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PHYTOCHEMICAL AND ANTIOXIDANT POTENTIAL OF TRIBULUS TERRESTRIS LEAF EXTRACTS

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ABSTRACT

Objectives: Many diseases are associated with the accumulation of free radicals and antioxidants can scavenge free radicals and minimize their impact. Therefore, the search for naturally occurring antioxidants of plant origin is vital. This study aimed to investigate the antioxidants and free radicals scavenging properties of ethanolic extracts from Tribulus terrestris leaf (TTL).

Materials & Methods: The antioxidant and free radical scavenging activities were measured by using standard protocols against phytoextract used at different concentrations. Total phenolic and flavonoid contents were estimated using Folin- Ciocalteu and aluminum-chloride reagent assay methods, respectively.

Results: TTL extract showed the highest total antioxidant ability and with regard to various scavenging activities, and showed the highest radical scavenging activity closely resembled the standards. The quantified phenolic and flavonoid contents of TTL ethanolic extract was19.0±0.16mg of gallic acid equivalent per gram extract and 26.6±0.15mg quercetin equivalent per gram extract respectively. A positive correlation ($p< 0.001$) was observed between phenolic content and free radical scavenging efficiencies.

Discussion: The results confirm that TTL is important sources of natural antioxidants and serves as effective free radical scavengers and/or inhibitors. Hence, TTL extract is suitable plant-based pharmaceutical product can be used to handle free radical-mediated diseases.

KEYWORDS: Tribulus Terrestris, Polyphenols, Antioxidants, Diseases.

INTRODUCTION

Recently, Ethnopharmacology has gained popularity in phytomedicines and much interest has been focused on the discovery of new biologically active molecules from medicinal plants. A current research on free radicals has confirmed that foods rich in antioxidants play an essential role in the prevention of adverse effects [1]. Resultantly, natural antioxidants, which are abundant in fruits, leaves, and flowers, have received great attention and have been studied extensively [2], since they are effective free radical scavengers and are accepted to be less toxic than synthetic antioxidants. Phytomedicines are well tolerated, with fewer side effects; in contrast, synthetic drugs can be highly effective, their usage is often hampered by severe side effects [3]. Therefore, considering the importance of natural product-based antioxidants in treating several human ailments, the present study was focused towards the evaluation of antioxidant properties of Tribulus terrestris leaf extract.

Tribulus terrestris (TT) known as Gokhru, a perennial creeping herb is an annual plant of the family Zygophyllaceae and being used for generations as traditional medicine to treat several

ailments due to its unique property of healing cuts, wounds and boils. The importance of antioxidants on human health has become increasingly clear owing to spectacular advances in understanding mechanisms of their reaction with oxidants. Though leaf extract contains a series of terpenoids and saponins as bioactive components along with coumaroylquinic acid derivatives [4], there is a lack of information on the antioxidant potential of its contents

MATERIALS AND METHODS

Chemicals

Nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), gallic acid, Folin-Ciocalteu reagent, quercetin and ascorbic acid were obtained from Merck India Ltd, Mumbai.

Plant Material

Tribulus terrestris leaves (TTL) were collected, cleaned, shade dried for around 15-20 days at room temperature and then crushed to fine powder. The plant sample was subjected to Soxhlet extraction system for 24 h using 70% ethanol and filtrates were made use for phytochemical analysis.

Quantification of phytochemical constituents

Total Phenolic content

The soluble phenolic content of TTL extract was determined according to the modified method of Singleton et al. [5] by using the Folin-Ciocalteu reagent. About 1 ml of plant extract was mixed with 0.5 ml of folin-ciocalteu reagent (1:10) followed by 1.5 ml of Na_2CO_3 (0.7 M). Subsequently, the mixture was shaken for 2 h at room temperature and absorbance was measured at 760 nm. The total phenolic content was calculated from standard calibration curve of gallic acid.

Total Flavonoid Content

The total flavonoid content was determined based on the formation of flavonoid - aluminum complex according to the modified method of Zhishen et al. [6]. To 1 ml of extract, 0.1 ml $NaNO_2$ (5%) was added and incubated for 5 min at room temperature, then 0.1 ml of $AlCl_3$ (10%) was added and continued incubation for further 5 min, later the reaction mixture was treated with 0.6 ml of NaOH (1 mM). Finally, the reaction mixture was diluted to 5 ml with distilled water and the absorbance was measured at 510 nm. The total flavonoid content was calculated from standard calibration curve of quercetin using the following equation,

DPPH Free Radical Scavenging Assay

Free radical scavenging activity of TTL extract against DPPH was investigated spectrophotometrically by a modified method of Chanet al. [7]. Different aliquots of both standard and sample solutions (150-250 µg/ml) were mixed with 1 ml of DPPH (0.2 mM) solution. The mixture was incubated in dark for 30 min at room temperature and the absorbance was measured at 517 nm. The absorbance of the control sample containing the same amount of solvent and DPPH solution was measured. Ascorbic acid was used as standard and the percent inhibition of activity was calculated using formula,

$$\text{DPPH scavening activity (\%)} = \frac{[\text{Abs (control)} - \text{Abs (test)}]}{[\text{Abs (control)}]} \times 100$$

Where Abs (control): Absorbance of the control and
Abs (test): Absorbance of the extract/standard.

