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RESEARCH ARTICLE
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ASSESSMENT OF PHENOLIC, FLAVONOID CONTENTS AND FREE RADICAL SCAVENGING ACTIVITY OF *RUELLIA TUBEROSA* L. STEM EXTRACTS

Daya L. Chothani
B K Mody government pharmacy college Rajkot Gujarat, India, Email: daya.herb@gmail.com

Abstract
The present study was aimed to evaluate antioxidant activity of the *Ruellia tuberosa* (Acanthaceae) stem by utilizing in vitro models, specifically 2, 2-diphenyl-1-picrilhydrazyl (DPPH) radical scavenging activity and reducing power. Moreover, the total phenol and total flavonoid content were assessed using Folin-Ciocalteau reagent and colorimetric techniques with aluminum chloride respectively. Protein, carbohydrates, alkaloids, phenolic and flavonoid compounds were found in the preliminary phytochemical screening. The results demonstrated that the extract from *R. tuberosa* stem demonstrated DPPH radical scavenging action, when compared with standard ascorbic acid. The total phenolic and total flavonoid contents were found higher concentration in methanol extract than ethyl acetate extracts. It has been revealed that the *Ruellia tuberosa* stem displays antioxidant activity. The additional research could be done to figure out the active principle producing this effect.

Keywords: Anti-Oxidant Activity, DPPH free radical scavenging activity, *Ruellia tuberosa*, Total phenolic

Introduction

Antioxidants are substances that protect biological systems against oxidative damage by neutralizing reactive oxygen species (ROS), reactive nitrogen species (RNS), and other reactive molecules. Reactive species are continuously generated during normal cellular metabolism and play important physiological roles in cell signaling, immune responses, and redox regulation. However, excessive production of reactive species or inadequate antioxidant defense can disturb cellular redox homeostasis, resulting in oxidative stress and damage to essential biomolecules such as lipids, proteins, and DNA. [1] The human body possesses an endogenous antioxidant defense system consisting of enzymatic and non-enzymatic components. Important antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), while non-enzymatic antioxidants include glutathione, vitamin C, vitamin E, and various polyphenolic compounds. These systems work together to maintain redox balance and limit oxidative damage. [2,3] Among the various natural antioxidants, phenolic compounds in herbs act as antioxidants due to their redox properties, allowing them to act as reducing agents,

hydrogen donors, free radical quenchers and metal chelators. [4-6] *Ruellia tuberosa* Linn. belongs to family Acanthaceae, a native of Central America, introduced into Indian garden as ornament. It is used medicinally in West Indies, Central America, Guiana and Peru. *R. tuberosa* is commonly known as 'Cracker plant' 30-45 cm height, erect, sub-erect or diffuse perennial herbs with tuberous roots. Leaves are opposite, inflorescence in axillary 1-3 flowered cymes, flower bluish, purple or deep blue; Fruit bluish, purple or deep blue seeds. [7-10] . In Siddha system of medicine, leaves are given with liquid copal as remedy for gonorrhea and ear diseases.[11.] *Ruellia tuberosa* used in stomach cancer. [12.] In Suriname's traditional medicine system, it is used as anthelmintic and also in management of joint pain and strained muscles. In folk medicine, it has been used as diuretic, anti-pyretic, anti-diabetic, antidotal, thirst-quenching agent, analgesic and anti-hypertensive activity. [13-14.] The present study was aimed on evaluating their phenolic constituents, flavanoid content, antioxidant potential and free radical scavenging capacity of stem of *R. tuberosa.*

Materials And Methods

Plant material:

A fresh specimen of *Ruellia tuberosa* was gathered from the campus of The M. S. University of Baroda in August 2008. The plant was verified at the Botany department of The M. S. University. A voucher specimen (PHR/HDT/DC-RT-08) was preserved in the herbarium of our laboratory. The stems were detached and dried in the sun individually. The dried plant material was then ground into a powder.

Chemicals:

All UV–Visible measurements were recorded on a Shimadzu UV–1800. Gallic acid, Folin Ciocalteu reagent, Sodium carbonate, Quercetin, aluminum chloride, Potassium acetate, Naringin, 2, 4-dinitrophenylhydrazine reagent, potassium hydroxide, α, α Diphenyl – β Picryl Hydrazyl (DPPH), Ascorbic acid was obtained from E. Merck (Darmstadt, Germany), Hi-Media lab. Ltd (Mumbai) and Sigma (Chemical Co, St. Louis, MO, USA). All other reagents were analytical grade.

Preparation of extracts:

Approximately 50 gm of powdered drug stems were extracted using a Soxhlet apparatus for 2 days with methanol and ethyl acetate separately. The extracts were filtered, concentrated through vacuum evaporation, and thoroughly dried in a vacuum.

