Pharmacological studies of *Adhatoda vasica* and *Calotropis procera* as resource of bio-active compounds for various diseases

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**Abstract**: *Adhatoda vasica* and *Calotropis procera* species were investigated as a resource for new diverse pharmacological agents including B complex, individual total phenolic compounds and antioxidants for curing and treatments of many infectious diseases in human through advanced analytical methods. These plants are abundant in Khyber Pukhtoon Khawa, Pakistan as well as in all over the world and famous for their unique medicinal importance. These herbaceous species are so far used for animals curing while current exploration of these species showed that these species are a precious resource of various compounds which can be employed in the formation of different drugs. The results showed that the leaf and flower extracts of *Adhatoda vasica* and leaf extract of *Calotropis procera* contained higher contents of bioactive compounds. The chemical analysis of the samples resulted in higher values of total phenolic compounds (71.32mg GAE/g), total antioxidants (651% DPPH inhibition), the enzyme catalase (4716µg/g), ash content (16.72%) and pH values in the *Calotropis procera*, whereas the total carotenoids (1987mg/100g), the enzymes, superoxide dismutase (4566µg/g) and peroxidase (1322µg/g) were higher in leaves of *Adhatoda vasica*. The flower extract of the *Adhatoda vasica* was rich in the flavonoids (0.87mg/100g) and organic matter (89.99%) as compared to *Calotropis procera*. The obtained data for each parameter was interpreted by applying Complete Randomized Design (CRD) along with factorial arrangements. The mean comparison was performed using LSD test at 5% probability level. The presence of these phytochemicals may lead to the conclusion that these herbal plants have the potential for formation of new drugs and can be used as herbal medicine for treatment of different cancer and viral diseases. These compounds are also useful in the treatment of the tumor.

**Keywords**: Total phenolic compound, antioxidant, potential elements.

**INTRODUCTION**

Infectious diseases are now a days considered as one of the leading causes of global morbidity and mortality, especially in developing countries (Yala et al., 2011). Many diseases caused by different agents (Andreotti et al., 2006). Plants are a vital part of the universe (Yala et al., 2011). Worldwide efforts are underway for the discovery of the new medicinal plants for their potential resistance against several diseases (Qureshi et al., 2006). The world is facing many new challenges regarding the health and cure of illness (Qureshi et al., 2006). The World Health Organization estimates that 80% of peoples in developing countries (65% of the world’s population) still rely on traditional medicine (Yala et al., 2011). Phytochemicals with potential antimicrobial activities isolated from plants are thus being explored in view of the possible therapeutic application to fight fatal opportunistic infections (Bakkali et al., 2008). The world focused the important discoveries of drugs with the help of medicinal plants (Pultrini et al., 2006). *Adhatoda vasica* belong to family Acanthaceae that commonly known as Malabar Nut plants (Irvine 1961). *Calotropis procera* is specie of flowering plant which belongs to the family Asclepiadaceae Lima). It is widely distributed in West Africa and other parts of the tropics (Irvine 1961). Flavonoids and phenolic compounds widely distributed in plants which have been reported to excess multiple biological effects, including antioxidant, free radicals scavenging abilities, anti-inflammatory, anti-carcinogenic (Miller 1996). Many experiments and observations on the medicinal plants were identified as the source of many medicines (Pultrini et al., 2006). Plants leave extract have a vast range of bioactive compounds in spite of the recent domination of the synthetic chemistry as a method to discover and produce drugs. The potential of bioactive plants or their extracts to provide new and novel products for disease treatment and prevention still enormous (Lima et al., 2011). Numerous groups with antitumor properties are plant derived natural products, including alkaloids, phenyl propanoids, and terpenoids (Akbar et al., 2010) are in use for curing. Maurya and Singh, (2010) accounted the highest amount of phenolic compounds which can scavenge the free radicals and exhibits greatest antioxidant activity for tumor cells (Roy et al., 2010). Parrotta, (2001) reported that the secretions from the root bark of *Calotropis procera* are used traditionally for the treatment of skin diseases, enlargements of abdominal viscera and intestinal worms, antitumor formation (Odugbemi & Akinsulire, 2006). In recent year, numerous
studies have shown that anthocyanin displays a broad range of biological activities including antioxidant, anti-inflammatory, anti-microbial and anti-carcinogenic, improvement of vision, and induction of apoptosis and neuroprotective effects on human bodies (Roy et al., 2010). They are also suggested to be iron chelator i.e. the unique natural’s antioxidant and radicals scavenging properties which act as an antitumor formation control in human bodies (Lima et al., 2011). Basic clinical and epidemiological research has suggested a potential protective effect of antioxidant nutrients such as (Vitamin C or Ascorbic acid), Anthocyanin, β carotene, Lycopene, chlorophyll, etc. on the risk of cardiovascular cancer diseases and aging effects (Lima et al., 2011). Pakistan has a diverse climate and is quite rich in medicinal herbs, in various scattered areas (Bakkali et al., 2008). Adhatoda and Calatropis are the two species widely distributed throughout the world as well as in Pakistan and particularly in Khyber Pakhtunkhwa region. Both the species contained tremendous valuable diverse medicinal bioactive compounds that act as anti-oxidant which is widely used all over the world for curing various viral diseases. On the basis of unique values of pharmacological agents and phytochemical properties of these herbaceous species, this study was planned to estimate the active phytochemicals and pharmacological agents to evaluate the health related properties of these species as an herball resource of antiviral and antitumor agents.

