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Pharmacological Screening of Leaf Extract of *Adhatoda vasica* for Therapeutic Efficacy

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Abstract: In the present study *Adhatoda vasica* was screened for therapeutic efficacy. The plant leaf extract was screened for phytochemicals, mineral elements, antioxidant and reducing power activity, foaming and swelling index properties. Both qualitative and quantitative analyses of phytochemicals were done. The result showed the presence of alkaloids, tannins, saponins, phenolics and flavonoids. Tannins were maximum among all the detected phytochemicals (61.38 ± 0.8 mg/g). Phenolics were minimum (1.30 ± 0.1 mg/g). Following the presence of phytochemicals the plant leaf samples were also screened for antioxidant and reducing power ability. The screening for mineral elements revealed the presence of macro and micro elements. Potassium (K), Calcium (Ca), iron (Fe), Copper (Cu), Zinc (Zn), Chromium (Cr), Vanadium and Manganese (Mn) were detected in the leaf sample. *Adhatoda vasica* showed highest (68070 ± 35.58 ppm) concentration of Ca. V was lowest among all the mineral elements (118 ± 6.03 pm). Since the plant contains important phytochemicals, mineral elements, antioxidant and reducing power analysis, it may be good source of future medicines.

Key words: Phytochemicals · Mineral Elements · Antioxidant · Reducing Power

INTRODUCTION

Adhatoda vasica (Family Acanthaceae) is commonly known as Malabar Nut, is distributed throughout India up to an altitude of 1300m. The leaves, flowers, fruits and roots are extensively used for treating cold, cough, whooping cough, chronic bronchitis and asthma as sedative, expectorant and antispasmodic in traditional medicines. It was also used by traditional midwives at the time of delivery [1]. The plant has been reported to have high medicinal value [2]

Every culture throughout the world has been using herbal and natural products of folk medicine from centuries. Various plant parts such as leaves, bark, fruits, roots and seeds are used in treatment of various diseases. For many years the role and metabolic functions of trace elements in human body have been investigated. The minerals are obtained from the earth's crust. Through the effects of the weather, rocks that contain minerals are ground into smaller particles, which then become part of the soil. The mineral content in the soil is absorbed by growing plants. The plants are consumed by both animals and human beings as food. This mineral becomes part of the food chain. The plants absorb much of the essential elements from the soil in which they grow and serve as indicators of the materialization and are in fact used for this purpose [3]. Heavy metals are the matter of concern in the herbal drugs as certain plants have the tendency to store them from the soil, polluted water and atmosphere [4]. Human body comprises chemical compounds such as water, proteins, fatty acids, nucleic acids and carbohydrates, these in turn consists of elements such as Carbon, Hydrogen, Oxygen, Nitrogen and Phosphorus and may or may not contain minerals such as Zinc, Calcium, Iron, Magnesium [5].

In recent years , there is an increasing interest in finding antioxidant phytochemicals, because they can inhibit the propagation of free radical reactions, protect human body from diseases [1, 6]. Polyphenols constitute a large group of naturally occurring substances in plant kingdom, which include the flavonoids. The plant phenolics are commonly present in fruits, vegetables, leaves, nuts, seeds, barks, roots and in other plant parts. These substances have considerable interest in the field of food chemistry, pharmacy and medicine due to wide rangeof favourablebiological effectsincluding antioxidant properties. The antioxidant properties of

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phenolics is mainly due to their redox properties. They act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [7].

