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Article

# Leaf phenolics and pigments along with their antioxidative potential in some medicinal plants

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**Abstract:** Medicinal plants play significant role in human health. Besides this all of the Unani, Herbal, Kobiraji, and Homeopath medicine are produced from medicinal plant. The aim of the present study was to evaluate the phytochemical constituents as total phenolics content, free radical scavenging capacity, chlorophyll a, chlorophyll b, total chlorophyll (a+b) and carotenoids. Leaves of seven important non-solanaceous medicinal plants namely Boerhavia diffusa (punarnava), Adhatoda vasica (basok), Paederia foetida (gandhabhaduli), Rauwolfia serpentina (sarpogandha), Catharanthus roseus (pink nayantara), Catharanthus alba (white nayantara) and Andrographis paniculata (kalomegh) were extracted for phenolics, chlorophyll a and b as well as carotenoids in this experiment. Total phenolics content in seven medicinal plants in this experiment ranged from 142.44 (C. roseus) to 416.25 (A. vasica) mg GAE/100 g leaf fresh weight. The IC<sub>50</sub> value for scavenging 2, 2- diphenyl-1- picrylhydrazyl (DPPH) free radicals ranged from 32.12 (C. roseus) to 38.40 (Boerhavia diffusa) mg mL<sup>-1</sup> leaf extract in seven different medicinal plants. Pigments content such as chlorophyll a, chlorophyll b, chlorophyll (a+b) and carotenoids were found highest in Adhatoda vasica leaf as 2.347, 0.900, 3.283, and 0.521 mg g<sup>-1</sup> fresh weight, respectively, among all the seven species. We may get highly valuable medicine from Adhatoda vasica (basok) leaf for its superior biochemical constituents, which will help our next generation to treat chronic diseases.

Keywords: phenolics; free radical; pigments; chronic diseases

#### 1. Introduction

The plants used for healing several diseases are called medicinal plant. They have different variety and diversity. Most of them have been used for Ayurvedic, Unani and folk medicine since the prehistoric time. They have been used all over the world for thousands of years as natural medicines possessing therapeutic and other pharmacological effect (Skrovankova et al., 2012). Traditional medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal preparations. Boerhavia diffusa of family Nyctaginaceae is a potential ethno pharmacological herb throughout the world that has been reported to use in treating fever, inflammations, constipation, anemia, cardiac ailments, jaundice, ophthalmia, bronchitis and dyspepsia (Mishra et al., 2014; Nadeem et al., 2012). Justicia adhatoda belonging to family Acanthaceae, commonly known as Basok, is a small, evergreen shrub with a multitude of uses in traditional Ayurveda (Sanjeev and Nayan, 2015). Also, Rauvolfia serpentina, a plant from Apocynaceae, is enriched with a large number of alkaloids, and over 30 alkaloids have been isolated from this plant so far (Rathi et al., 2013). Catharanthus roseus (pink) and Catharanthus alba (white), both are from Apocynaceae that have immense medicinal value in addition of being ornamental. They are used as a traditional folk remedy to cure diabetes and malaria and has been reported to contain anti-cancerous phyto-constituents (Sain and Sharma, 2013). Paederia foetida, from Rubiaceae has long been used as tonic, diuretic, and good for liver and stomach troubles and lumbago. It is regarded as a specific for treating rheumatic affections, in which it is administered both internally Asian Australas. J. Food Saf. Secur. 2018, 2 (2)

and externally (Yusuf *et al.*, 2009). *Andrographis paniculata* is an annual herbaceous plant in the family Acanthaceae which has several potential mechanisms of action relevant in the treatment of acute upper

respiratory tract infections including anti-inflammatory (Shen *et al.*, 2002). The plants are selected on the basis of their traditional use, chemical content and combination of several criteria. The most common strategy is careful observation of the use of natural resources in folk medicine in different cultures; which is usually known as ethnobotany or ethnopharmacology. Keeping these selection criteria in mind, I have selected seven medicinally important plants for my research work. Ceaselessly some of these seven important medicinal plants have been studied separately by the scientists for their phenolics content, DPPH radical scavenging capacity and pigment content. Analyzing these plants for their total soluble phenolics, anti-oxidative power to scavenge free radical and pigments content would provide a better understanding of relative importance of these medicinal plants in each group as natural antioxidants in the defense against oxidative stress. Keeping this view in mind laboratory studies were done to assess and compare total phenolics content in different medicinal plants; to determine radical scavenging ability of plant extracts as an index of antioxidative power of these plants against oxidative stress; and to quantify carotenoids content in these plant samples.