MATERIALS AND METHODS

Collection of herbaceous species and experimental site
Leaves of Adhatoda vasica and Calotropis procera and flowers of Adhatoda vasica were freshly plucked from Khanpur valley, Haripur Pakistan. The matured leaves were taken from the valley. The leaves were chosen of correct species and uniformity on the basis of its bright green color in which no pigmentation and blemishes were present during collection. These species were shifted into Horticulture laboratory of the University of Haripur for further analyzed the process.

Preparation of plant sample
The leaves and flower samples were washed thoroughly with tap water and crushed and peeled with the help of kitchen knife. The samples were then placed in a conventional oven at 73 °C for 48 hours. After the drying of the samples, the dried samples were grinded with a domestic electric grinder, and the powdered samples were then stored in air tight glass jar and were kept away from the direct sunlight for further processing.

Parts of the plant used during the experiment
- Leaves of Calotropis procera
- Flower of Adhatoda vasica
- Leaves of Adhatoda vasica

Pure sample preparation and extracts
The plant extract was prepared by taking one g of dry powdered sample and 10ml of distilled water. The sample was then mixed with the help of mechanical shaker and filtered through filter paper. The plant sample was then transferred to the separate bottle, and the clear solution was kept in the refrigerator (Anndy et al., 2003).

Separation and quantification of compounds
For individual phenolic compounds of both medicinal plants (Adhatoda vasica and Calotropis procera) extracts were subjected to HPLC 378 Coulter, Inc., Fullerton, CA equipped with a V System Gold 168 UV detector (Beckman Coulter, Inc.) at (260, 280, 520 nm). Separation was performed with a System Gold 168 UV detector (Beckman Counter, Inc.) at three different wavelengths (nm) with an HDO C-18, 5 columns. The mobile phase utilized a gradient consist of a 0.01M. The microliter of the various extracts was injected into HPLC.

Preparation of standard and sample solution
The standard solution was prepared by dissolving the compounds of interest in acetonitrile separately. The calibration solutions were developed by a stock standard solution with acetonitrile concentration; all standards were filtered through 0.45 µm. The linear regression analysis of the data for the calibration plots of both herball samples were noted with a relationship of r² values.

Chemical compositional analysis of herbaceous species

Ash Contents
One gram of both herbal plants samples were taken in a crucible before shifting sample into the dish to record the fresh weight. Then it was ignited in the muffle furnace at 550-600°C for 8 hrs. till ash formed. Finally, the weight of ash was recorded, and ash contents of samples were determined by the method described by (Anndy et al., 2003).

