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Research Article

Phytochemical screening and antioxidant activity of *Terminalia muelleri benth.* leaf extract

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ABSTRACT

In the present investigation, leaves of *Terminalia muelleri* were assayed for their phytochemical constituents, as well as its free radical scavenging activity. The antioxidant effect was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) radical scavenging assay, the phytochemical constituents were screened to assess their corresponding effect on antioxidant activity. The results showed that the ethanol soluble fraction of *T. muelleri* possesses a potent radical-scavenging activity using the DPPH[•] (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay (IC₅₀ value = 2.7 µg/mL, while the standard ascorbic acid has an IC₅₀ value = 10.5 µg/mL).

Keywords: *Terminalia muelleri*; DPPH; antioxidant; ascorbic acid.

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1. INTRODUCTION

Free radicals including superoxide anions, hydrogen peroxide, nitric oxide, and hydroxyl radical are chemically unstable atoms; they are generated during normal cellular function and are part of the natural physiological process of all living beings. The imbalance between the generation of reactive oxygen species (ROS) and the antioxidant enzymes, results in damage to lipid cells, proteins, and DNA [1]. ROS have a crucial role in the development of oxidative stress, which is considered as the major cause of numerous diseases, such as cancer, diabetes, liver and kidney impairment, cardiovascular diseases and aging.

Natural and synthetic antioxidants can scavenge these free radicals. Synthetic

antioxidants have been reported to be toxic. Therefore, natural antioxidants have gained much attention nowadays because they are considered safer. The high antioxidant capacity of plants may be due to certain phytochemical constituents, including flavonoids, tannins, and other phenolic compounds. These natural antioxidants act as reducing agents, hydrogen-donating antioxidants, and singlet oxygen quenchers owing to the presence of conjugated aromatic rings [2, 3].

Members of the Combretaceae family to which genus *Terminalia* belongs, are widely used in traditional medicine in southern Africa [4, 5]. Different *Terminalia* species have been used in traditional folk medicine in Egypt, India, and Pakistan for the treatment of various ailments, including abdominal disorders, bacterial

infections, cough, gastric ulcers, skin diseases and sore throats. Many pharmacological activities were reported for different *Terminalia* species including antioxidant [6], hepatoprotective [7], antihyperlipidemic, antidiabetic [8], anti-inflammatory [9] and cytotoxic activities [10]. The plants of the genus *Terminalia* are sources of diverse secondary metabolites, including triterpenes, flavonoids, tannins, and other phenolic compounds [4, 6].

The main objective of this study was to determine the phytochemical constituents of powdered leaves of *T. muelleri*, as well as investigate the free radical scavenging activity of *T. muelleri* leaf extract.

2. MATERIALS AND METHODS

2.1 Chemicals

2, 2-diphenyl-1-picrylhydrazyl (DPPH[•]) and L-ascorbic acid (Vitamin C) obtained from Sigma-Aldrich GmbH, Darmstadt, Germany.

2.2. Plant material

Fresh leaves of *Terminalia muelleri Benth.* (Combretaceae) were collected in November 2012 from trees grown in the Zoo Garden, Giza, Egypt. The plant was kindly authenticated by Eng. Therese Labib, the consultant at Orman Botanical Garden, Giza, and National Gene Bank at the Ministry of Agriculture, Egypt. A voucher specimen of *T. muelleri Benth* was deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt (ASU TMC2012).

2.3. Extract preparation

The air-dried powdered leaves of *T. muelleri* were extracted with 80% aqueous MeOH. The total extract was concentrated and freeze-dried to obtain a dry powder, then defatted with petroleum ether. The remaining part was concentrated and freeze-dried to obtain a dry powder, which was dissolved in absolute EtOH.

The EtOH-soluble portion was then concentrated and freeze-dried to obtain the total extract dry powder abbreviated as (TMEF).

