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ASSESSMENT OF ANTIOXIDANT ACTIVITIES OF METHANOL ETHANOL AND HEXANE EXTRACT OF *Urochloa ramose*

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ABSTRACT

Urochloa ramosa is a plant of the wet, seasonally dry and semi-arid tropics. It is also known as Bracharia ramosa or Brown top millet. In-vitro antioxidant assay was conducted by DPPH radical, NO. radical, superoxide anion radical scavenging assays and OH radical scavenging activity by DNA protection assay using BHT as the reference compound. IC50 value of methanol extract for each of these assays was calculated. IC50 of 10.10µg/ml, IC50 of 17.32µg/ml and IC50 of 25.12µg/ml demonstrated antioxidant activities of methanol extract of *U. ramosa* by scavenging DPPH and superoxide anion radicals respectively. At 100µg/ml concentration, methanol extract significantly protected DNA against the effect of hydroxyl radicalsThe leaves of Urochloa ramosa are basically enriched with natural phenolics with multitudinous biological potencies that can represent a promising class as bioactive molecules.

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

GENRAL INTRODUCTION

Urochloa ramosa (L.) T.Q. Nguyen, commonly known as 'Brown top millet' is an annual weedy grass in *Poaceae*. In India it is often cultivated for its high nutritional grains and used 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 due to its allelopathic effects [1,2]. The ash of the leaves is used 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 toxic heavy metals like zinc and lead from the soil and hence it can be a potential source for easy removal of toxic minerals [3]. *Urochloa ramosa* is also used as nurse crop, catch crop, cover crop and is beneficial in controlling root-knot nematodes in herbaceous crops like tomatoes, cucumbers, melons and pepper.

U.ramosa is an annual grass that originated in South-East Asia. It is now grown in Africa, Arabia, China and Australia as forage crop. It was introduced to the United States of America from India in 1915 and it is mainly grown in the South-East for hay, pasture and game bird feed [4,5]. The browntop millet commonly called korale in Kannada, is specially grown in rain fed areas of Tumakuru, Chitradurga and Chikkaballapura districts

of Karnataka state. The crop is popular in this region in terms of cultivation and consumption. This millet seed can grown in a variety of soils and different climatic conditions owing to its ability to acclimatize to various agroclimatic zones. Like other millets, it is a hardy crop and well suited for dry land agriculture practice.

Due to its rich nutritional content and the property of adapting to different agroclimatic zones especially arid zone, there is a huge demand among consumers in the Indian market. It is rich in protein, fibre and minerals. Nutritionists suggest that *U. ramosa* seeds as a solution for life style disease and problems such as diabetes, arthritis, and heart disease. Consumption of *U. ramosa* seeds as food is yet to gain popularity due to lack of awareness by consumers and the disinterest shown by food processing industries [6].

Plants play a major role in traditional as well as western medicines as they contain bioactive phytometabolites for the utilization of therapeutic purposes. They can also be used as precursors of chemo pharmaceutical semisynthetic drug. The plant *U.ramosa* which we have selected for our research work is used as a forage crop and no work is conducted and no literature is available on it for its biological activities. Other plants belonging to the same genus namely *Brachiaria brizantha, Brachiaria purpurascens* and *Brachiaria mutica* are found to be rich in proteins, carbohydrates, fibre, phenolics, tannins, lignins, saponins and the seeds of these plants posses high antioxidant properties. So far scientific investigations is not conducted on this plant so we evaluated and explored the antioxidant, anti-microbial, anti-inflammatory and antiangiogenic activities of leaf extract of *Urocloa ramosa* (*Bracharia ramosa*).

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. Methanol extracts were filtered and used for screening of phytochemicals and assessment of biological activities like antioxidant activities.

In-vitro Antioxidant assays

a. DPPH radical scavenging assay

Different aliquots containing reaction mixtures of 4-20µg of methanol, ethanol and hexane extracts of *U.ramosa*, 0.1mM DPPH solution were mixed thoroughly for the assessment of DPPH radical scavenging activity. The aliquots were shaken well and incubated for 20 minutes under room temperature condition. There will be decrease in absorbance of DPPH and it is read at 517nm with that of a blank was measured.

DPPH radical scavenging activity was calculated using the following equation.

