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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF LEAF EXTRACTS OF *RIVINA HUMILIS* L.

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ABSTRACT

Rivina humilis L. is a herbaceous plants commonly found in wastelands of gardens and plains. In the present study, preliminary phytochemical screening and *in vitro* antioxidant activity of ethyl acetate, chloroform and methanolic leaf extracts of *Rivina humilis* was investigated. Phytochemical analysis of the leaf extracts of *R. humilis* revealed the presence of carbohydrates, flavonoids, alkaloids, quinones, terpenoids, coumarins, steroids, tannins, saponins and cardiac glycosides. The results showed that the phytochemical properties of the leaves can be used for curing various diseases. The strongest radical scavenging activity (60.13%) was exhibited by the methanolic extract, moderate activity (44-45%) was recorded in chloroform extract and weakest activity (20.06%) was exhibited by the ethyl acetate extract. The IC_{50} value of ethyl acetate, chloroform and methanolic extracts were found to be 432.78, 173.93 and 61.74 µl/L respectively. The results showed that the methanolic extract at 200 µg has higher total flavonoid content and 200 µg of methanolic extracts showed higher of total tannin content. The data obtained in the present study suggests that the extracts of *R. humilis* leaves have potent antioxidant activity against free radicals with the presence of active phytochemicals that can be used in disease prevention.

INTRODUCTION

Rivina L. is monotypic genus, native to Mexico, West Indies, Central and South America and now it is wildly spread in China, Pacific Islands and Africa (Tseng *et al*., 2008). In early times *Rivina humilis* L. (Phytolaccaceae) was introduced to Taiwan for cultivated as a greenhouse plant or as a garden ornamental and introduced in Taiwan very recently. The plant is commonly known as pigeon-berry or rouge plant. With its colorful, bright red and shiny berries, it is more attractive in fruit than in flower. In India the plant is commonly available in wastelands of gardens and plains. According to Nellis (1997), consumption of *Rivina* fruits causes numbness of the mouth within 2 hours, with a feeling of warmth in the throat and stomach and those symptoms are followed by coughing, thirst, tiredness with yawning, vomiting, and diarrhea, sometimes bloody. Leaves and roots contain more toxins, and cattle eating them yield tainted milk, and stools that smell of *Rivina* (Burkill, 1966). The responsible toxins in *Rivina* have not been identified, but they may be similar to those in *Phytolacca* sps. (Nellis, 1997). It should be noted that *Rivina* possibly contains mitogens, but there is evidence that they are useful against microbial pathogens (Salvat *et al*., 2001). However, their effectiveness against bacterial infections is slight and use should be mediated by the awareness of their potential dangers. The majority of the antioxidant properties of plants may be from compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechins and isocatechins rather than from vitamins C, E or ß carotene (Kahkonen *et al*., 1999) and most of these phytochemicals may help to protect cells against the oxidative damage caused by the free radicals. Plants are the potential sources of natural antioxidants and much concern has been specified to natural antioxidant and their association with health benefits in recent years and these natural antioxidants has been devoted for use in nutraceutical and therapeutic materials to replace synthetic antioxidants which are being restricted due to their carcinogenicity (Arnous *et al*., 2001; Zheng and Wang, 2001). It has been noticed that damages caused by free radical-induced oxidative stress is the major causative agent of many disorders including various cancers, tissue injuries, neurological diseases, cardiovascular diseases, aging and etc (Patil and Patil, 2011). Information on *R. humilis* is scanty in available literatures thus suggesting that not much work has been done on the phytochemical contents and antioxidant potentials of this species. This present study, therefore investigated for the phytochemical compositions, the *in vitro* antioxidant and free radical scavenging potential of this plant.

MATERIALS AND METHODS

Plant Material

The leaves of *Rivina humilis* were collected in the month of August, 2011 from campus of Government Arts College,

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Chennai, Tamil Nadu, India. It was taxonomically authenticated at the Department of Botany. A Voucher specimen was deposited in the department for future reference. The leaves were shade dried for a month and ground into powder. 500 g of powdered leave sample was extracted sequentially with methanol, chloroform and ethyl acetate. The extract was filtered using Whatman filter paper No. 1 and concentrated with rotary evaporator, which provideed 35.5 g of the crude extract.