Organic matter
Organic matter (OM) was calculated using the following formula:
Organic Matter (%) = 100 – % Ash (Anndy et al., 2003).

pH of herbal extracts
Soil pH was measured in 1:5 soil water suspensions with a pH meter Jackson, (1962).

Body defense enzymes of herbal species
Peroxidase activity (POX)
Peroxidase activity was determined using the guaicol oxidation method (Anndy et al., 2003). The 3 ml reaction mixture contains 10mM potassium phosphate buffer (pH 7.0), 8mM guaicol and 100µL enzyme extract was used. The reaction was initiated by adding of 1% H₂O₂. The absorbance was recorded within 30 S at 430/470 nm. The unit of peroxidase activity was expressed as the change in
absorbance per min and particular activity as enzyme units per mg soluble protein (extinction coefficient 6.39 mM⁻¹cm⁻¹).

**Catalase activity (CAT)**
Catalase activity was estimated as described by Anndy et al. (2003). To initiate the reaction; 1ml of the reaction mixture which contain potassium phosphate buffer (pH 7.0), 250µl of enzyme extract and 60 mM H₂O₂ was taken. The activity was measured at a wavelength of 240 nm for 3min in which H₂O₂ consumption was determined through molar extinction coefficient, 39.4mM⁻¹cm⁻¹.

**Assay of super oxide dismutase (SOD)**
Super oxide dismutase activity was examined through spectrophotometrically by the method of Chen and Tarchitzky, (2009). For the preparation of incubation medium; then 3ml of reaction mixture contain, 50mM potassium phosphate buffer (pH 7.8), 5.3mM riboflavin, 45µM methionine, 84µM NBT and 20µM potassium cyanide was taken. The tubes were placed in an aluminum foil-lined box maintained at 25°C and equipped with 15W fluorescent lamps. After exposure to light, the reduced NBT was measured at 600nm.

**Determination of total flavonoids content**
For estimation of total flavonoids contents method of Kim, (2003) was used.

**Determination of total phenolic compounds and total antioxidants**
Total Phenolic contents (TPC) were estimated by using Folin-Ciocalteu reagent method as described by (Anndy et al., 2003). While total antioxidants activities of the herbal extracts were assessed by measuring their scavenging abilities to 2, 2-diphenyl-1-picrylhydrazyl stable radicals (Amira et al., 2012).

**Determination of total carotenoids**
Total carotenoids contents were assessed using the method of Amira et al. (2012).

**Sample preparation for HPLC analysis**
For HPLC analysis, flavonols and phenolic acids were extracted / hydrolyzed according to a reported method of Tokusoglut et al. (2000).

**B-Complex studies in herbal species**
B complex studies were conducted by the method of Halliwell and Gutteridge (1999).

**STATISTICAL ANALYSIS**
The obtained data for each parameter was interpreted by applying Complete Randomized Design (CRD) along with factorial arrangements. The mean comparison was performed using LSD test at 5% probability level as described by Steel et al. (1997).

**RESULTS**

**pH and organic content as an important indicator in herbal medicinal plant species**
The pH of both *Calotropis procera* and *Adhatoda vasica* were determined in various parts as an important biological factor which determined the activities of various important compounds found in the plants (Table 1). The results indicated that there was a broad range of variation in pH of *Calotropis procera* and *Adhatoda vasica* extracts. The pH was higher (7.22) in the leaf extract of *Calotropis procera*, followed by the flower extract of *Adhatoda vasica* with a pH value of (6.8). The least pH value (6.03) was recorded in the leaf extract of *Adhatoda vasica*. The organic matter contents were recorded higher (91.25%) in the flower extract of *Adhatoda vasica*, followed by the leaf extract (89.99%) of *Adhatoda vasica*. The leaf extract of *Calotropis procera* was recorded minimum (83.27%). The higher ash content (16.72%) was recorded in the leaf extract of *Calotropis procera*, followed by the leaf extract (10.01%) of *Adhatoda vasica*. The flower extract of *Adhatoda vasica* had the least ash content (8.75%). Higher values of protein contents were noted in *Adhatoda vasica* (Flower) while lowers contents of protein contents were found in *Calotropis procera* (leaf). The higher contents of fibers were in *Calotropis procera* (leaf) however 10% fiber was measured in *Adhatoda vasica* (Flower).