2.4. Methods for phytochemical screening

Powdered leaves of *T. muelleri* were screened for the following phytoconstituents: carbohydrates and/ or glycosides, flavonoids, sterols and/ or triterpenes, saponins, tannins, alkaloids, and anthraquinones according to the standard procedures [11, 12].

2.5. Methods for DPPH[•] radical scavenging assay

The antioxidant effect of the TMEF and pet. ether-fraction was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) radical scavenging assay, according to the method described by Bourgou, Ksouri [13] with slight modification. Tested samples and L-ascorbic acid (standard), both prepared at different concentrations or ethanol in case of control, were added to 0.25 mM freshly prepared DPPH[•] ethanolic solution. The mixture was slightly shaken and kept in dark for 30 min at room temperature, the absorbance was determined against a blank at 517 nm using a UV Spectrophotometer. All assays were conducted in triplicates.

Percentage inhibition of free radical DPPH[•] was calculated as follow:

$$\text{Inhibition \%} = [(A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}] \times 100.$$

Where: A_{Control} is the absorbance of the control reaction.

A_{Sample} is the absorbance in the presence of the tested samples.

To calculate the IC₅₀ [14] the concentration of the substrate that causes 50% loss of the DPPH[•] activity (color), different concentrations of the tested samples were used and the percentage inhibition was calculated. The IC₅₀ values were calculated according to the equation for Boltzmann sigmoidal concentration-response

curve using the nonlinear regression fitting models (Graph Pad, Prism Version 6, La Jolla, CA, USA).

3. RESULTS AND DISCUSSION

3.1. Results of phytochemical screening

Table 1 Results of phytochemical screening

Constituents	Results
Carbohydrates and/or glycosides	+
Flavonoids	+
Sterols and/or triterpenes	+
Saponins	-
Tannins	+
Alkaloids	-
Anthraquinones	-

(+) present, (-) absent

From the table, it can be concluded that the phytochemical constituents of *T. muelleri* include tannins, flavonoids, sterols and /or triterpenes and carbohydrates and/or glycosides. Also, results revealed that this species most probably doesn't contain alkaloids, anthraquinones, and saponins.

3.2. Results of the antioxidant activity of ethanol-soluble fraction (TMEF)

The IC₅₀ (the concentration that inhibits 50% of the absorbance of DPPH[•]), was determined from the graph plotted for the % inhibition against the concentration (**Fig. 1**). TMEF IC₅₀ was 2.7 µg/mL compared to 60 µM ≈ 10.5 µg/mL for ascorbic acid.

It could be concluded from the obtained values that the ethanol-soluble fraction (TMEF) of *T. muelleri* leaf extract showed a more potent antioxidant activity when compared to the standard ascorbic acid.

4. CONCLUSION

This study demonstrated the potent antioxidant activity of *T. muelleri* leaf extract which might be attributed to its high tannins and

flavonoids content. The wide use of genus *terminalia* in traditional medicine for the treatment of various diseases may be in part due to their antioxidant potency.

Conflict of Interest

We declare that we have no conflict of interest.

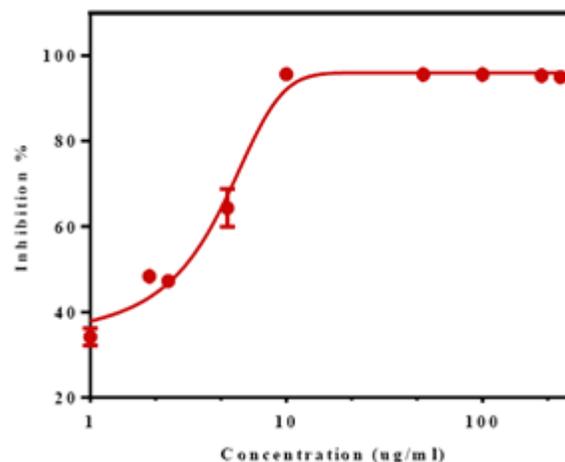


Fig. 1 Antioxidant activity of the TMEF (% inhibition against concentration in µg/ml).

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