Phytochemical screening

Phytochemical screening of the leaf extract of *R. humilis* was carried out to identify the secondary metabolites and other phytoconstituents such as carbohydrates, tannins, saponins, flavonoids, alkaloids, quinines, glycosides, cardiac glycosides, terpenoids, triterpenoids, phenols, coumarins, proteins, steroids and phytosteroids phlobatannins and anthraquinones by following standard phytochemical methods (Harborne, 1973; Trease and Evans, 1987; Harborne, 1987).

Test for carbohydrates

To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.

Test for tannins

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

Test for flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for alkaloids

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for quinones

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

Test for glycosides

To 2ml of plant extract, 3ml of choloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Test for cardiac glycosides

To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides.

Test for terpenoids

To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates presence of terpenoids.

Test for triterpenoids

To 1.5ml of extract, 1ml of Libemann –Buchard Reagent (acetic anhydride + conc. sulphuric acid) was added. Formation of blue green color indicates presence of triterpenoids.

Test for phenols

To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

Test for coumarins

To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins.

Test for proteins and aminoacid: Ninhydrin test

To 2ml of plant extract, few drops of 0.2% Ninhydrin was added and heated for 5 minutes. Formation of blue color indicates the presence of proteins.

Steroids and phytosteroids

To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

Phlobatannins

To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of phlobatannins.

Total flavonoids

Aluminium chloride colorimetric method was used for flavonoids estimation. Each plant extracts (1mg/ml) were prepared and 100 µl, 150 µl and 200 µl of each sample was taken in separate tubes and made up to 2ml with methanol, 0.1 ml of 10% aluminium chloride and 0.1 ml of 1M potassium acetate. 2.8 ml of methanol was added and kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. The content of flavonoid was expressed in mg/g.

Total Tannin

Estimation of extracted tannin concentration in the extract was measured by Folin–Denis method with minor modifications. The extracts were liquated in required concentrations and made upto 1 ml with distilled water and then mixed with 0.5 ml of Folins–Ciocalteau reagent. The reaction mixture was alkalinized by the addition of 1 ml of 15% (w/v) sodium carbonate solution and kept in dark for 30 min at room temperature. The absorbance of the solution was read at 700 nm using spectrophotometer, and the concentration of tannin in the extract was determined using pure tannic acid as standard (10mg/100ml).

Anthraquinones

To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones.

Free radical scavenging activity

Antioxidant activity or free radical scavenging activity of the methanolic extract against DPPH (2,2-diphenyl-1-picrylhydrazyl) was measured according to Blois (1958). The percentage inhibition of DPPH radical by the sample was calculated using the following formula:

$$\text{Inhibition \%} = \frac{(\text{Absorbance of control (A517)} - \text{Absorbance of sample (A517)})}{\text{Absorbance of control (A517)}} \times 100$$

Various concentrations of 50, 100, and 150 µl of sample extract in ethyl acetate, chloroform and methanol were prepared. To 3.0 ml of solutions of DPPH, 1 ml of each of the extracts was added. After 30 min, the absorbance was measured at 517 nm. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. The actual decrease in absorption was measured against the control and the percentage inhibition was also calculated. The same experiment was carried out using butylated hydroxy toluene which is known as an antioxidant (control). The activities were determined as a function of their % inhibition (% I). The stable DPPH is known to generate free radicals was used as the radical source. From analysis, a larger percentage of the samples exhibited the ability to scavenge the free radical used in a concentration dependent manner, as their % of inhibition decreased with decrease in concentration.

RESULTS AND DISCUSSION

Phytochemical screening

Preliminary Phytochemical screening of the leaf extracts of *Rivina humilis* revealed the presence of different kind of chemical groups that are summarized in table 1. Ethyl acetate and chloroform extracts of leaves of *R. humilis* contain carbohydrates, flavonoids, alkaloids, quinones, terpenoids, coumarins and steroids. Methanol extract of leaves contain carbohydrates, tannins, saponins, flavonoids, cardiac glycosides, alkaloids, quinones, terpenoids, coumarins and steroids. All the tested extracts failed to detect glycosides, triterpenoids, phenols, proteins, phlobotannins and anthraquinones. Among the observed phytoconstitents, carbohydrates and quinines are abundantly present in all the tested extracts of leaves when compared to all other constituents which showed low percentage of presence. These phytochemical compounds are known to support bioactive activities in medicinal plants and thus responsible for the antioxidant activities of this plant extract used in this study. Through phytochemical prospecting of the extracts, it was possible to determine the presence of diverse classes of secondary metabolites that shows a wide variety of biological activities. Polyphenols are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer (Barreiros *et al*., 2006; Okuda *et al*., 1989). Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2 (Li *et al*., 2003), and this property may explain the mechanisms of antioxidative action of *R. humilis*.

