. Therefore, this study indicates that *U. ramosa* may be used as potent anti-microbial drugs of natural origin. Plants are backbone of sophisticated traditional medicine systems dating back to hundreds of years and persist to supply human mankind with new remedies. The use of synthetic chemical compounds has led to decline in the use of plants in contemporary medicine over years (29). Botanicals are considered as best source of medicine, as they are ecofriendly, more

affordable than conventional medicine, easier to obtain than prescribed medicine, strength in immune system, fewer side effects and cost effective (30). Upon qualitative analysis hexane extract of *U. ramosa* confirmed the presence of biologically active and medicinally important active compounds in both ethanol and hexane extracts proving potent antimicrobial other biological activities.

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