Superoxide Radical (O_2^-) Scavenging Assay

Superoxide radical scavenging activity of TTL extract was evaluated using nitro blue tetrazolium (NBT) reduction using the modified method of Nishikimi et al. [8]. In the assay, auto-oxidation of phenazine methosulphate (PMS, in phosphate buffer pH 7.4) generates superoxide anions which reduce the yellow dye nitro blue tetrazolium to blue colored formazan. The reaction mixture consisted of 1 ml NBT solution (156 µM) and sample solutions of different concentrations (150-250 µg/ml). The reaction was started by adding 100 µl of PMS (60 µM) to the reaction mixture and incubated for 5 min at 25 ^{o}C, absorbance was measured at 560 nm against blank. Ascorbic acid was used as the standard and percent inhibition activity was calculated using the formula,

$$\text{Superoxide scavenging activity (\%)} = \frac{[\text{Abs(control)} - \text{Abs(test)}]}{[\text{Abs(control)}]} \times 100$$

Where Abs (control): Absorbance of the control and
Abs (test): Absorbance of the extract/standard.

Statistical Analysis

Statistical analysis was performed using one-way Analysis of Variance (ANOVA) with least significant difference (LSD) post hoc (at $P<0.01$) by SPSS software package 20.0. Linear regression analysis was used to calculate IC_{50} values by using 'Graph pad prism software 6.0'. Results are shown as the mean ± SEM of six measurements.

RESULTS

Total Phenolic and Flavonoid Content

From the results, it was evident that both, TTF and MFF extracts were found to be a good source of polyphenols. In comparison, MFF extract showed more amount of phenolic and flavonoid contents compared to TTF extract. The quantified phenolic contents of TTL ethanol extract was found to be 19.0±0.16 mg gallic acid equivalent per gram extract; similarly, the quantified flavonoid contents were found to be 26.6±0.15mg quercetin equivalent per gram extract respectively.

DPPH Free Radical Scavenging Activity

The antioxidant potential of TTL extract was analyzed by DPPH radical scavenging assay and the plant extract exhibited scavenging ability in a concentration-dependent manner. TTL

extract exhibited a higher scavenging ability (50.12%) with IC_{50} value of 151.2 µg/ml at 250 µg/ml concentration and the standard (ascorbic acid) value being 123.2 µg/ml.

Superoxide Radical Scavenging Activity

The percent inhibition of superoxide radical generation by TTL ethanolic extract was found increasing in a concentration-dependent manner showing IC_{50} value of 134.2 µg/ml (39.22% inhibition) at 250µg/ml concentration when compared to the standard, ascorbic acid IC_{50} value (103.3 µg/ml).

DISCUSSION

Plant polyphenols are significant compounds that act as free radical scavengers and can exert multiple biological effects, including antioxidant and free radical scavenging abilities [4-5]. Similarly, flavonoids are of great importance and help in fighting against diseases. The ability of flavonoids to act as potent antioxidants depends on their molecular structures, the position of the hydroxyl group and other features in its chemical structure. It is evident from present study results that TTL extract is a good source of polyphenols. In brief, TTL extract showed high levels of total phenolic content of 19.0±0.16mg of equivalent gallic acid per gram followed by total flavonoids of 26.6±0.15mg of equivalent quercetin per gram extract.

The antioxidant and radical scavenging properties of plants are based on their medicinal value. In this study, the ethanolic extracts of both plants exerted a significant scavenging activity on the DPPH radical which was found to be increasing with the increasing concentration. At 250 µg/ml concentration, the exerted value of TTL extract was found to be 50.12% (with IC_{50} 151.2 µg/ml) when compared to ascorbic acid, the standard (123.2µg/ml). The preponderance of studies on ethyl acetate and methanolic extracts of TT leaf exhibited a dose-dependent increase in scavenging activity [8]. Thereby, the results of the present study confirm that the ethanolic extract of TTL is effective with respect to DPPH radical scavenging activity and has better antioxidant potential.

Superoxide anion radical is generated by four-electron reduction of molecular oxygen into water. In living organisms, O_2^- is removed by the enzyme called superoxide dismutase (SOD). In this study, assessment was made to confirm in vitro superoxide anion scavenging activity of TTL extract. At 250 µg/ml concentration, the exerted value of TTL extract was found to be 39.22% (with IC_{50} 134.2 µg/ml)when compared to ascorbic acid, the standard (76%).Similar results were observed in earlier investigations wherein TT leaf extract exhibited superoxide radical scavenging activity when tested with different solvents such as aqueous, hexane and methanol [9-10].The present study results rely only on ethanol solvent wherein a concentration-dependent increase in quenching superoxide radicals witnessed to ahigh extent and extract possess strong radical scavenging ability. The correlation coefficient (r) assessed indicates the existence of a linear relationship between the amount of total phenolic content and antioxidant properties of TTL extract.

CONCLUSION

The ethanolic extract of TTL possess high total phenolic and flavonoid contents and exhibited significant antioxidant potential in scavenging free radicals. The results confirm that TTL extract is important source of natural antioxidants and help to curb free radicals and highly recommended as substitutes to handle oxidative stress-related diseases.

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