## 2. Materials and Methods

#### 2.1. Plant materials and experimental sample collection

Seven important medicinal plants, namely*Boerhavia diffusa* L. (punarnava), *Paederia foetida* L. (gandhabhaduli), *Justicia adhatoda L.* (basok), *Catharanthus roseus* L. (pink nayantara), *Catharanthus alba* L. (white nayantara), *Andrographis paniculata* Nees. (kalomegh) and *Rauvolfia serpentina* L. (sarpogandha) were collected from the medicinal plants repository of Botanical Garden of Bangladesh Agricultural University, Mymensingh. For each plant species studied, three different plants were considered as three replicates. The plants shoots were collected in the Ziploc bag and brought to the laboratory for chemical analyses. Tender leaves of each replicate were chopped to make a composite sample.

#### 2.2. Phytochemical and biochemical characterization

#### 2.2.1. Total phenolics content assay

Total phenolics compound assayed with the method modified after Albano and Miguel (2011). Exactly 5 g of working samples for leaf or fruit were taken to a 250 mL beaker and 100 mL ice cooled methanol were added to it and then the samples were homogenized for 2 minutes using OV-5 Homogenizer, VELP, Italy. The mixture was kept for 30 minutes in the dark condition and centrifuged for 5 minutes at 1500 rpm and then its supernatant was treated as working sample extract. An aliquot amount of extract was used for determining total phenolics content or DPPH scavenging activity. Gallic acid was used here as standard. Exactly, 330  $\mu$ L different concentrations of gallic acid solutions or suitable amount of plant extracts were taken into a 50 mL test tube. Then 0.16 mL of Folin-Ciocalteu reagent and 3 mL of Na<sub>2</sub>CO<sub>3</sub> (10%) solution was added to 1 mL of gallic acid solution. The mixture was kept in dark condition for half an hour at room temperature (250C).

Then absorbance was measured at 760 nm. The absorbance value is the reflection of the total phenolic content of the compound. After plotting the absorbance in ordinate against the concentration a linear relationship was obtained which was used as a standard curve for the determination of the total phenolics content of the test samples.

#### 2.2.2. DPPH radical scavenging assay

Free radical scavenging activity of the medicinal plant extracts were determined by using a stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) (Brand-Williams *et al.*, 1995). DPPH is a free radical of violet colour. The antioxidants in the sample scavenge the free radicals and turn it into yellow colour. The change of colour from violet to yellow is proportional to the radical scavenging activity. Briefly, the assay contained 2.7 mL of 0.1 mM DPPH in methanol and made up to 3 mL with 300 µL plant extracts (working sample). The contents were mixed well immediately and then incubated for 30 min at room temperature (25°C). The degree of reduction of absorbance was recorded at 517 nm using DR 6000 UV Spectrophotometer. The percentage of scavenging activity was calculated as:

 $(Ac - As)/Ac \times 100$ 

where 'Ac' is the absorbance of control (without extract) and 'As' is the absorbance of sample with plant extract. Percentage of radical scavenging activity was plotted against the corresponding concentration of the extract to obtain IC50 value. IC50 is defined as the amount of antioxidant material required to scavenge 50% of

free radical in the assay system. The IC50 values are inversely proportional to the antioxidant activity (Nisha *et al.*, 2009).

## 2.2.3. Pigments determination

This protocol describes how the contents of chlorophyll-a, b and total carotenoids can be determined in a whole pigment extract of green plant tissue by spectrophotometer (Lichtenthaler, 1987). From the fresh composite leaf or fruit samples, 50 mg were taken in glass bottles and 200  $\mu$ L distilled water were added to it. Then 16 mL ethanol were added and shaken properly and finally the content was kept in dark condition for 24 h. Absorbance reading was taken in the following day in a spectrophotometer (DR 6000, Hach, USA) at 470, 649, 664 and 750 nm wave lengths. Afterward, amount of total carotenoids (sum of carotene and xanthophylls) were calculated using the following formulae:

Carotenoids (C x+c)=(4.785 A470+3.657 A664-12.76 A649)×16.2/FW

## Where,

A649 = Absorbance at 649 nm A664 = Absorbance at 664 nm A470 = Absorbance at 470 nm FW = Fresh weight of plant tissue (mg)

## 2.2.4. Statistical analysis

Mean values of each parameter studied for each group of medicinal plants were subjected to one way ANOV Analysis using Minitab 17.3 to determine whether significant differences in the group existed or not. In case of having significant *F-ratio*, means were subjected to Tukey's post-hoc test to observe the significant differences among the mean values.