**Anti-tumor and anti-cancerous compounds of Calotropis procera and Adhatoda vasica**
The total phenolic compounds of *Calotropis procera* and *Adhatoda vasica* species were presented in table 2. Higher contents of phenolic compounds (71.32 mg GAE/g) were observed in the leaf extract of *Calotropis procera*, which was followed by the phenolic compounds (65.77 mg GAE/g), found in leaf extract of *Adhatoda vasica*. The total phenolic compounds (51.41 mg GAE/g) were lower in the flower extract of *Adhatoda vasica*. The antioxidant content (651 % DPPH inhibition) of the leaf extract of *Calotropis procera* was recorded higher as compared to the leaf (251% DPPH inhibition) and flower (239 % DPPH inhibition) extract of *Adhatoda vasica*.

**The antioxidant activities of both herbal species**
Studies on these two herbal species showed that both plants of *Calotropis procera* and *Adhatoda vasica* showed higher contents of antioxidant enzymes which are essential for reducing internal inflammation and lessening pain associated with conditions such as arthritis. The higher content of superoxide dismutase (4566 µg/g) was recorded in the leaves’ extract of *Adhatoda vasica* and the superoxide dismutase content (2100 µg/g) of flower extract of *Adhatoda vasica* was ranked 2nd among *Calotropis procera* and *Adhatoda vasica*. Lower contents of superoxide dismutase (1061 µg/g) were recorded in the leaf extract of *Calotropis procera* (table 3). The catalase
contents were higher (4716 µg/g) in the leaf extract of *Calotropis procera* and almost similar trend showed in catalase content (4629 µg/g) was found in the extract of *Adhatoda vasica*. The catalase contents were lower (2100 µg/g) in the flower extract of *Adhatoda vasica*. The presence of catalase enzyme indicated the ability of the plant to be beneficial for medicinal purpose.

The higher peroxidase content (1322 µg/g) was found in the leaf extract of *Adhatoda vasica*, followed by the flower extract (1288 µg/g) of *Adhatoda vasica*. The lower peroxidase content (1107 µg/g) was found in the leaf extract of *Calotropis procera*. Polyguthionase was higher in *Calotropis procera* (leaf) while lowers values were noted in *Adhatoda vasica* (leaf).

Vitamin B complex’s studies of *Calotropis procera* and *Adhatoda vasica*

The estimation of vitamin B complex from these two species was conducted as these species are not reported yet as a herbal resource of vitamin B complexes. The quantitative data of Vitamin B complexes were tabulated in Table 4. The results reported in Table 4 demonstrated the significance of these herbaceous species. The standard curves of (figs. 2 and 3) B complex showed Thiamin, Riboflavin, Niacin, Pantothenate, Vitamin B6. Our results (table 4) showed maximum contents of B complex in *Adhatoda vasica* (leaf) and *Calotropis procera* (leaf). *Adhatoda vasica* (leaf) showed higher contents of Thiamin (0.44 mg) while *Calotropis procera* (leaf) contained Thiamin contents were (0.12 mg). Maximum contents of Riboflavin were obtained by *Adhatoda vasica* (Flower). Lower contents of Riboflavin (0.34mg) were showed in *Adhatoda vasica* (leaf). While higher contents of Niacin were noted in *Adhatoda vasica* (Flower), however Niacin contents of *Calotropis procera* (leaf) were lowers. *Adhatoda vasica* flowers contains higher contents of Pantothenate (0.56 mg) while the lowers contents of Pantothenate (0.22 mg) in a leaf of *Calotropis procera*. The higher contents of Vitamin B6 (0.60 mg) showed in flower of *Adhatoda vasica*. However, the lower contents of Vitamin B6 (0.23 mg) showed in a leaf of *Calotropis procera*. Flower of *Adhatoda vasica* contained higher contents of Folate (0.71 mg) while lowers values of Folate were displayed in Table 4.

