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Research Article

EVALUATION OF ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITIES OF DIFFERENT SOLVENT EXTRACTS OF LEAVES OF *PIPER UMBELLATUM*

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ABSTRACT

Objective: This study was designed to determine the total phenol, flavonoid content, antioxidant, and free radical scavenging properties of different solvent extracts of *Piper umbellatum*.

Methods: Different solvent extracts evaluated with 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity and reducing power activity.

Results: The presence of phenol and flavonoid showed highly in the methanol extracts than ethyl acetate and acetone extracts. All the extracts have various level of antioxidant activity. Methanol solvent extract has good extraction and shows significant antioxidant activity. The effect of reducing the power of methanol extract found good antioxidant activity compared with other tested solvent extracts.

Conclusion: On the basis of the above results, we concluded that methanol extract of *P. umbellatum* whole plant extracts shows a significant antioxidant activity than ethyl acetate and acetone extracts.

Keywords: *Piper umbellatum,* Antioxidant, 1, 1-diphenyl-2-picrylhydrazyl, Ferric reducing antioxidant power assay, Polar, Nonpolar solvents.

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INTRODUCTION

Free radical is the primary harmful health factor in the human systems, when the oxidative molecule formation the loss of electron from one atom due to the oxidative process. Free radical formation is the long chain reaction. Free radical formation due to environmental pollutants, radiation, chemicals and toxins, deep fried, and spicy foods as well as physical stress and depletion of immune system of the human body [1-3]. The oxidative reaction is the crucial role to formation human disorders. They are cancer, emphysema, cirrhosis, atherosclerosis, and arthritis all are correlated with oxidative damage [4]. Free radical formation controlled by such artificial and plant derivate compounds such as vitamin A, C, beta-carotene, and polyphenolic compounds. Phenolic group of compounds possesses the good and properties and used to various diseases.

Piper umbellatum belongs to the family Piperaceae. *P. umbellatum* originated from tropical America but is now found in tropical rain forest in Africa, Japan, and the Indian Ocean Islands [5]. The genus *Piper* comprises about 1000-2000 species of shrubs, herbs, and lianas that have economic and ecological values of which *P. Umbellatum* and Piper guineense [6]. The important phytoconstituents isolated from leaves of *P. umbellatum* L. revealed three pure compounds were isoasarone, 2-(4'-methoxyphenyl)-3-methyl-5-propenylbenzofuran, and 2,3-dihydro-2-(4-hydrophenyl)-3- methyl-5-propenylbenzofuran [7]. The aim of this study was to determine for the first time, comparison of phytochemical analysis and antioxidant activity of three different solvent extracts of *P. umbellatum* leaves. Spectroscopic analysis was determined of the total phenol and total flavonoid content from the leaves extract. Furthermore, total antioxidant activity value was quantified by the ascorbic acid equivalents. Free radical scavenging activity was also determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay compared to ascorbic acid. Since this plant is used to cure various therapeutic purposes by indigenous people, we have chosen this plant for our investigation.

METHODS

Plant material

Leaves samples of *P. umbellatum* plants were collected from Courtallam hills, Tirunelveli District, Tamil Nadu, India, during the month of October 2015. The plant was identified, and the herbarium specimen was prepared and deposited at Botany Research Laboratory, V.H.N.S.N. College (Autonomous) for future references.

Preparation of leaves extracts

The fresh leaves were air dried under the fan, and the leaves extracts were prepared by sequential extraction method using three organic solvents on the basis of the polarity of solvents (acetone, ethyl acetate, and methanol). 30 g of the dry leaves sample was taken in a conical flask and 200 ml of acetone was added. The conical flask was kept on mechanical shaker for 24 hrs, after that the extract was filtered through Whatman filter paper 1 and the pellet was allowed to drying, and this pellet was used for the next solvent extraction (ethyl acetate and methanol). The dried extracts were recovered and stored in the refrigerator for further analysis.

