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 *Asclepaiadaceae 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

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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 herbals 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 herbals samples were noted with a relationship of r^2 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% H_2O_2. 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 $mM^{-1}cm^{-1}$).

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 H_2O_2 was taken. The activity was measured at a wavelength of 240 nm for 3min in which H_2O_2 consumption was determined through molar extinction coefficient, $39.4mM^{-1}cm^{-1}$.

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 Tokusogluet *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 leaf 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).

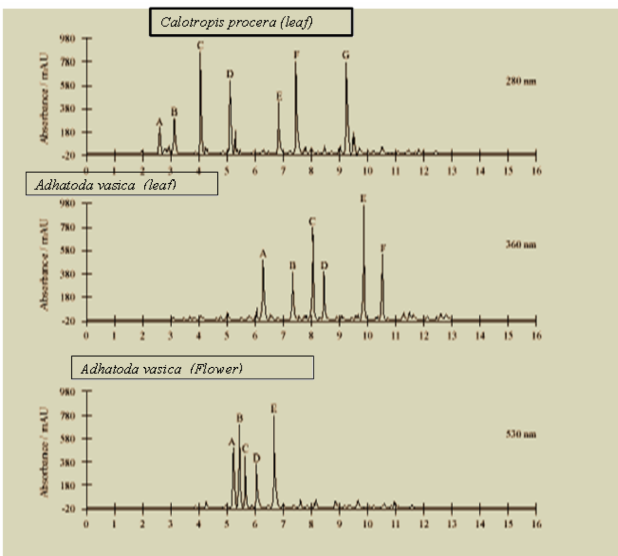

Fig. 1: Chromatogram of ethanolic extract of *Calotropis procera* (leaf), *Adhatoda vasica* (leaf) and *Adhatoda vasica* (Flower) obtained by HPLC-DAD-MS (A) p-coumaric (B) p-hydroxy-benzoic (C) Chlorogenic; (D) Ferulic acid (E) Gallic (F) Vanillic; (G) Σ HBA at 280 nm, 360nm, 530nm.

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 B_6 (0.60 mg) showed in flower of *Adhatoda vasica.* However, the lower contents of Vitamin B_6 (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.

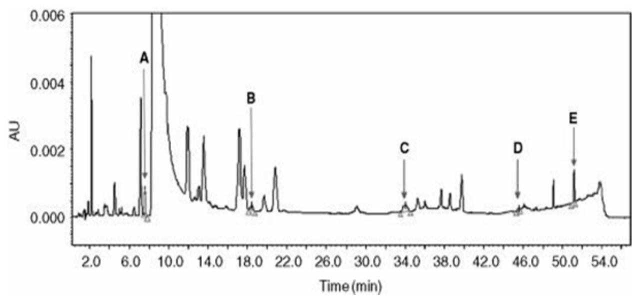

Fig. 2: Chromatogram of ethanolic extract of *Calotropis procera* (leaf), *Adhatoda vasica* (leaf) and *Adhatoda vasica* (Flower) obtained by HPLC-DAD-MS (A) Thiamin; (B) Rboflavin (C) Niacin (D) Pantothenate (E) Vitamin B6 at 280nm.

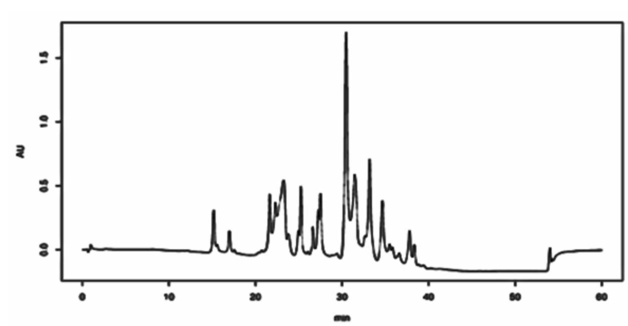

Fig. 3: Vitamin B Complex study on *Calotropis procera* and *Adhatoda vasica* herbal plants

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. Conventional solvent extraction: m/v: 5

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

Chemical composition analysis					
Herbal Medicinal Species	pH	Organic matter (%)	Ash contents (%)	Protein contents (%)	Fiber contents (%)
Calotropis procera (leaf)	7.22a	83.27 c	16.32 a	23 c	14 a
Adhatoda vasica (leaf)	6.02 b	89.99b	10.01 b	32 b	11 b
Adhatoda vasica (Flower)	6.81b	91.25 a	8.75 c	45 a	10 c

