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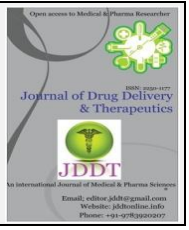

Open Access Research Article

PHENOLIC, FLAVONOID AND TANNIN CONTENT DETERMINATIONS AND *IN-VITRO* ANTI-OXIDANT ACTIVITY OF ROOT EXTRACTS OF *SACCHARUM MUNJA* ROXB.

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ABSTRACT

Free radicals are reactive molecules involved in many physiological processes and have been associated with many diseases, such as ageing, cancer, arthritis, liver injury and cardiac complications. The fact between anti-oxidant potential and amount of polyphenolics compounds of *Saccharum munja* is very crucial co-relation because of its ehnomedicnal uses and experimental values. The total phenolics content were found to be 10.15±2.45 mg gallic acid equivalent (GAE)/g total flavonoids contents and total tannin contents were 11.34±1.20 mg RE/g and 14.54±0.52 mg GAE/g respectively of *Saccharum munja*. The ability of *Saccharum munja* root extract was found to inhibit reactive oxygen species (ROS) free radicals. In the present study, the relative antioxidant ability of *Saccharum munja* was investigated through two *in-vitro* models, such as antioxidant capacity by radical scavenging activity using, α, α-diphenyl-β-picrylhydrazyl (DPPH) and nitric oxide (NO) methods. The extracts were used at concentration 20, 40, 60, 80 and 100 µg/ml concentrations and radical scavenging activity was determined in terms of inhibition percentage. The IC_{50} (concentration required for 50% inhibition) were calculated for each radicals. The *In-vitro* free radical DPPH activities was found to 73.45 ± 0.25 and NO antioxidant activity were found to be 57.20 ± 1.15 at maximum concentration of 100µg/ml. This study proven the significant role of hydro-alcoholic extract of *Saccharum munja* Roxb. as a potential source of natural antioxidants.

Keywords: polyphenolic, anti-oxidant, free radical etc.

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INTRODUCTION

Climatic changes are giving rise to a variety of free radicals, in which plants have to deal with them in order to survive. Many reactive oxygen species, such as singlet oxygen, superoxide ion, hydroxyl ion and hydrogen peroxide, are highly reactive, toxic molecules, which are generated normally in cells during metabolism. They cause severe oxidative damage to proteins, lipids, enzymes and DNA by covalent binding and lipid peroxidation, with subsequent tissue injury. Natural antioxidant agents have attracted much interest because of their ability to scavenge free radicals.[1-2]

Saccharum munja (sar) root is sweet acrid, cooling, aphrodisiac useful in burning sensation thirst, erysipelas blood troubles, liver, urinary complains, eye disease and is a good source of polyphenolics.[3]

Figure 1: Morphology of (a) root (b) entire plant

MATERIAL AND METHODS

Collection and preparation

The medicinal plant material was collected from different geographical area of district Agra, Uttar Pradesh, India. The medicinal plant was authenticated from National Institute of Science Communication and Information Research (NISCAIR), New Delhi, India under supervision of scientist Dr. Sunita Garg.

Plant Extraction

The plant part was dried for two weeks under shade, then at room temperature and were subjected to size reduction with a crusher and then passed through sieve no. 40 to get uniform powder. Around 250 g of powdered plant material was subjected to extraction with solvent such as petroleum ether (for the purpose of defatting), alcohol (60%). The hydro-alcoholic (40:60) extracts were subjected for maceration process of cold extraction. The extract was then distilled to dryness under reduced pressure using rotatory evaporator to yield the respective dried extract.[4]

Determination of Total Phenolic Content:

The total phenolic content of the plant extract was determined by the Folin-Ciocalteu method.

Quantitative analysis/total phenolic content of plant was performed by Spectrophotometric method and determination was done on the basis of a standard curve of gallic acid. Absorbance of the standards (Gallic acid and Ascorbic acid) were measured at 765 nm using UV/VIS spectrometer (Shimadzu, Japan) against blank, i.e., distilled water [5]. The total phenolic content of plant extract displayed in figure 2.

Determination of Flavonoid Contents:

Individual plant extract of 1g with aluminium chloride for determination of flavonoids content was performed[6]. The total flavonoid content of plant extract displayed in figure 2.

Determination of Tannin Content

The tannin was determined by Folin-Ciocalteu method. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer.[7] The tannin contents were expressed in (table 2) terms of mg of GAE/g of extract.