Total flavonoids and total tannins

The amounts of total flavonoids and total tannins in the extracts were determined spectrometrically according to the Folin–Ciocalteu procedure. The amounts of total flavonoids and total tannins found in the plant extracts are shown in figure 1 & 2 respectively. The results showed that the methanolic extract at 200 µg has higher total flavonoid content (16.2) than the other extracts. The total tannin and contents of the methanolic extracts of *R. humilis* were 44.5, 60 and 80 µg/g at the concentrations of 100, 150 and 200 µg respectively.

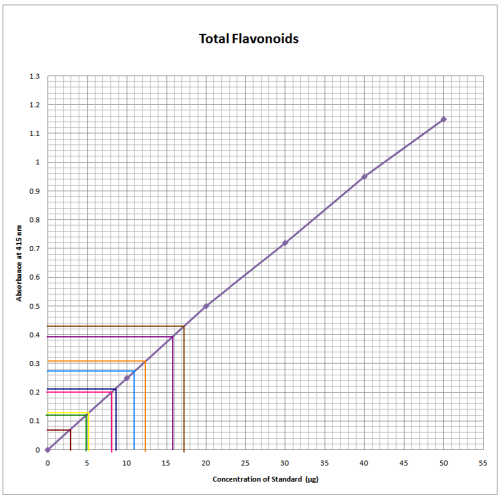

Figure 1. Estimation of total flavonoid contents in leaf extracts of *Rivina humilis*

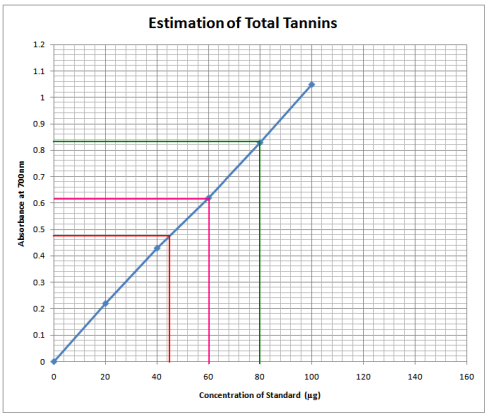

Figure 2. Estimation of total tannins in in leaf extracts of *Rivina humilis*

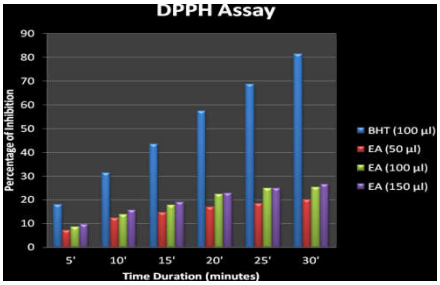

Figure 3. DPPH free radical scaven ging activity of ethyl acetate extract of leaves of *R. humilis*

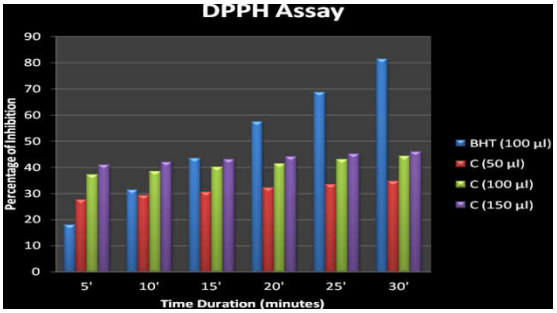

Figure 4. DPPH free radical scavenging activity of chloroform extract of leaves of *R. humilis*

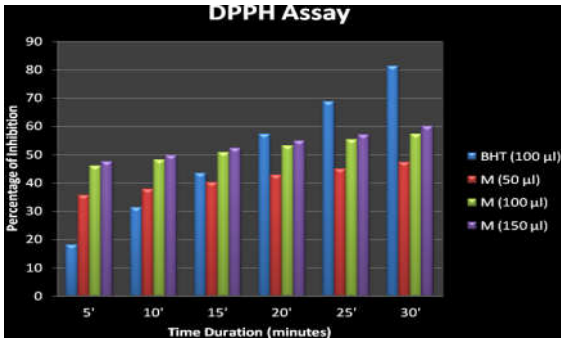

Figure 5. DPPH free radical scavenging activity of methanol extract of leaves of *R. humilis*