Quantitative phytochemical analysis

Estimation of total phenol content

The amount of total phenol was determined using the Folin–Ciocalteu reagent method of Lister and Wilson, 2001 [8]. A standard curve was prepared using gallic acid. Different concentrations of gallic acid were prepared in 80% methanol, and their absorbance was recorded at 760 nm. 100 µl of sample was dissolved in 500 µl of Folin–Ciocalteu reagent (1/10 dilution) and 1 ml of distilled water. The contents were mixed and incubated at room temperature for 1 minute. After 1 minute, 1.5 ml of 20% sodium carbonate solution was added. The final mixture was shaken well and incubated for 2 hrs in the dark at room temperature. The absorbance of all samples was measured at 760 nm using an ultraviolet and visible (UV-Vis) spectrophotometer. The results

were expressed in mg gallic acid equivalents (GAE) per milligram of dry weight of the plant.

Estimation of total flavonoid content

The flavonoid content in the extract was determined spectrophotometrically by the method of Quettier-Deleu *et al*., 2000 [9]. This method was based on the formation of a complex, flavonoid-aluminum, with the absorbance maximum at 430 nm. Rutin was used as standard to make the calibration curve. 1 ml of diluted sample was separately mixed with 1 ml of 2% aluminum chloride methanolic solution. After incubation at room temperature for 15 minutes, the absorbance of the reaction mixture was measured at 430 nm in a UV-vis spectrophotometer. The flavonoid content was expressed in mg per mg of rutin equivalent.

Free radical scavenging ability (DPPH)

The scavenging ability of methanol extract on DPPH free radicals was estimated according to the method of Shimada *et al*. (1992) [10]. This method depends on the reduction of purple DPPH to yellow-colored diphenyl picryl hydrazine. 2 ml of various concentrations (10-100 µg/ml) of the test sample was mixed with 0.5 ml of 0.01 M DPPH in methanol. An equal amount of methanol and DPPH served as a control. The mixture was shaken vigorously and then steadily kept for 30 minutes at room temperature in the dark. The absorbance of the resulting solution was measured at 517 nm against a blank using UV-vis spectrophotometer. The experiment was performed in triplicates. The DPPH radical scavenging activity was calculated by the following equation:

% DPPH radical scavenging activity=$(A_o-A_1)/A_o\times100\%$.

Where, A_o is the absorbance of the control reaction and A_1 is the absorbance of the sample of the tested extracts. The percentage of free radical activity was plotted against the corresponding antioxidant substance concentration to obtain the half maximal inhibitory concentration (IC_{50}) value, which is defined as the amount of antioxidant substance required to scavenge the 50% of free radicals present in the assay solution. IC_{50} values are inversely proportional to the antioxidant potential.

Reducing power ability

The reducing power ability of methanol extract was determined by the method given by Oyaizu (1986) [11]. Reaction mixtures were prepared by adding 2.5 ml of phosphate buffer (0.2 M, pH 6.6), 2.5 ml potassium ferricyanide (0.1%), and varying concentrations of extracts (10-250 µg/ml). Then, the reaction mixtures were incubated at 50°C in water bath for 30 minutes and allowed to cool at room temperature. Then, 2.5 ml of 10% TCA (trichloroacetic acid) were added to each reaction mixture and centrifuged at 2000 rpm for 10 minutes. The supernatant (2.5 ml) was separated in the test tube and added with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1.0%). After 10 minutes incubation at room temperature, the absorbance was measured at 700 nm. The ascorbic acid solution was used as standard.

Statistical analysis

All the data were reported as mean±standard deviation of three replicates. The IC_{50} values were calculated using the ED_{50} plus v 1.0 programme. Statistical analysis was performed using Microsoft Excel.

RESULTS

The phenolic compounds may contribute directly to antioxidants activities. The total phenolic content of the extracts was determined as GAE using Folin–Ciocalteu reagent. A calibration curve with different concentration of gallic acid was formed as y = 0.2976x+0.0567, R^2=0.9704 (Fig. 1). According phenol content in methanol extract 0.178 mg/GAE mg followed by ethyl acetate extract showed 0.120 mg/GAE mg and acetone extract revealed 0.094 mg/GAE mg.