Estimation of Individual phenolic compounds in two herbal species

The results of the various phenolic compounds of *Calotropis procera* and *Adhatoda vasica* herbal medicinal plants were reported in table 5 and figs. 1-3. The individual phenolic profile of *Calotropis procera* (leaf), *Adhatoda vasica* (leaf) and *Adhatoda vasica* (Flower) was determined by using high performance liquid chromatography coupled with mass spectrometry (HPLC-MS). The chromatogram of the ethanolic extract of herbals plants presented in fig. 1. Seven peaks were detected; viz (A) p-coumaric; (B) p-hydroxy-benzoic; (C) Chlorogenic; (D) Ferulic acid; (E) Gallic; (F) Vanillic; (G) Σ HBA at different wavelength 280 nm, 360nm, 530nm.

Fig. 1: Chromatogram of ethanolic extract of *Calotropis procera* (leaf), *Adhatoda vasica* (leaf) and *Adhatoda vasica* (Flower) obtained by HPLC-DAD-MS (A) p-coumaric; (B) p-hydroxy-benzoic; (C) Chlorogenic; (D) Ferulic acid; (E) Gallic; (F) Vanillic; (G) Σ HBA at different wavelength 280 nm, 360nm, 530nm.

Fig. 2: Chromatogram of ethanolic extract of *Calotropis procera* (leaf), *Adhatoda vasica* (leaf) and *Adhatoda vasica* (Flower) obtained by HPLC-DAD-MS (A) Thiamin; (B) Rboflavin (C) Niacin (D) Pantothenate (E) Vitamin B6 at 280nm.

Fig. 3: Vitamin B Complex study on *Calotropis procera* and *Adhatoda vasica* herbal plants
The higher contents of Chlorogenic and Gallic acid from *Calotropis procera* (leaf) and *Adhatoda vasica* (leaf) respectively were observed followed to the flower of *Adhatoda vasica*. The flower of *Adhatoda vasica* contained higher contents of p-coumaric (16 mg/100g) while in a leaf of *Calotropis procera* it was 12mg/100g followed by the leaf of *Adhatoda vasica* 14mg/100g. Higher contents of p-hydroxy-benzoic were disclosed in flower of extract of *Adhatoda vasica*. However, the leaf of *Calotropis procera* showed lower contents of p-hydroxy-benzoic 0.14 mg/100g. The standard curve is presented in (Fig 4) which showed the peaks of p-coumaric (A), p-hydroxy-benzoic (B), Chlorogenic acid (C), Ferulic acid (D), Gallic acid (E), Vanillic F Σ HBA G. Higher contents of Chlorogenic were observed in *Adhatoda vasica* (Flower). While lower contents of Chlorogenic were observed in leaf extract of *Calotropis procera*. Higher contents of Ferulic acid were found in *Adhatoda vasica* (Flower) however the leaf of *Calotropis procera* showed lower contents of Ferulic acid 25mg/100g. Gallic acid (19mg/100g) was higher in flowers of *Adhatoda vasica*. However, the contents of gallic acid were lower in *Calotropis procera* (leaf). *Adhatoda vasica* (leaf) contained Vanillic 22mg/100g although the contents of Vanillic were showed in similar trends in *Adhatoda vasica* (Flower) and *Calotropis procera* (leaf). Sum of benzoic acid derivatives was higher in herb of *Adhatoda vasica* (Flower) while lower contents of the sum of benzoic acid derivatives 160 showed in a leaf of *Calotropis procera*. The *Adhatoda vasica* (leaf) contained the sum of benzoic acid derivatives (170). The results showed that these two species are the best resource of Phenolic compounds, especially in leaves as reported earlier by Burt and Reinders (2004).