An antioxidant is a molecule that inhibits the oxidation of other molecules and oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reaction can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Reactive oxygen species (ROS) include free radicals such as superoxide (O_2), Hydroxyl radical (OH-), Peroxyl radical (ROO) [8]. The oxidation induced by reactive oxygen species(ROS) can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disorders. Although the body possess defense mechanisms such as enzymes and antioxidant nutrients which arrest the damaging properties of ROS [9]. Reducing power is associated with antioxidant activity and may serve as significant reflection of the antioxidant activity [10]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes [11]. Plants have great importance due to their nutritive value and continue to be a major source of medicines as they have been found throughout human history [12], 30-40 % of today's conventional drugs used in the medicinal and curative properties of various plants are employed in herbal supplements, botanicals, nutraceuticals and drugs [13]. All human beings require number of complex organic compounds as added [14] caloric requirements to meet the need for their muscular activities. Carbohydrates, Fats and Proteins comprise major part while vitamins and minerals form comparatively minor part of plant parts [15]. Human body comprises chemical compounds such as water, proteins, fatty acids, nucleic acids and carbohydrates

MATERIALS AND METHODS

The fresh tender leaves of *Adhatoda vasica* were collected from Ranchi district of Jharkhand State of India. The plant leaves were washed with deionised water and disinfected with 0.1% $HgCl_2$ solution for 5min and dried in shade for 15 days. The dried materials were ground to fine powder with the help of electrical grinder [16,17].

Determination of Swelling Index: One gram of powder was weighed, placed into 25 ml measuring cylinder. 25 ml of water was added and shaken thoroughly in every 10 min for 1 hr. and then allowed to stand for 3 hrs. at room temperature. The volume occupied by the plant material was measured and compared to that of the dry powder [18].

Determination of Foaming Index: One gram of powder was taken in a 500 ml conical flask containing 100 ml water and boiled for 30 min, cooled and filtered into a 100 ml volumetric flask and made the volume with water. The decoction was poured into 10 test tubes in successive portion of 1ml, 2ml, 3ml and so on, up to 10ml. and adjusted the volume of each test tube with water to 10ml. The test tubes were shaken lengthwise for 15 sec and allowed to stand for 15 min. and the height of foam was measured and foaming index was calculated using following formula as suggested in the Quality control methods for medicinal plant materials [18].

$$Foa\min g\ index = \frac{1000}{a}$$

Where, "a" is the volume of decoction used for preparing the dilution in tube.

Antioxidant Activity: Theantioxidantproperties of plantsamples were determined by Spectrophotometric quantitation method [19]. Various concentrations of samples (5 µg, 50 µg, 100 µg) were taken in a series of test tubes. The 1.9 ml of reagent solution (0.6 M Sulphuric acid, 28 mM Sodium phosphate & 4 mM Ammonium molybdate) was added to the test tubes. The tubes were incubated at 95°C for 90 min and allowed to cool down. The absorbance of aqueous solution of each was measured at 695 nm against blank. Antioxidant capacities were expressed as equivalents of ascorbic acid. Butylated hydroxyl anisole (BHA) was used as reference standard.

Reducing Power: Spectrophotometric quantitation [20, 21] method was used for the determination of reducing power activity. 2.5 ml of each of the extracts was mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1 % potassium ferricyanide (10 mg/ml). The mixture was incubated at 50°C for 20 min and cooled down. Then 2.5 ml of 10 % trichloroacetic added to the test tube and centrifuged at 6500 rpm for 10 min. An aliquot (2.5 ml) of

supernatant was diluted with distilled water (2.5 ml) and then ferric chloride (0.5 ml, 0.1 %) was added and allowed to stand for 10 min. The absorbance was recorded spectrophotometrically at 700 nm. Ascorbic acid was used as standard.

Determination of Mineral Elements: Various essential and trace elements such as K, Ca, Fe, Cu, Zn, Cr, Cl, V, Mn and Ti in plant samples were analyzed using atomic absorption spectrophotometer (AA 6300, Shimadzo, Japan) equipped with flameandgraphitefurnace [22]. Air-acetylene flame was used for determination of metal content. The instrument was operated with the following conditions in flame mode: acetylene 2.0 L/min, air 15 L/min, the inert argon gas flow and the temperature parameters were followed as recommended by manufacturer. The absorption wavelength for the determination of each metal together with its linear working range and correlation coefficient of calibration graphs are given in Table 1. Data were rounded off suitably according to the value of standard deviation from measurements in triplicate.