The total flavonoid content of the extracts was determined as rutin acid equivalent. The calibration curve with different concentration of rutin acid was created as y=0.3351x-0.1085; R^2=0.9905 (Fig. 2). Methanol extract showed a significant amount of flavonoid content 0.225 mg/mg RAE followed by ethyl acetate extract revealed 0.155 mg/RAE mg and acetone extract possess 0.136 mg/RAE mg.

In this study, percentage of inhibition of different extracts were calculated and compared with ascorbic acid as a standard. Methanol extract shows a higher percentage of inhibition (64.39%) than other solvent extract ethyl acetate revealed (58.21%) and acetone extract possesses (49.40%). All the extracts have lower % of inhibition compare with ascorbic acid (91.94%) (Fig. 3).

Fig. 4 showed the reducing ability of ascorbic acid as a standard. Different solvent extracts of *P. umbellatum* leaves of reducing power compared to ascorbic acid. Among the tested solvent, methanol extract exhibited higher reducing activity (0.435 mg/AA mg) followed by ethyl acetate extract shows 0.341 mg/AA mg and acetone extract revealed (0.308 mg/AA mg).

DISCUSSION

Flavonoids are a group of polyphenolic compounds known properties which include free radical scavenging and inhibition of hydrolytic and oxidative enzymes and inflammatory activity [12]. In this study, the quantitative analysis of phenol and flavonoid content of the extracts highly correlated to the previous results [13]. The previous reports of *P. umbellatum* revealed steroid, flavonoid, tannin, alkaloid, saponin, and phenol in the leaves extract [14]. The activity of free radical scavenging activity of the plant extracts at different concentrations increases with increasing concentrations. Similar observation was made in the methanolic extract of *Piper longum* [15]. The results showed that the antioxidant activity was higher for methanol extract of *P. umbellatum* as followed by ethyl acetate and acetone extracts. DPPH is a nitrogen-

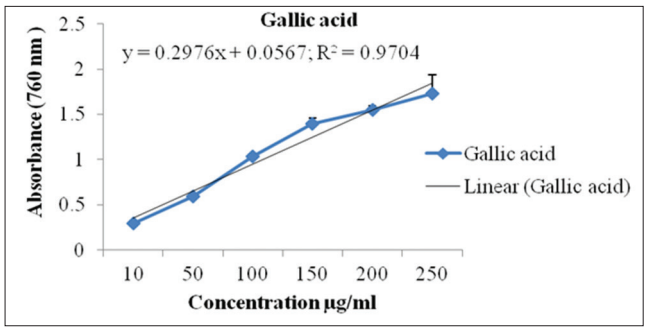

Fig. 1: Standard curve of gallic acid for total phenol estimation. All values are reported as mean±SD (n=3). SD: Standard deviation

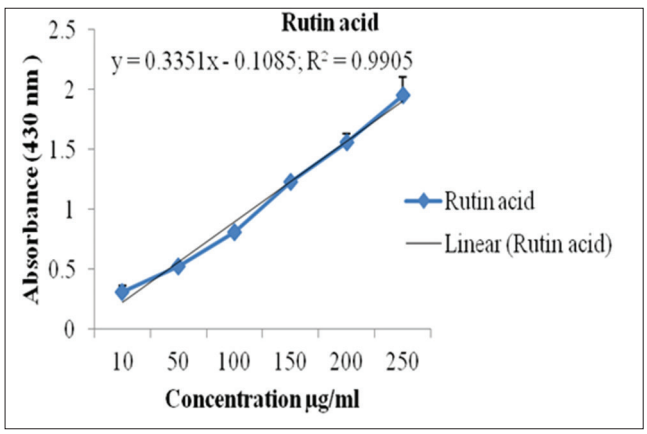

Fig. 2: Standard curve of rutin for total flavonoid estimation. All values are reported as mean±SD (n=3). SD: Standard deviation