**Table 1:** Estimation of chemical composition analysis of *Calotropis procera* and *Adhatoda vasica* of medicinal species

<table>
<thead>
<tr>
<th>Herbal Medicinal Species</th>
<th>pH</th>
<th>Organic matter (%)</th>
<th>Ash contents (%)</th>
<th>Protein contents (%)</th>
<th>Fiber contents (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Calotropis procera</em> (leaf)</td>
<td>7.22a</td>
<td>83.27 c</td>
<td>16.32 a</td>
<td>23 c</td>
<td>14 a</td>
</tr>
<tr>
<td><em>Adhatoda vasica</em> (leaf)</td>
<td>6.02 b</td>
<td>89.99b</td>
<td>10.01 b</td>
<td>32 b</td>
<td>11 b</td>
</tr>
<tr>
<td><em>Adhatoda vasica</em> (Flower)</td>
<td>6.81b</td>
<td>91.25 a</td>
<td>8.75 c</td>
<td>45 a</td>
<td>10 c</td>
</tr>
</tbody>
</table>

Different letters in superscript within the same row indicate significant difference among the herbal plants species at p<0.05 by LSD test. 0.012

**Table 2:** Human health potential elements of *Calotropis procera* and *Adhatoda vasica* medicinal species as antitumor and antiviral activity

<table>
<thead>
<tr>
<th>Herbal Medicinal Species</th>
<th>Total phenolic contents (mg GAE/g)</th>
<th>Total antioxidants (%DPPH)</th>
<th>Total carotenoids (mg/100g)</th>
<th>Total flavonoids contents (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Calotropis procera</em> (leaf)</td>
<td>71.32a</td>
<td>651a</td>
<td>702 c</td>
<td>0.59 c</td>
</tr>
<tr>
<td><em>Adhatoda vasica</em> (leaf)</td>
<td>65.55b</td>
<td>251b</td>
<td>1987 a</td>
<td>0.76b</td>
</tr>
<tr>
<td><em>Adhatoda vasica</em> (Flower)</td>
<td>51.41 c</td>
<td>239 c</td>
<td>1361 b</td>
<td>0.87a</td>
</tr>
</tbody>
</table>

Different letters in superscript within the same row indicate significant difference among the herbal plants species at p<0.05 by LSD test. 0.011

DISCUSSION

Herbal plants had a significant role in diminishing many infectious diseases of human body (Yala et al., 2011). In this study the variations in pH showed that the particular metabolism is available at some pH ranges which are not available for other. Xianquan and Kakuda (2005) reported that the herbal plants are abundant resources of different essential nutrients. Amira et al., (2012) observed the...
Pharmacological studies of Adhatoda vasica and Calotropis procera as resource of bio-active compounds

Table 3: Enzymes studies of Calotropis procera and Adhatoda vasica herbal medicinal species for controls of different chronic diseases.

<table>
<thead>
<tr>
<th>Herbal Medicinal Species</th>
<th>SOD (µg/g)</th>
<th>CAT (µg/g)</th>
<th>POX (µg/g)</th>
<th>Polyguthionase (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calotropis procera (leaf)</td>
<td>1061 c</td>
<td>4716 a</td>
<td>1107 c</td>
<td>1622 a</td>
</tr>
<tr>
<td>Adhatoda vasica (leaf)</td>
<td>4566 a</td>
<td>4629 b</td>
<td>1322 a</td>
<td>1244 c</td>
</tr>
<tr>
<td>Adhatoda vasica (Flower)</td>
<td>2100 b</td>
<td>2100 c</td>
<td>1288 b</td>
<td>1577 b</td>
</tr>
</tbody>
</table>

Different letters in superscript within the same row indicate significant difference among the herbal plants species at p<0.05 by LSD test. 0.001

Table 4: Vitamin B complex’s studies of Calotropis procera and Adhatoda vasica herbal plants species

<table>
<thead>
<tr>
<th>Medicinal herbal Species</th>
<th>Thiamin (mg)</th>
<th>Riboflavin (mg)</th>
<th>Niacin (mg)</th>
<th>Pantothenate (mg)</th>
<th>Vitamin B6 (mg)</th>
<th>Folate (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calotropis procera (leaf)</td>
<td>0.12 c</td>
<td>0.56 b</td>
<td>0.44 c</td>
<td>0.22 c</td>
<td>0.23 c</td>
<td>0.44 c</td>
</tr>
<tr>
<td>Adhatoda vasica (leaf)</td>
<td>0.44 a</td>
<td>0.34 c</td>
<td>0.71 b</td>
<td>0.33 b</td>
<td>0.45 b</td>
<td>0.50 b</td>
</tr>
<tr>
<td>Adhatoda vasica (Flower)</td>
<td>0.25 b</td>
<td>0.65 a</td>
<td>0.77 a</td>
<td>0.56 a</td>
<td>0.60 a</td>
<td>0.71 a</td>
</tr>
</tbody>
</table>

