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In vitro Antioxidant and free radical scavenging activity of *Macrotyloma uniflorum* dal from Kumauni region

Renu Singh, Manoj Kumar Singh, Lovy Raj Chandra, Deepa Bhat, Manmeet Singh Arora, Tapan Nailwal, Veena Pande*

Department of Biotechnology, Bhimtal Campus, Kumaun University, Nainital-263136, Uttarakhand, India.

Abstract

Background & Aim: The present study was carried out to evaluate the *in vitro* antioxidant activities of Methanol extract of *Dolichos biflorus* dal ((DME)) commonly edible food from central Himalayans. **Methods:** This was achieved by screening of the plant extracts at varying concentrations (20-200µg/ml), using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity, reducing power assay and hydrogen peroxide radical scavenging activity. **Results:** Total phenol and flavonoid contents (92.10 ± 8.11 mg/ml GAE per 100 mg plant extract and 139.5 ± 55.09 mg/ml QE equivalent per 100 mg plant extract) were found respectively. Scavenging effect of DME was 4 times greater than that of the synthetic antioxidant ascorbic acid. **Conclusion:** Result also suggests a close relations in between total phenolic content and antioxidant activity, reducing power and radical scavenging effect on DPPH radicals, which proves *Dolichos biflorus* has a potential source of useful natural antioxidants.

Keywords: *Dolichos biflorus ; antioxidants; Phenol; Flavonoid; DPPH.*

1. Introduction

Gratis radicals are essential part of aerobic life and modulate diverse physiological functions[1]. Their excessive generation may disrupt the body's antioxidant system which might lead to "oxidative stress". This situation contributes to a variety of diseases like diabetes[2]. Although the development of some synthetic antioxidants in the past few years has flourished, they are not yet widely used as therapeutic agents due to their possible toxicity. As a result of which the development of natural antioxidant has now drawn the attention of scientific community and different kinds of plant material have already been reported as natural antioxidant[3]

Ethno-botany has emerged as an important branch of study, which focuses on the utility of different plant species and their properties as food, medicine and other uses. Plant species of the Himalaya as medicine has been known for a long time. *Macrotyloma uniflorum* (Old name *Dolichos biflorus* Linn) (Fabaceae), is commonly known as Kulthi or Gahat in Uttarakhand and horse gram in English. It is widely used in kidney stone, Inflammed joints, sudation therapy, fever, Musculoskeletal disorder, breast milk purifier, sinus wounds, tumours, ascites and localized abdominal tumor.[4,5,6]

Therefore, this study, is aimed to evaluate the correlation between phytochemicals and antioxidant activity of the *Macrotyloma uniflorum* extract.

2. Material and Methods

2.1 Chemicals and reagents

2,2-diphenyl-1-picryl-hydrazyl (DPPH), quercetin, sodium nitrite ($NaNO_2$), trichloroacetic acid (TCA), ascorbic acid, Ferric chloride ($FeCl_3$), gallic acid were obtained from Himedia Laboratories Pvt. Ltd, Mumbai, India. Potassium di-hydrogen phosphate (KH_2PO_4), di-potassium hydrogen phosphate (K_2HPO_4), sodium hydroxide (NaOH), potassium ferricyanide ($K_2Fe(CN)_6$), sodium carbonate (Na_2CO_3), Hydrogen peroxide (H_2O_2) and Methanol were procured from Merck, Mumbai, India. Folin-Ciocalteu reagent from Sisco research laboratory, Mumbai, India. Aluminium chloride ($AlCl_3$) was obtained from Sd fine chemicals limited, Mumbai, India. All chemicals and solvents are analytical grade.

2.2 Plant material and extraction

Macrotyloma uniflorum dal was collected from the Bhimtal market in Sep, 2011 and were authenticated by a taxonomist. A voucher specimen (KU/D001) is deposited at Botany department herbarium, Kumaun University, Nainital, Uttarakhand.