In-vitro antioxidant Activity

DPPH Test

The ability of *individual plant* extracts to scavenge the DPPH$^{\bullet}$ radicals were assessed by using given method.[8] The stable DPPH radical was used for determination of free radical scavenging activity of test sample.[9-10] The absorbance was recorded at 517 nm using UV/Vis spectrophotometer against blank (using methanol). The measurements were taken thrice, and scavenging effect was calculated based on the percentage of DPPH scavenged.[11-12]

Nitric oxide scavenging activity

Nitric oxide radical generated from sodium nitroprusside was measured.[13] Briefly, the reaction mixture (5.0 mL) containing sodium nitroprusside (5 mM) in phosphate-buffered saline (pH 7.3), with or without plant extract at different concentrations, were incubated at 25°C for 3 hours. The nitric oxide radical thus generated and reacted with oxygen to produce the nitrite ion, which was assayed and incubated at 30-minute intervals by mixing 1.0 mL incubation mixture with an equal amount of *Griess reagent*. The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulfanilamide and subsequent coupling with naphthyl-ethylene di-amine di-hydro chloride (NEDD) was measured at 546 nm.[15-17] (Table 2)

RESULTS AND DISCUSSION

Total Phenolic, Flavonoids & Tannin Contents:

Phenolic compounds have redox properties, which allow them to act as antioxidants. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolics concentration could be used as a basis for screening of anti-oxidant activity. The polyphenolics are plant secondary metabolites, the anti-oxidant activity of which depends on the presence of free OH groups, especially 3-OH. The total phenolics content was 10.15±2.45 mg gallic acid equivalent (GAE)/g, total flavonoids contents and total tannin contents were 11.34±1.20 mg RE/g and 14.54±0.52 mg GAE/g respectively shown in figure 2.

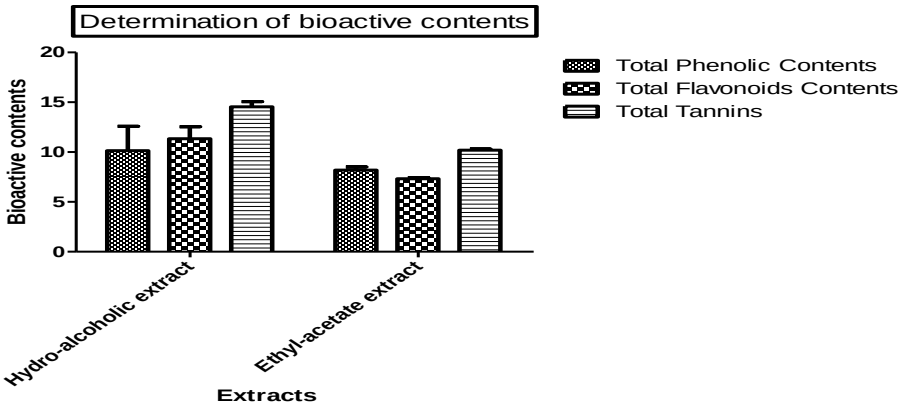

Figure 2: *Total phenolic, total flavonoid & total tannins contents of Saccharum munja*

Anti-oxidant activity:

The DPPH assay is one of the most commonly used methods for measuring antioxidant activity. The DPPH radical is widely employed in assessing free radical scavenging activity because of the ease of the reaction. The *In-vitro* free radical DPPH activity was found to 73.45 ± 0.25 at maximum concentration of 100µg/ml shown in table 1.

In the present study, the nitric oxide radical quenching activity of the polyphenolics extract was detected and compared with the standards Rutin and ascorbic acid. The NO methods revealed that the scavenging of the free radicals was found to be 57.20 ± 1.15 at maximum concentration of 100µg/ml shown in table 2

Statistical analysis

The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates (Triplicate).

Table 1: *In-vitro* free radical scavenging activity of *Saccharum munja* Roxb. against DPPH model

PLANT NAME		CONCENTRATION				
S.N.		**20(µg/ml)**	**40 (µg/ml)**	**60 (µg/ml)**	**80(µg/ml)**	**100(µg/ml)**
1	***Hydro-alcoholic extract***	22.0 ± 0.08	38.37 ±0.040	53.47 ± 0.02	65.0 ± 0.04	73.23 ± 0.01
2	***Ethyl-acetate extract***	21±0.15	30.45±1.54	42.0±1.25	56.5±1.10	65.25±1.45
3	***Ascorbic acid (Standard)***	45.86±0.178	63.78±0.156	74.34±0.123	82.79±0.149	89.17±0.180
4	***Rutin (Standard)***	30.51±8.92	42.05±5.61	65.53±1.68	79.98±6.39	91.46±2.64

Table 2: Nitric oxide free radical scavenging activity of *Saccharum munja* Roxb.

		CONCENTRATION				
S.N.	**PLANT NAME**	**20(µg/ml)**	**40 (µg/ml)**	**60 (µg/ml)**	**80(µg/ml)**	**100(µg/ml)**
1	***Hydro-alcoholic extract***	32.50 ± 0.02	45.17 ±0.050	47.0 ± 0.012	53.0 ± 0.042	72 ± 0.012
2	***Ethyl-acetate extract***	27.20±1.02	37.20±1.10	42.10±1.20	46.0±0.50	60.10±0.20
3	***Ascorbic acid (Standard)***	45.86±1.02	63.78±1.20	74.34±1.21	82.79±1.30	91.17±1.20
4	***Rutin (Standard)***	30.51±1.05	42.0±1.12	65.0±1.21	79.0±1.20	89.0±1.25

CONCLUSION

The results indicated a direct correlation between the antioxidant activity and the polyphenolics contents of the *Sacchrum munja*, which may the foremost contributors to the antioxidant activity of *Sacchrum munja*. On the basis of all finding the present study confirmed that *S. munja* are potential source of natural antioxidants.

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