DPPH assay. Table 2 demonstrates DPPH scavenging activity, expressed in percentage and nm, caused by different concentrations of solvent extracts from *R. humilis*. DPPH is a purple colour dye having absorption maxima of 517 nm and upon reaction with a hydrogen donor the purple colour fades or disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance. The weakest radical scavenging activity (20.06%) was exhibited by the ethyl acetate extract of 50 µl/L (Fig. 3), and chloroform extract showed moderate radical scavenging activity (Fig. 4) whereas the strongest activity (60.13%) was exhibited by the methanolic extract at a concentration of 150 µl/L (Fig. 5).

The IC_{50} value of ethyl acetate, chloroform and methanolic extracts were found to be 432.78, 173.93 and 61.74 µl/L respectively. Plants with antioxidant activities have been reported to possess free radical scavenging activity and free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defence mechanism (Das and Pereira, 1990; Parr and Bolwell, 2000). As shown in table 2, the antioxidant activity of extracts increased with an increase in their concentrations. However, it remained lesser than BHT which was used as control. At higher concentrations, the antioxidant activity of extracts was closer to the scavenging effect of BHT (control). For example, at 100 µl/L, the scavenging activity of BHT was 81.33%, and a methanolic

Table 1. Preliminary Phytochemical screening of *Rivina humilis* leaf extract

Phytochemical tests	Ethyl acetate		Chloroform		Methanol	
Carbohydrates	Red color	++	Red color	++	Red color	++
Tannins	No color change	–	No color change	–	Greenish black	+
Saponins	No color change	–	No color change	–	Foam observed	+
Flavonoids	Yellow color	+	Yellow color	+	Yellow color	+
Alkaloids	White precipitate	+	White precipitate	+	White precipitate	+
Quinones	Red color	++	Red color	++	Red color	++
Glycosides	No color change	–	No color change	–	No color change	–
Cardiac glycosides	No color change	–	No color change	–	Brown ring	+
Terpenoidss	Red brown color	+	Red brown color	+	Red brown color	+
Triterpenoids	No color change	–	No color change	–	No color change	–
Phenols	No color change	–	No color change	–	No color change	–
Coumarins	Yellow color	+	Yellow color	+	Yellow color	+
Proteins	No color change	–	No color change	–	No color change	–
Steroids and Phytosteroids	Brown ring	+ (Steroids)	Brown ring	+ (Steroids)	Brown ring	+ (Steroids)
Phlobatannins	No color change	–	No color change	–	No color change	–
Anthraquinones	No color change	–	No color change	–	No color change	–

++ : Present in high concentration, + : Present in moderate concentration and - : Absent

Table 2. DPPH free radical scavenging activity of leaf extracts of *Rivina humils*

Samples	Concentration (µl)	Control	Absorbance at 517 nm (after 30 minutes)	% of inhibition (after 30 minutes)	IC_{50} value (µl)
BHT (control)	100	0.150	0.028	81.33	-
Ethyl acetate	50	0.927	0.741	20.06	
	100	0.927	0.690	25.26	432.78
	150	0.927	0.680	26.64	
Chloroform	50	1.046	0.684	34.60	
	100	1.046	0.584	44.16	173.93
	150	1.046	0.566	45.88	
Methanol	50	1.046	0.551	47.32	
	100	1.046	0.447	57.26	61.74
	150	1.046	0.417	60.13	

extract at a concentration of 150 µl/L had a scavenging activity of 60.13%. The next highest value (57.26%) was observed in methanolic extract at a concentration of 100 µl/L. It was reported that, the DPPH assay has been largely used as a rapid, consistent and reproducible parameter to search for *in vitro* antioxidant activity of pure compounds as well as plant extracts (Koleva *et al*., 2002; Goncalves *et al*., 2005).

Conclusion

The present results will serve as the preliminary findings for selection of potential plant species for further investigation, especially in isolating new bioactive compounds, which have strong antioxidant activity. Considering the phytochemical screening, total phenolics, reducing capacity and the DPPH radical scavenging activity as indices of antioxidant activity of the extract, these results revealed the potential of *R. humini* as a source for natural antioxidants. However, the components responsible for the antioxidant activities of the extracts were not identified and furthermore, detailed studies on the isolation and characterization of the plant extract as well as studies with other models such as lipid peroxidation and *in vivo* assays will be interesting in discovering new biological antioxidants.

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