Shade dried, ground dal of *Dolichos biflorus*(10 g each), passed through a 40 mesh sieve, were extracted by Soxhlation using 70% aqueous methanol (DME) (Plant: Solvent-1:15 w/v) by continuous hot percolation method[2] for 18 hours. The extracts were stored at -20°C. Before use, the extracts were dissolved in double-distilled water (DDW) in desired concentrations. The methanolic extract of *D. biflorus* was subjected to preliminary phytochemical screening to find out the presence of active principles[7].

Corresponding author

Full Address :

Department of Biotechnology, Bhimtal Campus, Kumaun University, Nainital.

Phone no. +911594 2248042

E-mail: *veena_kumaun@yahoo.com*

2.3 Phytochemical Estimations

The yield of evaporated extract[7] based on dry weight, *Total Phenolic Content,*[8] *Total flavonoid content*[9] were as previous prescribed methods. All tests were performed in triplicates.

2.4 In vitro Antioxidant properties of the extracts

2.4.1 Free Radical Scavenging Activity (FRSA)

FRSA was assessed using ***2,2-Diphenyl-1-picrylhydrazyl (DPPH)* method** as per the modified protocol by Goyal *et al.*[7]

2.4.2 Reducing Power Assay

The reducing power of the extracts was determined according to the method of Oyaizu.[10]

2.4.3 Scavenging of Hydrogen Peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch.[11]

2.4.4 Statistical Analysis

Results were calculated as the mean ± SD (standard deviation) for each sample. Statistical analysis was done with one way analysis of variance using Graph pad Prism, Version 4.0 (Graph Pad Software, San Diego, CA, USA). The correlation coefficient (R^2) was used to show correlations. A significant difference was judged to exist at a level of $p< 0.05$ and $p<0.01$.

3. Results and Discussion

3.1 Plant Yield

The plant yield of DME was found to be 8.13% w/w.

3.2 Determination of total phenolic contents

The total phenolic content was found in DME with 92.10 ± 8.11 mg/ml GAE per 100 mg plant extract.

High phenolic contents show that 70% methanol could be a suitable solvent for the preparation of extracts since it also inhibit the degradation of polyphenols present in the plants by neutralizing the activity of polyphenol oxidase[12].

3.3 Determination of total flavonoids contents

The total flavonoid content was found to be maximum in case of DME of *Macrotyloma* with 139.5 ± 55.09 mg/ml QE equivalent per 100 mg plant extract. Higher level of flavonoids in DME can be attributed to the fact that methanol is less polar than water and thus has the potential to release the bound flavonoids and polyphenols from the cell wall of the plant[13,14].

3.4 DPPH scavenging activity

DPPH antioxidant assay is the most commonly used assay to evaluate the antioxidant activity. It is based on the ability of DPPH to decolorize from violet to yellow in presence of antioxidants thus leads to decrease in absorbance at 517nm. The screening results of the DPPH activity along with standard ascorbic acid are epitomized and scavenging effect of DME extract at 0.3 $mgml^{-1}$was similar to ascorbic acid at 1.2 $mgml^{-1}$.

3.5 Scavenging of Hydrogen Peroxide

H_2O_2 scavenging activity of *M. uniflorum* dal extract is illustrated in figure 1, which proves the various extracts as good scavenger of H_2O_2 as compared to ascorbic acid as standard. Antioxidants of *M. uniflorum* dal has been reported to be capable of blocking chain reactions of lipid auto-oxidation, chelating