Preliminary Phytochemical Screening: Qualitative phytochemical screening tests were conducted on the *Adhatoda vasica* and *Vitex negundo* leaf samplewith previously published standards [23].

Quantitative Analysis of Detected Phytochemicals: The quantitative analysis of detected phytochemicals in the preliminary phytochemical screening were done as per the previously published standards of Kumar *et al.* [24].

RESULTS AND DISCUSSIONS

Mineral Elements and Nutritive Value: The result of mineral elements analysis of *Adhatoda vasica* is given in Table-1. While the result of various nutrients and nutritive value is summarized in Table-3.Results showed that chromium was very low (41ppm) in comparison with other mineral elements; Cr is vital element as it works with insulin to stabilize blood sugar level, helps to absorb energy from blood and increase muscle mass by reducing fat mass in human body [25]. Deficiency of Cr results in growth failure, cataract, hyperglycaemia, neuropathy, atherosclerosis and leads to diabetes in humans [26]. Potassium was higher (Table - 1). Sodium and Potassium take part in ionic balance of the human body and maintains tissue excitability, carry normal muscle contraction and help in formation of gastric juice in stomach [27]. K helps in release of chemicals which acts as nerve impulses, regulate heart rhythms, deficiency causes nervous irritability, mental disorientation, low blood sugar, insomnia and coma [28]. Iron sufficient in *Adhatoda vasica* (Table - 1) is involved in making of body tendons and ligaments, certain chemicals of brain are controlled by presence or absence of iron. It is essential for formation of hemoglobin, which carry oxygen around the body [29]. Iron deficiency causes anemia, weakness, depression, poor resistance to infections [30]. Ca was sufficient in *Adhatoda vasica* (68070 ppm). Calcium play important role in building and maintaining strong bones and teeth and also large part of human blood and extra cellular fluids. It is also necessary for normal functioning of cardiac muscle,bloodcoagulation, milk clotting and regulation of cell permeability [31]. Calcium deficiency causes rickets, back pain, osteoporosis, indigestion, irritability, premenstrual tension and cramping of uterus [1]. Zinc was found to be 67 ± 6.25 ppm, Cu is an important component of many enzyme systems such as cytochrome oxidase, lysyl oxidase and ceruloplasmin, an iron oxidizing enzyme in blood [32]. Cu deficiency has been associated with cardiac abnormalities in human and animal; causes anaemia and neutropenia [32]. Zn maintain various reaction of body, which help to construct and maintain DNA, required for growth of body tissues, important element of ligaments and tendons [33]. Zn deficiency causes clinical consequences, including growth delay, diarrhoea, pneumonia, disturbed neurophyshological performance and abnormalities of foetal development [33]. Vanadium, its deficiency causes reduced growth and impaired reproduction. Vanadium isrelatively controversial dietary supplement,primarily for increasing insulin sensitivity [22]. Mn was 118 ± 6 ppm in *Adhatoda vasica* (Table -1).

Table 1: Mineral elements from *Adhatoda vasica*

ELEMENTS	Concentration present in *Adhatoda vasica* mg/g
K	31190 ± 20.31
Ca	68070 ± 35.58
Fe	705 ± 8.95
Cu	64 ± 5.86
Zn	67 ± 6.25
Cr	41 ± 3.67
V	19 ± 1.11
Mn	118 ± 6.03