Different letters in superscript within the same row indicate significant difference among the herbal plants species at p<0.05 by LSD test. 0.01

Table 5: Estimation of Individual phenolic compounds of Calotropis procera and Adhatoda vasica herbal plants

<table>
<thead>
<tr>
<th>Medicinal herbal Species</th>
<th>p-coumaric A</th>
<th>p-hydroxybenzoic B</th>
<th>Chlorogenic C</th>
<th>Ferulic acid D</th>
<th>Gallic E</th>
<th>Vanillic F</th>
<th>Σ HBA G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calotropis procera (leaf)</td>
<td>12 c</td>
<td>0.14 c</td>
<td>17 c</td>
<td>25 c</td>
<td>15 c</td>
<td>20 b</td>
<td>160 c</td>
</tr>
<tr>
<td>Adhatoda vasica (leaf)</td>
<td>14 b</td>
<td>0.65 b</td>
<td>20 b</td>
<td>27 b</td>
<td>17 b</td>
<td>22 a</td>
<td>170 b</td>
</tr>
<tr>
<td>Adhatoda vasica (Flower)</td>
<td>16 a</td>
<td>0.90 a</td>
<td>24 a</td>
<td>31a</td>
<td>19 a</td>
<td>21 b</td>
<td>188a</td>
</tr>
</tbody>
</table>

Different letters in superscript within the same row indicate significant difference among the herbal plants species at p<0.05 by LSD test. 0.012

Σ HBA = Sum of benzoic acid derivatives

Phytochemical are natural substance produce by plants and have potential role for cure different chronic diseases of human (Cakir et al., 2004). The role of plants phenolic compounds as a promising tool in eradicating the causes and effects of skin damage, aging, skin diseases, and damage to skin cells, including wounds and burns. The higher contents of phenol which were reported in this research, directing the way of using these two plant species as a resource of secondary metabolites for controlling various types of viral and tumor activities. Carotenoids are good for human health like pro-vitamin A, antioxidant, anti-obesity and anti-cancerous which promote resistance against oxidative stress through scavenging the singlete oxygen. Various types of carotenoids like α-carotene, lutein, zeaxanthin, lycopene, β-cryptoxanthin, fucoxanthin, astaxanthin, as well as β-carotene, are famous for cancer prevention. Calotropis procera showed higher contents of total phenolic compounds due to its unique ability. Our results are in agreement with those of (Katalinic et al., 2006) who reported that the amount of total phenolic compound varied widely with Calotropis procera herbal species. Total antioxidants have ability to reduce the diseases (Akbar et al., 2010). Dorman and Dean, (2000) reported that herbal species leaves were rich source of phytochemicals. The results of current (table 2) research are in agreement to those of (Skaltsa et al., 2003, Cakir et al., 2004 ; Hosni et al., 2010; Khan et al., 2012 Wong et al., 2006) who observed that these species contain higher contents of phytochemicals with variation in antioxidant activities (Khan et al., 2012).
Superoxide dismutase plays a significant role in curing oxidative stress implicated in atherosclerosis and other life-threatening diseases and found to be one of the body’s primary interior anti-oxidant defensive components. Brand (2012) reported that the herbal plants act as a major supplier for different enzymes activities. The estimation of SOD in these species is in accordance with work of Brand (2012) and (Brinda et al., 2013) who reported the higher activity of superoxide dismutase in *Adhatoda vasica*. The variation in catalase enzyme of *Calotropis procera* and *Adhatoda vasica* may be related to the variation in plant species and their biochemical modifications in plant and their parts (Pham-Huy et al., 2008). Peroxidase contents were increased in *Adhatoda vasica* (Table 3). (Singh et al., 2011) reported that *Adhatoda vasica* Nees (Acanthaceae) is a well-known medicinal plant from which certain alkaloids, phenolics, flavonoids, sterols and their glycoside derivatives have been isolated (Pham-Huy et al., 2008). Maurya and Singh, (2010) conducted an experiment on the quantitative determination of flavonoid (flavonols) contents calculated in terms of quercetin equivalent in various extracts of *Adhatoda vasica*. The vitamin B complex plays a vital role in the development of immune system, healthy brain, healthy skin (Pultrini et al., 2006; Gislen et al., 2011). Vitamin B complex is a cell membrane phospholipid which is transformed in choline and used for acetylcholine synthesis which is a major Neurotransmitter to improve the brain and human skin cells (Brand, 2012; Lichtenthal and Buschmann, 2001). These complexes have been used many years for treatment of monotherapy with several drugs such as anti-inflammatory drugs, in several clinical situations, such as degenerative spinal diseases, rheumatologic diseases, polyneuropathies (especially diabetic neuropathy) and in different postoperative periods. The analysis revealed that these herbal plants have a rich source of Vitamin B complexes. These essential vitamins were used in various drug formulations (Brand 2012). These extracts have the potential role for the creation of new synthetic drugs and make their byproduct (Cakir et al., 2004).