Phytochemical screening:[15]

Chemical tests were used to screen the prepared extracts for the presence of several classes of phytoconstituents. It was further confirmed by thin later chromatographic studies.

Determination of total phenolics content: [16,17]

The Folin-Ciocalteau reagent was used to quantify the total phenolic content using gallic acid as a reference phenolic compound, in accordance with the method provided by Singleton and Rossi (1965). The calibration curve of Gallic acid was taken using methanol. About 1.0 ml of extract solution containing 1mg extract in a volumetric flask was combined with 10ml of distilled water. To this, 1.5 ml

of folin ciocalteu reagent was added. The above mixture was kept for 5 min. and then 4 ml of 20 % sodium carbonate solution was added and the total volume was adjusted to 25 ml using distilled water. This mixture was kept for 30 minutes the absorbance of the resulting blue hue was recorded at 765 nm with a Shimadzu 1800 spectrophotometer. The total phenolics percentage was determined using the calibration curve for Gallic acid, and total phenolics were reported as % Gallic acid.

Determination of total Flavonoid content: [18]

The total flavonoids content was determined using aluminum chloride method in Methanol extract and ethyl acetate extracts of stem of *Ruellia tuberosa*.

Aluminum chloride colorimetric method:

The calibration curve was made using quercetin. 1 mg of Quercetin was dissolved in 100 ml methanol to produce (10µg/ml). From this solution different concentrations were prepared. The standard solution was combined with 2.8 ml of distilled water, 1.5 m of 95% ethanol (methanol), 0.1 ml of 10% aluminum chloride, and 0.1 ml of 1 M potassium acetate. After 30 minutes of incubation at room temperature. Using a Shimadzu 1800 spectrophotometer, the absorbance of the reaction mixture was determined at 415 nm. In the blank, the same volume of distilled water was used in place of 10% aluminium chloride. As mentioned in the preceding technique, 1.0 ml of extract solution containing 4 mg of extracts was reacted with aluminium chloride to determine the flavonoid content; the calibration curve was used to compute the percentage of total flavonoids.

Antioxidant activity in vitro:

DPPH free radical scavenging activity: [19,20]

Required quantity of Ascorbic acid (standard) was dissolved in methanol and different concentrations were prepared. 1.3 mg of precisely weighed DPPH was dissolved in 1 ml of methanol, protected from the light by covering the test tube with aluminum foil.

Protocol for estimation of DPPH free radical scavenging activity

After adding 75µl of DPPH solution to 3ml of methanol, the absorbance at 516 nm was measured right away for the control reading. 75µl of DPPH was added after varying quantities of standard and test samples were diluted with methanol up to 3 ml. Using a Shimadzu 1800 spectrophotometer, the absorbance was measured at 516 nm as soon as the DPPH solution was added. Methanol was used as a blank. Decrease in absorbance in presence of test samples at different concentration was noted after 10, 20 and 30 minutes. The % reduction and IC50 were calculated as follows

% Antioxidant activity = Control absorbance – Sample absorbance X 100Control absorbance

Reducing power by $FeCl_3$ [21,22]

Required quantity of Ascorbic acid (standard) and test samples were dissolved in methanol. The reducing power of methanolic, and ethyl acetate extracts of the stem of R. tuberosa was determined according to the method of Oyaizu (1986). Sample solutions at different amounts were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1%). After the mixture was incubated at 50 °C for 20 min, 2.5 mL of TCA (10%) were added and the mixture was centrifuged at 3000 rpm for 10 min. Supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL of

ferric chloride (0.1%), kept for 10 min. Control was prepared in similar manner excluding samples and the absorbance was measured at 700 nm and compared with standard. Higher absorbance of the reaction mixture indicates greater reducing power.

Results:

Phytochemical Screening

It was found that stem contain carbohydrates, proteins, alkaloid, flavonoid, phenolic compound and sterols.

Total phenolics content:

The percentage of total phenolics was calculated from calibration curve (figure 1) of Gallic acid and total phenolics were expressed as % Gallic acid. The components of the Folin-Ciocalteu reagent are phosphomolybdate and phosphotungstate. Under alkaline circumstances, phenolic compounds reduce the phosphotungstate-phosphomolybdate complex to blue reaction products. It calculates the quantity of the material required to prevent the reagent from oxidizing. The total phenol concentration in the *R. tuberosa* stem methanol extract and ethyl acetate extract were found to 0.82% and 0.89% respectively.