## 3. Results

## **3.1.** Total soluble phenolics

Methanolic extracts of leaves of seven important medicinal plants, namely *Boerhavia diffusa* L. (punarnava), *Paederia foetida* L. (gandhabhaduli), *Justicia adhatoda* L. (basok), *Catharanthus roseus* L. (pink nayantara), *Catharanthus alba* L. (white nayantara), *Andrographis paniculata* Nees. (kalomegh) and *Rauvolfia serpentina* L. (sarpogandha) were tested to determine total phenolics as gallic acid equivalent (GAE) per 100 g fresh weight (Figure 1). Phenolics content in leaves of seven different medicinal plants varied significantly and ranged from 142.44 to 416.25 mg GAE/100 g fresh weight (Figure 1). The highest amount of total soluble phenolics was recorded in *Justicia adhatoda* leaf (416.25 mg GAE/100 g FW) followed by *Boerhavia diffusa* (264.25 mg GAE/100 g FW) and *Rauvolfia serpentina* (245.21 mg GAE/100 g FW). Statistically the lowest amount of phenolics was shared by *Paederia foetida* (165.81 mg GAE/100 g FW). Considering the phenolics content of *Justicia adhatoda* leaves (416.26 mg GAE/100 g FW) as 100%, the relative phenolics contents in *Boerhavia diffusa*, *Paederia foetida*, *Catharanthus roseus*, *Catharanthus alba*, *Andrographis paniculata* and *Rauvolfia serpentina* were calculated as 63.5, 39.8, 34.2, 39.9, 45.4 and 58.9%, respectively.

#### 3.2. DPPH scavenging capacity of seven different medicinal plants

The IC<sub>50</sub> value (the amount of antioxidant material required to scavenge 50% of free radical in the assay system) of leaf extract to scavenge DPPH radical varied significantly among the tested seven species of medicinal plants in experiment-1 and ranged from 32.12 (*Catharanthus roseus*) to 38.40 (*Boerhavia diffusa*) mg mL<sup>-1</sup> leaf methanolic extract (Figure 2). Justicia adhatoda, Andrographis paniculata, Rauvolfia serpentina, Catharanthus roseus and Catharanthus alba shared lowest and statistically identical IC<sub>50</sub> values for leaf methanolic extracts to scavenge DPPH. Considering IC<sub>50</sub> values of leaf methanolic extract of *Justicia adhatoda* leaves (35.15 mg mL<sup>-1</sup>) to scavenge DPPH as 100%, the relative IC<sub>50</sub> in *Boerhavia diffusa*, *Paederia foetida*, *Catharanthus roseus*, *Catharanthus alba*, *Andrographis paniculata* and *Rauvolfia serpentina* were 109.3, 107.1, 91.4, 92.4, 94.1 and 94.1%, respectively.

# 3.3. Pigments content

# 3.3.1. Leaf pigments in seven different medicinal plants

Chlorophyll a content in leaves varied significantly among the tested seven different species of medicinal plants and ranged from 1.151 to 2.347 mg g<sup>-1</sup> FW (Figure 3). *Justicia adhatoda* leaf showed the highest amount of

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chlorophyll-aa content (2.347 mg g<sup>-1</sup> FW) followed by the second highest in *Andrographis paniculata* leaf (1.639 mg g<sup>-1</sup> FW). The lowest value of chlorophyll a content was recorded in *Rauvolfia serpentina* leaf (1.151 mg g<sup>-1</sup> FW). Chlorophyll a content in *Boerhavia diffusa*, *Paederia foetida*, *Catharanthus roseus*, *Catharanthus alba*, *Andrographis paniculata* and *Rauvolfia serpentina* were 54.7, 58.7, 56.6, 56.2, 69.8 and 48.9% of that in *Justicia adhatoda* leaf.