The results reported in table 5 showed maximum contents of p-coumaric in *Adhatoda vasica* (Flower) which was comparable to the finding of Burt and Reinders (2004) who explain that p-coumaric have a potential role in the conquest of tumor cell (Muthuraman et al., 2011). Polyphenols are a large family of natural compounds widely distributed in plant foods particularly in herbal plants (Gislen et al., 2000). Herbal plants have antioxidative properties due to the presence of phenolic compounds (Dorman and Deans 2000). Kim et al. (2003) showed maximum contents of Ferulic acid in herbal species. Ferulic acid has the potential for suppression of tumor cells in human body. Gislen et al. (2000) investigated the phytochemical activities and wound healing properties of *Adhatoda vasica*. Singh et al. (2011) reported that Amla fruit is rich in quercetin, phylaembolic compounds, gallic acid, tannins, flavonoids, pectin and vitamin C and also contains various polyphenolic compounds. Hismath et al. (2011) studied the extraction conditions for phenolic compounds from neem (*Azadirachta indica*) leaves using response surface methodology (RSM). Ferulic acid (4-hydroxy-3-methoxycinnamic acid, FA) is a widely dispersed in herbal plants (Dorman and Deans 2000; Manoj et al., 2014). Spengler et al. (2000) reported that Ferulic acid has the potential to remove infections in the urinary tract. It was also reported that free FA or FA linked to simple sugars had a higher absorption rate when compared to FA bound with more complex matrices (Gislene et al., 2000). These matrices had a role in kidney metabolic rate (Singh et al., 2011). The role of FA arose in the liver and regulated intentional juice and glands human function (Spengler et al., 2004; Hismath et al., 2011; Burt and Reinders, 2004).

**CONCLUSIONS**

The investigation based on the solvent extraction of leaves of *Calotropis procera* and *Adhatoda* displayed that it contained higher contents of phenolic compounds, antioxidants, catalase flavonoids, superoxide dismutase, catalase, peroxidase, organic matters and ash contents with variable pH values respectively. These substances are active in antitumor activity and controls of viral diseases. The results suggested that these herbal species were an abundant source of potential health elements and can play a role in the developments of new drugs formation and have the possibility of resistance to many chronic diseases of human body. Moreover, these two herbaceous species investigated the first time for their health potential elements and a vast range of Pharmacological agents such as P-hydroxy-benzoic acid and Chlorogenic, etc are present. These agents have an extensive range of suppression activity against different chronic diseases. According to the estimation of these medicinal compounds, it is proposed that these two species must be taken under investigation for the biological activates for various diseases.

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**REFERENCES**


Amira EA, EB Saafi, B Mechri, L Lahouar and M Issaoui (2012). Effects of the ripening stage on phenolic...
Pharmacological studies of Adhatoda vasica and Calotropis procera as resource of bio-active compounds