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

(Phytochemicals) Human health related elements				
Herbal Medicinal Species	Total phenolic contents (mg GAE/g)	Total antioxidants (%DPPH)	Total carotenoids (mg/100g)	Total flavonoids contents (mg/100g)
Calotropis procera (leaf)	71.32a	651a	702 c	0.59 c
Adhatoda vasica (leaf)	65.55b	251b	1987 a	0.76b
Adhatoda vasica (Flower)	51.41 c	239 c	1361 b	0.87a

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

g/50 ml, 30 min, 35°C, 80% ethanol, mechanical agitation in the dark and three successive extractions were used. 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 (C), Vanillic F and Σ HBA G and lower activity of p-coumaric (A), p-hydroxy-benzoic acid (B), Ferulic acid (D), Gallic acid (E) from *Calotropis procera* (leaf) at 280 nm. *Adhatoda vasica* (leaf) showed maximum contents of Chlorogenic (C), Gallic acid (E) at 360nm. The higher contents of p-hydroxy-benzoic acid (B) and Gallic acid (E) from leaves of *Adhatoda vasica* were displayed in Fig 1. Maximum contents of Chlorogenic were observed in *Adhatoda vasica* (Flower). While lower contents of Chlorogenic were obereved 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).

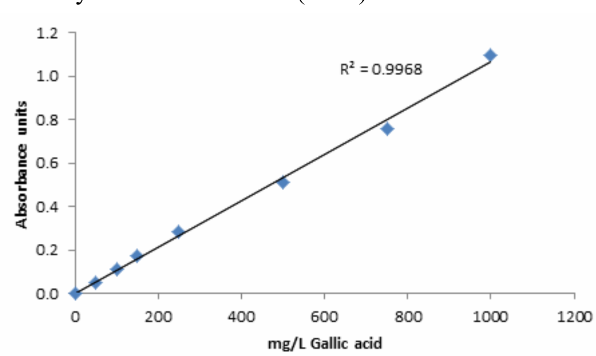

Fig. 4: Standard curve of gallic acid drawn for TPC calculation

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

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

Enzymes studies for Health related elements of various Diseases				
Herbal Medicinal Species	SOD (µg/g)	CAT (µg/g)	POX (µg/g)	Polyguthionase (µg/g)
Calotropis procera (leaf)	1061 c	4716 a	1107 c	1622 a
Adhatoda vasica (leaf)	4566 a	4629 b	1322 a	1244 c
Adhatoda vasica (Flower)	2100 b	2100 c	1288 b	1577 b

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

Vitamin B-complex's						
Medicinal herbal Species	Thiamin (mg)	Rboflavin (mg)	Niacin (mg)	Pantothenate (mg)	Vitamin B6 (mg)	Folate (mg)
Calotropis procera (leaf)	0.12 c	0.56 b	0.44 c	0.22 c	0.23 c	0.44 c
Adhatoda vasica (leaf)	0.44 a	0.34 c	0.71 b	0.33 b	0.45 b	0.50 b
Adhatoda vasica (Flower)	0.25 b	0.65 a	0.77 a	0.56 a	0.60 a	0.71 a

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

Individual phenolic compounds (mg/100g)							
Medicinal herbal Species	*p*-coumaric A	*p*-hydroxy-benzoic B	Chlorogenic C	Ferulic acid D	Gallic E	Vanillic F	Σ HBA G
Calotropis procera (leaf)	12 c	0.14 c	17 c	25 c	15 c	20 b	160 c
Adhatoda vasica (leaf)	14 b	0.65 b	20 b	27 b	17 b	22 a	170b
Adhatoda vasica (Flower)	16 a	0.90 a	24 a	31a	19 a	21 b	188a

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
Peak observation in various wavelength (280, 360, 530 nm).
Alphabetic letter showed in different individual phenolic compounds were noted.

significance of the pH and indicated that the pH values and organic contents showed the importance of herbal species in the medical treatment. The leaf extract of *Calotropis procera* was recorded minimum organic matters (83.27%). These results are in line with those of Chen and Tarchitzky (2009) who reported that the there is a negative response of ash with organic matter and both have an inverse relationship. Dorman and Deans (2000) reported that increase in ash content decreased the content of biological matter. Xianquan & Kakuda (2005) indicated that protein is an abundant source of these herbal species due to its unique character. Similar results were reported by (Akbar *et al*., 2010) in *Adhatoda vasica* (Flower) which showed higher contents of protein.

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 (Acanthaceace) 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; Gislene *et al.,* 2000). 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; Lichtenthaler 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 formations (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 anti-oxidative 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. Gislene *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, phyllaemblic 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 *Calotrapis 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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