Chlorophyll b content in leaves varied widely among the tested seven species and ranged from 0.498 to 0.900 mg g<sup>-1</sup> FW (Figure 4). *Justicia adhatoda* leaf showed the highest amount of chlorophyll b content (0.900 mg g<sup>-1</sup> FW) followed by the second highest in *Andrographis paniculata* leaf (0.795 mg) and *Paederia foetida* leaf (0.780 mg g<sup>-1</sup> FW). The lowest content of chlorophyll b was found in *Rauvolfia serpentina* leaf (0.498 mg g<sup>-1</sup> FW). Other species showed the chlorophyll b content as: *Boerhavia diffusa* (0.543 mg g<sup>-1</sup> FW), *Catharanthus roseus* (0.541 mg g<sup>-1</sup> FW) and *Catharanthus alba* (0.521 mg g<sup>-1</sup> FW). Considering the chlorophyll b content in *Justicia adhatoda* leaf as 100%, chlorophyll b content in *Boerhavia diffusa*, *Paederia foetida*, *Catharanthus roseus*, *Catharanthus alba*, *Andrographis paniculata* and *Rauvolfia serpentina* were 60.4, 88.3, 60.2, 57.9, 86.7 and 55.4 %, respectively.

Chlorophyll (a+b) content in leaves of seven different medicinal plants varied widely among the tested seven species and ranged from 1.619 to 3.283 mg g<sup>-1</sup> FW (Figure 5). *Justicia adhatoda* leaf showed the highest amount of chlorophyll (a+b) content as 3.283 mg g<sup>-1</sup> FW followed by the second highest in *Andrographis paniculata* leaf (2.420 mg g<sup>-1</sup> FW). The lowest value of chlorophyll (a+b) content found in *Rauvolfia serpentina* leaf (1.619 mg g<sup>-1</sup> FW). The other leaves showed the chlorophyll (a+b) content as *Boerhavia diffusa* (1.831 mg g<sup>-1</sup> FW), *Paederia foetida* (2.174 mg g<sup>-1</sup> FW), *Catharanthus roseus* (1.871 mg g<sup>-1</sup> FW) and *Catharanthus alba* (1.841 mg g<sup>-1</sup> FW). Considering the value of chlorophyll (a+b) content in *Justicia adhatoda* leaf as 100%, chlorophyll (a+b) content in *Boerhavia diffusa*, *Paederia foetida*, *Catharanthus roseus*, *Catharanthus alba*, *Andrographis paniculata* and *Rauvolfia serpentina* were found as 55.8, 66.3, 57.0, 56.2, 73.8 and 49.4%, respectively.

Figure 1. Total soluble phenolics content in the leaves of seven different medicinal plants. It is expressed as mg gallic acid equivalent (GAE)/100 g of leaf fresh weight (FW). Each data point is the average of three replicates  $\pm$  SEM. Bars sharing common letter are statistically identical but those having different letters are significantly different from each other at P  $\leq$  0.05.









Figure 3. Chlorophyll-a content in leaves of seven different medicinal plants. Each data point is the average of three replicates  $\pm$  SEM. Bars sharing common letter are statistically identical but those having different letters are significantly different from each other at P  $\leq$  0.05.



Figure 4. Chlorophyll-b content in leaves of seven different medicinal plants. Each data point is the average of three replicates  $\pm$  SEM. Bars sharing common letter are statistically identical but those having different letters are significantly different from each other at P $\leq$  0.05.



Figure 5. Chlorophyll-(a+b) content in leaves of seven different medicinal plants. Each data point is the average of three replicates  $\pm$  SEM. Bars sharing common letter are statistically identical but those having different letters are significantly different from each other at P  $\leq$  0.05.



Figure 6. Amount of carotenoids in leaves of seven different medicinal plants. Each data point is the average of three replicates  $\pm$  SEM. Bars sharing common letter are statistically identical but those having different letters are significantly different from each other at P  $\leq$  0.05.

Total carotenoids content in leaves varied widely among the tested seven species and ranged from 0.206 to 0.521 mg g<sup>-1</sup> FW (Figure 6). *Justicia adhatoda* leaf showed the highest amount of carotenoids content as 0.521 mg g<sup>-1</sup> FW followed by the second highest in *Andrographis paniculata* leaf (0.382 mg g<sup>-1</sup> FW). The lowest value of carotenoids content found in *Paederia foetida* leaf (0.206 mg g<sup>-1</sup> FW). The other species showed the carotenoids content as: *Boerhavia diffusa* (0.258 mg g<sup>-1</sup> FW), *Rauvolfia serpentina* (0.290 mg g<sup>-1</sup> FW), *Catharanthus roseus* (0.325 mg g<sup>-1</sup> FW) and *Catharanthus alba* (0.336 mg g<sup>-1</sup> FW). Carotenoids in *Boerhavia diffusa*, *Paederia foetida*, *Catharanthus roseus*, *Catharanthus alba*, *Andrographis paniculata* and *Rauvolfia serpentina* were calculated as 47.7, 39.8, 62.5, 64.6, 73.5 and 55.9%, to that in *A. vasica*, respectively.