Table 2: Swelling Index and foaming index

Attributes	*Values in percent*
Swelling Index	34 ± 2.66
Foaming index	90 ± 3.54

Phytochemicals: The results on phytochemical analysis of the leaf samples of *Adhatoda vasica* and has been presented in fig - 1. The result reveals that tannin was highest in *Adhatoda vasica* i.e. 61.3 ± 0.8 mg/g in *Adhatoda vasica*; phenols were recorded lowest among all the studied phytochemicals i.e. 1.3 ± 0.1 mg/g in *Adhatoda vasica*. Soladoye and Chukwana [34] reported tannin (4.98%), saponin (47.3%), alkaloid (2.49%), flavonoids (6.48%) in *Cissuspopulnea.* Khan *et al.* [35] reported alkaloid content (1.13%) in *A. vasica,* 1.11% in *P. harmala*, 1.036% in *W. fruticosa* and 0.90%. in*V. cotinifolium*. 0.87% Phenolic content was observed in methanolic extract of *W. fruticosa.* Tannin content was recorded 15.75% in *M. rubicaulis,*14.16%, *W. fruticosa,* 13.4% in *C. grata* , 12.33% in *V. cotinifolium* , 11.2% in *E. hirta*, 10.56% in B. papyrifera and 10.2% in *P. harmala*.Flavonoid content has been reported to be 10.95% in V. negundo. Saponin content was recorded 5.06% in methanolic extract of *T. officinale.*

Antioxidant Activity: The results on antioxidant activity of the *Adhatoda vasica* have been presented as fig - 2. The results clearly reveals that the absorbance is directly proportional to the antioxidant activity (Fig -1, 2) i.e. more the absorbance, more is the antioxidant activity of the extract. The total antioxidant activity of the *Adhatoda vasica* extracts expressed as the mg of ascorbic acid/100mg, which showed that methanolic extracts of *Adhatoda vasica* have good antioxidant capacity (Fig - 1) which underlines its suitability as antioxidant supplement. Tiwari and Tripathi [36] reported that non-polar fractions of *V. negundo* leaf trapped free radicals and thereby inhibited lipid peroxidation which is reflected as antioxidant activity. Several reports emphasize that the type of solvents is also associated with antioxidant activities. Antioxidant activity of methanolic and hexane extracts of *Cordiawallichii*were examined and results showed the methanolic extract to be more effective (28.2%) than hexane extract (16.7%). Sheikh *et al*. [37]

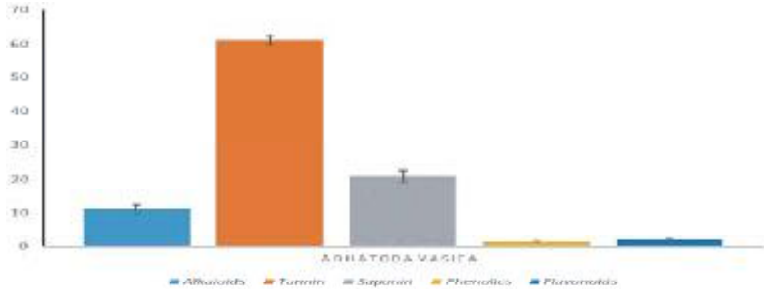

Fig 1: Quantitative analysis of phytochemicals in *Adhatoda vasica* (values in mg/g; n=3; M ± SD)