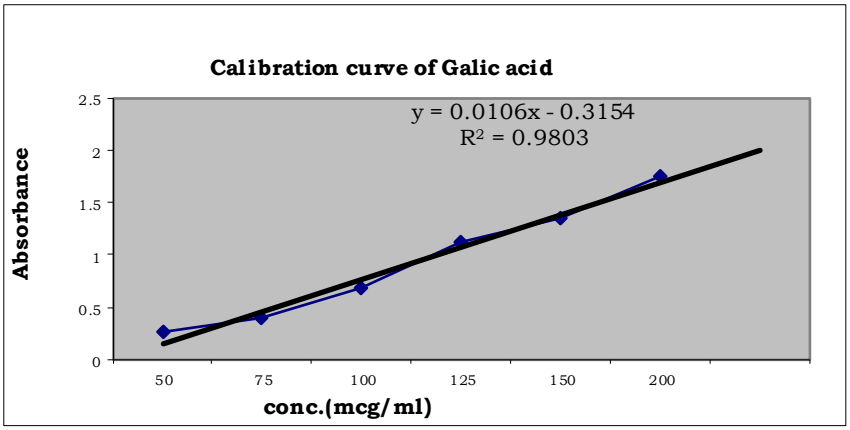

Figure 1. Calibration curve of Gallic acid

Flavonoid Content

The percentage of Flavonoid was calculated from calibration curve (figure 2) of quercetin and Flavonoids were expressed as % quercetin. Aluminum chloride (AlCl3) with the C-4 keto molecule and the C-3 or C-5 hydroxyl group interact to form acid stable complexes for flavones, isoflavons, and flavonols. Moreover, AlCl3 generates acid-labile substances with the ortho-dihydroxyl groups present in the A or B rings of flavonoids. Using standard quercetin as a reference, the flavonoid concentration stem of *R. tuberosa* methanol extract and ethyl acetate extract was found to be 0.43% and 0.58%.

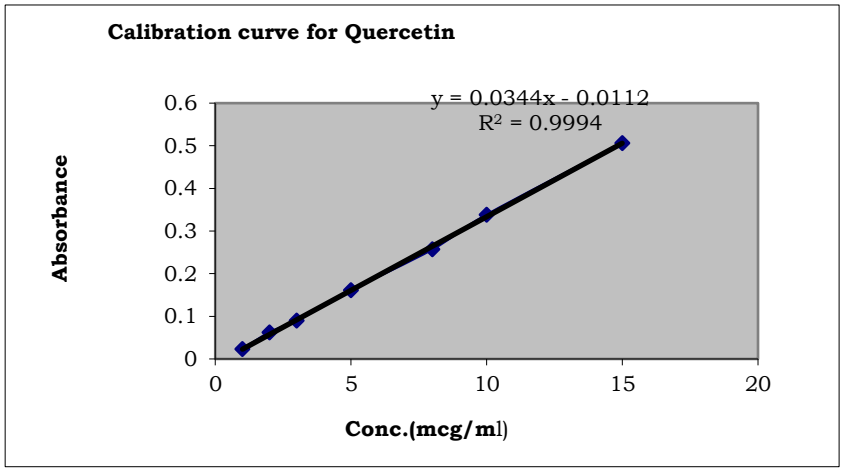

Figure 2. Calibration curve of quercetin

Radical scavenging activity in vitro:

In vitro antioxidant activity for methanol extract and ethyl acetate extract of stem of *Ruellia tuberosa* were carried out as per reported method.

DPPH free radical scavenging activity

This activity was expressed as decrease in absorbance of the samples at a different concentration level. Comparison data of various extract of *R. tuberosa* stem of DPPH free radical scavenging activity was shown in figure 3. The IC_{50}: radical-scavenging activity (concentration in µg required for 50% inhibition of DPPH radical) was calculated from the graph. The IC_{50} value found for ascorbic acid 33.33± 0.21 µg/ml, ethyl acetate extracts 75.5± 0.521 µg/ml and methanol extract 95± 0.26 µg/ml.