## 4. Discussion

Phenolic chemical compounds are the most important groups of secondary metabolites in medicinal herbs and dietary plants that are characterized by having at least one aromatic ring (C6) bearing one or more hydroxyl groups. Various phenolic compounds (e.g., benzoic and cinnamic acid, coumarins, tannins, lignins, lignans, flavonoids and etc.) have a wide range of beneficial biological functions, including antioxidant activity. Unique structure and a high tendency of phenolic compounds for metal chelation and their redox properties allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers which could lead to anti-oxidant activity (Khoddami *et al.*, 2013; Bhatt and Negi, 2012).

In this study, total phenolics content in the methanolic extracts of leaf was found in the order as: Adhatoda vasica (basok) >Boerhavia diffusa (punarnava) >Rauvolfia serpentina (sarpogandha) >Andrographis paniculata (kalomegh) >Paederia foetida >Catharanthus alba (white nayantara) >Catharanthus roseus (pink nayantara) (Figure 1). Adhatoda vasica is an ayurvedic medicinal plant which is a home remedy for several diseases related to respiratory disorders such as asthma, acute and chronic cough, and bronchitis (Mamta *et al.*, 2015). In the present study, the highest phenolics content in Adhatoda vasica leaves among the seven important medicinal plants reveals the importance of this plant in favour of being strong position in folk medicine. Presence of high quantity of total polyphenolic compounds in Adhatoda vasica leaf gives significant antidiabetic, hypolipidemic, expectorant and emulsifying agent (Kumari *et al.*, 2013). In our study, second highest content of phenolics was observed in *Boerhavia diffusa* and *Rauwolfia serpentina* leaves. Studies have revealed that *Boerhavia diffusa* is an excellent diuretic, anti-inflammatory, mild laxative and is a heart tonic. *Boerhavia diffusa* is also used in treating obesity, improving appetite, jaundice and general fever (Bhowmik *et al.*, 2012).

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The antioxidants in the sample scavenge this free radical. This method is widely used to evaluate the free radical scavenging capacity of natural antioxidants (Maharana *et al.*, 2010). In this study IC<sub>50</sub> (IC<sub>50</sub> is defined as the amount of antioxidant material required to scavenge 50% of free radical in the assay system) of plant extracts to scavenge DPPH were assayed for the studied medicinal plants. The IC<sub>50</sub> values are considered to be inversely proportional to the antioxidant activity of plant extract (Nisha *et al.*, 2009).

Among the seven different medicinal plants studied basok (*Adhatoda vasica*), pink nayantara (*Catharanthus roseus*), white nayantara (*Catharanthus alba*), kalomegh (*Andrographis paniculata*) and sarpogandha (*Rauwolfia serpentina*) possessed statistically highest amount of antioxidative activity, while punarnava (*Boerhavia diffusa*) and gandhabhaduli (*Paederia foetida*) were ranked as second highest.

Among the seven different medicinal plants studied in the experiment, the highest chlorophyll-a, chlorophyll-b, chlorophyll-(a+b) and carotenoids content was detected in *Adhatoda vasica* leaf (Figures 3-5). Chlorophyll is a green pigment consists of tetrapyrrole ring with a central magnesium ion. It has a long hydrophobic phytol chain in its structure. It is found in some varieties in plants and algae (Aminot and Rey, 2000). In tumor or cancer therapy chlorophyll or chlorophyll derivatives can be utilized as a photodynamic agent (Brandis *et al.*, 2006).

# **5.** Conclusions

From the above summary of results for seven different medicinal plants *Adhatoda vasica* and *Catharanthus roseus* leaf contained the highest and lowest amount of phenolics, respectively. While highest radical scavenging ability was detected in *A. vasica*, *C. roseus*, *C. alba*, *A. paniculata* and *R. serpentina* leaves; the lowest was detected in *B. diffusa* and *P. foetida*. *A. vasica* leaf showed the highest amount of chlorophyll-a, chlorophyll-b, chlorophyll-(a+b) and carotenoids. In contrast, lowest amount of chlorophyll-a, chlorophyll-b, chlorophyll-(a+b) and carotenoids were detected in the *P. foetida* leaf.

# **Conflict of interest**

None to declare.

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