Scavenging effect (%) = 1- sample absorption / control absorption X100 at 517nm

b. Superoxide radical scavenging assay

For the assessment of scavenging of superoxide radicals by crude extracts, different aliquots of 10-50µg of methanol, ethanol and hexane extracts of *U.ramosa* were mixed with 1mM NADH, 0.1mM Phenazinemethosulphate and 1mM Nitrobluetetrazolium chloride dissolved in 0.1M phosphate buffer

adjusting the reaction mixture pH to 7.4. The reaction mixture was further incubated under room temperature condition for 5 minutes and absorbance reading was taken against the blank at 560 nm.
The superoxide scavenging effect was calculated by
Scavenging effect (%) = [1- sample absorption / control absorption] X100 at 560nm

Results and Discussion

Antioxidant assay

Table 1: Antioxidant activity of crude extracts from *U. ramosa*

Antioxidant activity	**BHT (IC_{50} value)** µg/ml	**M*UR* (*IC_{50}* value)** µg/ml	**E*UR* (IC_{50} value)** µg/ml	**H*UR* (IC_{50} value)** µg/ml
DPPH radical scavenging assay	7.8± 0.00^{c}	10.10± 0.00^{d}	10.02± 0.00^{c}	9.04 ± 0.00^{c}
Superoxide radical scavenging assay	19.00± 0.20^{b}	25.12± 0.05^{b}	23.14± 0.00^{c}	21.00± 0.30^{b}

MUR- Methanol extract of *Urochloa ramosa* , EUR- Ethanol extract of *Urochloa ramosa*, HUR- Hexane extract of *Urochloa ramosa*

Standard deviation is denoted by the symbol ±. The notation n=3 signifies that three independent experiments were conducted, with the values presented representing the average of these experiments. Based on Tukey's HSD analysis, the means obtained do not exhibit significant differences ($p \leq 0.05$), as indicated by the means sharing the same letter within the same column. Table 4.5 presents the IC_{50} values for DPPH radical scavenging, superoxide anion radical scavenging activity, and nitric oxide radical scavenging for standard butylated hydroxytoluene, as well as methanol, ethanol, and hexane extracts of *U. ramosa*. The hexane extract demonstrated superior scavenging activity for DPPH, superoxide anion radicals, and nitric oxide radicals compared to the methanol and ethanol extracts.

1. DPPH radical scavenging activity

Natural antioxidants are capable of neutralizing free radicals. The methanol and ethanol extracts of *U. ramosa* demonstrated lower DPPH radical scavenging activity, with IC50 values of 10.10 µg/ml and 10.02 µg/ml, respectively, in comparison to BHT, which had an IC50 value of 7.8 µg/ml. The hexane extract presented an IC_{50} value of 9.04 µg/ml. The protonated DPPH radical exhibits maximum absorbance at 517 nm. This absorbance may decline due to the scavenging effects of antioxidants, particularly phytochemicals in the extracts that possess hydrogen-donating capabilities.

b. Superoxide anion radical scavenging activity

In the present investigation, extracts of *U. ramosa* obtained using methanol, ethanol, and hexane demonstrated moderate activity in scavenging superoxide radicals. Superoxide anions are generated in small amounts during

normal metabolic processes. Although they are relatively weak oxidants, they can lead to the production of highly reactive hydroxyl radicals, which are implicated in oxidative stress. Therefore, it is essential to neutralize and eliminate the detrimental effects of superoxide radicals. The methanol and ethanol extracts of *U.ramosa* exhibited superoxide anion radical scavenging activities with IC50 values of 25.12 µg/ml and 23.14 µg/ml, respectively, in comparison to BHT, which had an IC_{50} value of 19.00 µg/ml. The hexane extract displayed an IC_{50} value of 21.00 µg/ml.

CONCLUSION:

IC_{50} value of DPPH radical scavenging, superoxide anion radical scavenging activity and Nitric oxide radical scavenging of standard butylated hydroxyl toluene and methanol extract of UR were assessed and it was found that methanol extract of *U.ramosa* showed better Nitric oxide radical scavenging activity when compared to BHT. IC50 value of methanol extract for each of these assays was calculated. IC_{50} of 10.10µg/ml, IC_{50} of 17.32µg/ml and IC_{50} of 25.12µg/ml demonstrated antioxidant activities of methanol extract of *U. ramosa* by scavenging DPPH and superoxide anion radicals respectively. At 100µg/ml concentration, methanol extract significantly protected DNA against the effect of hydroxyl radicalsThe leaves of Urochloa ramosa are basically enriched with natural phenolics with multitudinous biological potencies that can represent a promising class as bioactive molecules.Antioxidant activities of methanol extract of leaf of *Urochloa ramosa* is mainly because of the presence of phenolics in the methanol extract. In the present study we could able to partially purify the phenolics from the methanol extract, further purification and elucidation of the structure is under progress.

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