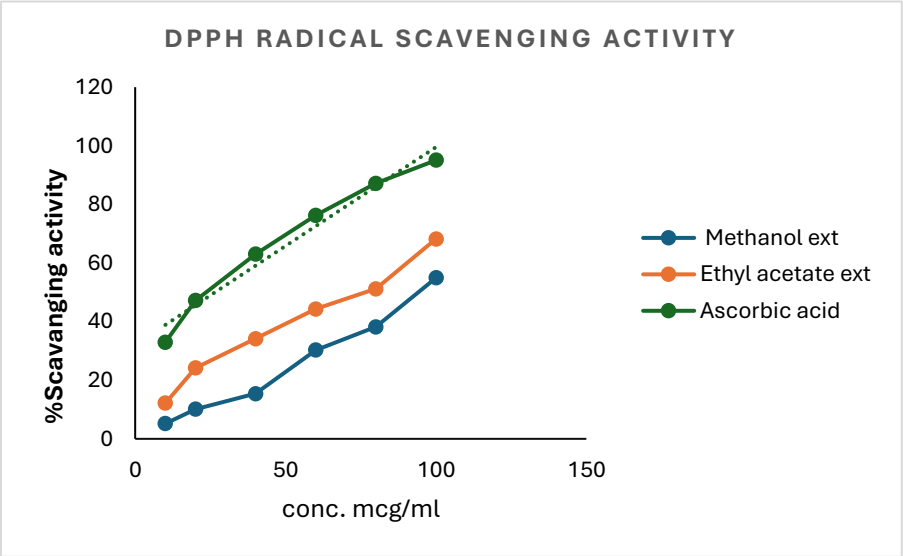

Figure 3. DPPH free radical scavenging activity of stem *R. tuberosa*

Reducing power by FeCl3:

Figure 4 shows the reducing power of the extracts of *R. tubeosa* and standards, using the potassium ferricyanide reduction method. The reducing power of the extracts increased with increasing concentration.

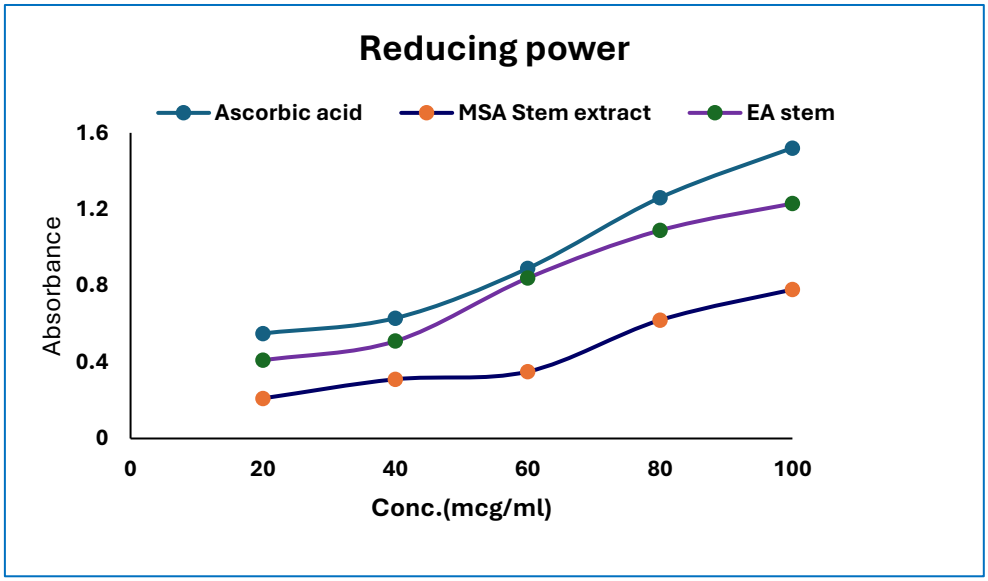

Figure 4. Reductive potential of *R. tuberosa* stem extracts and standard

Discussion:

Phenolic compounds are known as powerful chain breaking antioxidants. Phenolic compounds are very important constituents of plants and their radical scavenging ability is due to their hydroxyl groups.[23] Total phenolic compounds were determined by Folin-Ciocalteu reagent; it is mixture of phosphomolybdate and phosphotungstate. Phenolic compound causes the reduction of phosphotungstate- phosphomolybdate complex to blue reaction products in alkaline conditions and the estimation of phenolics involves the measurement of the absorbance of this colored complex at 765nm. It measures the amount of the substance need to inhibit the oxidation of the reagent.[24] The reduction capability of DPPH radical is determined by the decrease in absorbance at 516 nm induced by antioxidants. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, flavonoids, tannins, and aromatic amines (p-phenylene diamine, p-aminophenol, etc.), reduce and decolourise DPPH by their hydrogen donating ability. A higher DPPH radical scavenging activity is associated with a lower IC_{50} value.[25] DPPH (1, 1-diphenyl-2-picrylhydrazyl) is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule yellow-colored diphenyl picrylhydrazine.[26] The scavenging effect of extracts and standards with the DPPH radical is in the following order: Ascorbic acid > Ethyl acetate extract >Methanol extract. Ethyl acetate showed higher radical-scavenging activity as compared to methanol as compared with Ascorbic acid.

Conclusion:

The present study demonstrated that the *Ruellia tuberosa* stem has showed in vitro antioxidant activity when performed using DPPH free radical scavenging assay and reducing power by $FeCl_3$. The antioxidant activity of methanol extract and ethyl acetate was comparable with standard. The present study demonstrated that the stem extracts of *Ruellia tuberosa* L. contain appreciable amounts of phenolic and flavonoid compounds which might responsible for free radical scavenging activity.

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