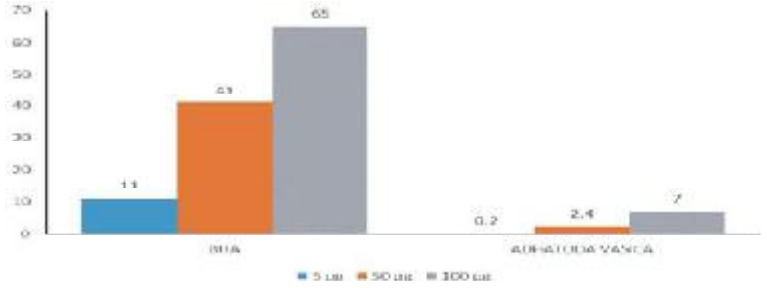

Fig 2: Antioxidant activity of *Adhatoda vasica* in comparison

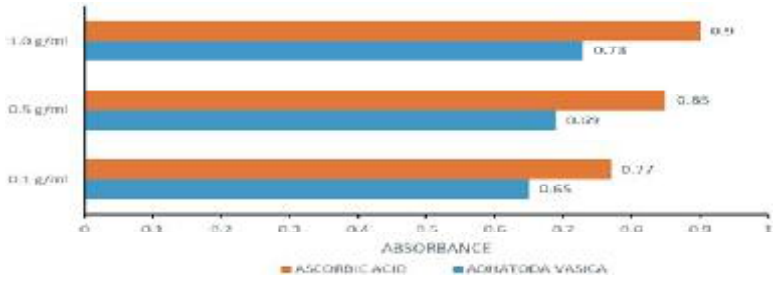

Fig 3: Reducing power of *Adhatoda vasica* in comparison with ascorbic acid

reported that antioxidant activity of some marine macro-algae depends on type of solvent used for extraction apart from other condition and non-polar solvents were more effective over aqueous solvents. Hence the methanolic solvent was used for the present extraction. Mshvildadze *et al.* [38] reported that antioxidant activities are directly related to the saponins content. Rodrigues *et al.* [39] reported that the beneficial effects of saponin on serum lipids were related to a direct antioxidant activity of saponins. Elekofehinti *et al*. [40] concluded that *Solanum anguivi* saponins were capable of improving the antioxidant defense in rats. The antioxidant activity of Malasian *D. grandis*is mainly due to the saponin (18.9 mg/g) content of the plant. Satayanshu kumar [41] reported phenolic content in *Vitex trifolia (*74.5 GAE gm-1), *T. chebula*(531.5 74.5 GAE gm-1), *T. bellerica*(362.5 74.5 GAE gm-1), *E. officinalis*(221.6 74.5 GAE gm-1), *A racemosus*(10.0 74.5 GAE gm-1) and found a liner relation between antioxidant activity and phenolic contents of plants. Joabe Gomes de Melo *et al.* [42] screened some plants for their antioxidant activity and Tannin content. They reported highest tannin content in *Pyramidalis queiroz* (8.17 ± 0.64 µg/g) and lowest in *Cyperus distans* (1.22 ± 0.02 µg/g), they attributed the antioxidant activity of studied plants to their Tannin content.

The antioxidant activity *Adhatoda vasica* in present study seems to be due to its high content (Fig -1) of tannins and Saponins. Flavonoid, phenols and alkaloids content (Table-1) are known to pose antioxidant properties to the plants [43, 44]. Anti-dysenteric and anti-diarrhoeal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids [45]. Various workers reported that there is a liner relation between the antioxidant activity and saponin, flavonoid, phenols, tannins and alkaloid contents [36, 46]. Thus *Adhatoda vasica* can be used as antioxidant supplement.

Reducing Power: The reducing ability of Ascorbic acid in comparison with *Adhatoda vasica* is shown in fig-3. The plant leave sample was found to have high reducing ability. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [10]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary processes [11]. Tannic acid and Quercetin had highest reducing activity than ascorbic acid.

Swelling and Foaming Index: Swelling and foaming indices indicators of the drug release characteristics. The release of drug from hydrophilic matrices occurs as a result of complex interaction between diffusion, dissolution and erosion mechanisms. On coming in contact with water, hydrophilic matrices undergo gel formation and progressive phase transition from glassy to rubbery state takes place. This results in solvation of individual polymer chains. As the swelling continues, the swollen matrix retains more water until the shear forces in the dissolution medium disentangle the individual polymer chains from the matrix. Direct relationship has been observed between swelling index and polymer concentration, as polymer concentration increases, swelling index also increases [47]. Medicinal plants are known to contain saponins that cause persistent foam when an aqueous decoction is shaken which is indicated by the foaming index [48]. Not much work has been done on foaming index and of those available, none has reported any significant value against the index.

CONCLUSION

The plant leaf has good phytochemical contents and exhibits strong antioxidant and reducing power activity, the plant also contains major macro and micro elements, Although low nutritive value suggest it being unfit to be used as food and fodder, but owing to former characteristics, the plant can be good source of future medicine.

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