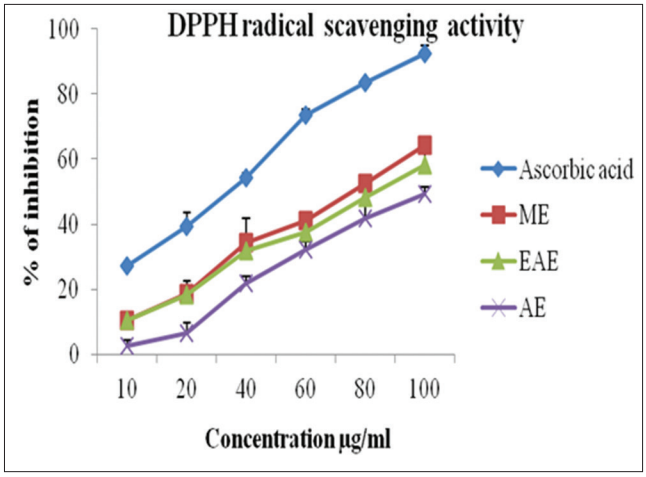

Fig. 3: Percentage inhibition of 1, 1-diphenyl-2-picrylhydrazyl by ascorbic acid and different solvent extracts of *Piper umbellatum*. ME: Methanol Extracts, EAE: Ethyl Acetate Extract, AE: Acetone Extract. All values are reported as mean±Standard deviation (n=3)

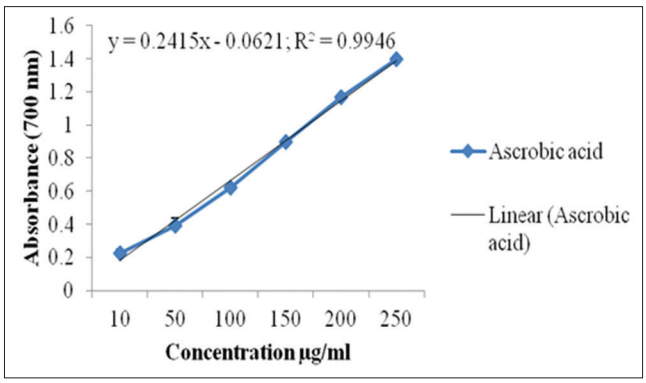

Fig. 4: Reducing power capacity of ascorbic acid. All values are reported as mean±SD (n=3). SD: Standard deviation

centered free radical molecules [16]. The previous researcher reported on extract react with a free radical molecule (single electron - DPPH) after reaction free radical convert to nonfree radical when tested extract give one electron changes to pair electron (DPPH+H) and the presence of phenol and flavonoid content in tested extracts. Before testing, the DPPH dark brown color after dilution with ethanol changes to deep violet color. Deep violet color DPPH solution tested the different concentration of extract and the color changes to yellow. During the reaction, violet color solution suddenly changes to yellow color because the tested extract found a high amount of antioxidant properties [17,18]. Reducing power was measured the reductive ability of antioxidant, and transformation of Fe^{+3} to Fe^{+2} in the presence of the extract [19]. Increasing reducing power was increased when the concentration increased. A higher absorbance indicates a higher reducing power. In earlier researcher reported, the similar antioxidant activity possesses in the methanolic extract *Baccaurea courtallensis* [20]. Several reports indicated the phenolic content of the plant extracts had the reducing power of bioactive compounds and associated with antioxidant activity [21]. Our results revealed that the leaves of different solvent extracts of *P. umbellatum* showed same scavenging activity compared with in earlier report [22].

CONCLUSION

In this study, indicated the three extracts of *P. umbellatum* have potent antioxidant capacity in DPPH and ferric reducing antioxidant power assay methods. Thus, it can be concluded that considered good sources of natural antioxidants substances to health systems.

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