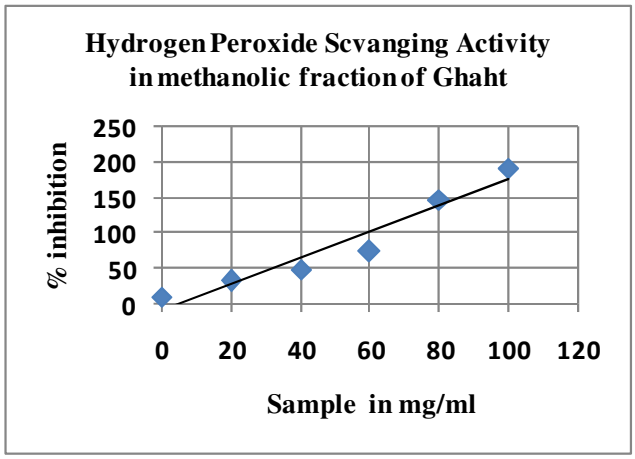

Figure 1: H_2O_2 scavenging activity of *M. uniflorum* extracts. Values are in triplicates ± standard deviation.

transient state metal ions, scavenging nitrite compounds, H_2O_2 and blocking the synthetic reaction of nitrosamine[15].

3.6 Reducing power assay

Figure 2 depicts the reductive capabilities of the various plant extracts and fractions compared with ascorbic acid used as positive control.

The reducing power was found to be directly proportional to the concentration of the extract and was found to increase steadily with increase in concentration. The reductive capability is determined by the transformation of Fe^{3+} to Fe^{2+} in presence of the extract. The absorbance of plant extract

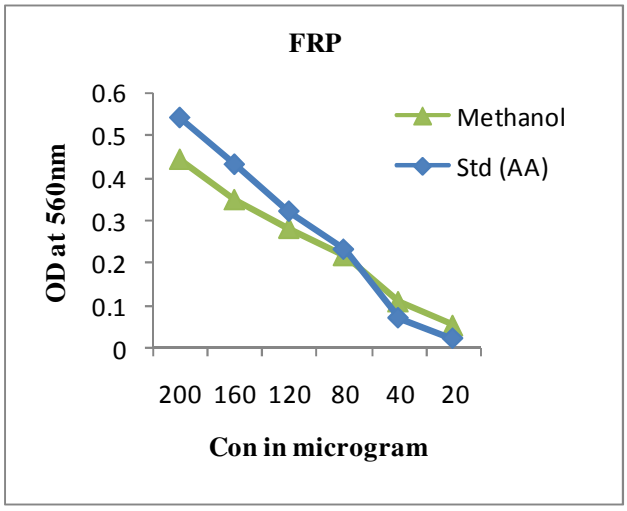

Figure 2: Reducing power activity of different extracts of *M. uniflorum* compared to ascorbic acid (AA) as standard. Each value in the graph was obtained by calculating the average of three experiments ± standard deviation.

and ascorbic acid showed parallelism at 120-200µg/ml. At 80 µg/ml concentration the absorbance of the DME and ascorbic acid was found to be almost similar i.e. 0.216 and 0.23 respectively.

3.7 Linear correlation between different parameters of *M. uniflorum*

Linear correlation between the phytochemical constituents and total antioxidant activity was established in order to determine how the antioxidant activity and total phenols or flavonoids level are related to extract of *M. uniflorum* .

A positive linear correlation was found between the phenol and DPPH scavenging activity DME (R^2= 0.796). Our

experimentation on the correlation between the total phenol and reducing power also led to similar results in case of DME (R^2= 0.754). Correlation between the total flavonoids and the DPPH was also established for DME (R^2= 0.502) as well as between flavonoids and reducing power and was found to be DME (R^2= 0.841). The positive linear correlation between total contents of phenolics and DPPH free radical scavenging activities were in accordance of previous studies[13].

CONCLUSION

To conclude, this is first report to concur the quantitative correlations between the polyphenols and the DPPH, H_2O_2 scavenging activity and reducing power of *Macrotyloma uniflorum* and a close linear correlation among each other were established. This study substantiates utilization of this plant as an antioxidant in future. On the other hand, further studies should be continued to obtain appropriate information about the role of *Macrotyloma uniflorum* in the other dose dependent processes. However, further studies are needed to isolate the active principles, elucidate their structures, and determine their pharmacological activities.

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