

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT POTENCY OF ADHATODA VASICA AND VITEX NEGUNDO

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ABSTRACT

The leaf samples of *Adhatoda vasica* and *Vitex negundo* were subjected to phytochemical analysis. Both the plants contained antioxidant phytochemicals such as alkaloids, tannin, saponins, phenolics and flavonoids; which were present in comparatively higher amount in *Vitex negundo*. Tannins were recorded highest among all the phytochemicals (93.9 ± 0.8 and 61.3 ± 0.8 mg/g in *Vitex negundo* and *Adhatoda vasica* respectively). Phenolics were recorded lowest ($13. \pm 0.1$ and 8.1 ± 0.5 mg/g in *Adhatoda vasica* and *Vitex negundo* respectively). The methanolic extracts of the plants were also analyzed for antioxidant and reducing power potentiality. Both plants showed strong antioxidant and reducing power ability. The strong antioxidant and reducing power ability of the plant underlines their use as antioxidant supplement against diseases such as typhoid during which antioxidant system fails; cardiovascular diseases which are caused due to accumulation of Reactive oxygen species; ageing related diseases, Alzheimer, Prkinson's disease, Amytrophic lateral sclerosis, cataractogenesis and other diseases.

INTRODUCTION

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. It had been reported that aqueous and methanolic extracts from plants used in allopathic medicines were potential sources of antiviral, antitumour and antimicrobial agents (Vlietinck *et al.*, 1995). Herbal drugs have become increasingly popular and their use is widespread. Clear cut proof of their efficacy in microorganisms including pathogenesis is yet to be explored. There is a need to develop alternative drugs for the treatment of infectious diseases from medicinal plants (Mohanta *et al.*, 2007). Natural antimicrobials can be derived from plants (Zaika, 1975). The side effects of drugs available today compel the discovery of new pharmaco-therapeutic agents from medicinal plants (Cordell, 1993).

The review of literature revealed that considerable contributions have been made on medicinal plants by many workers (Dadsena *et al.*, 2013; Dandapat *et al.*, 2013; Kullu *et al.*, 2013; Kumar *et al.*, 2013; Mahato *et al.*, 2013; Tabassum *et al.*, 2013; Toppo *et al.*, 2013; Sahu *et al.*, 2013).

The generation of reactive oxygen species (ROS), which are normally produced in mitochondria (Evans and Halliwell, 2001; Sastre *et al.*, 2003) and other free radicals (FR) during metabolism is a necessary (ex: it helps the body's immune system to kill microorganism) and normal process that ideally is compensated for by an elaborate endogenous antioxidant system. But due to lifestyle, pathological situations, excess radicals can accumulate, resulting in oxidative stress (Willcox *et al.*, 2004). Oxidative stress has been related to cardiovascular

disease, cancer, and other chronic diseases that is a major cause of death today. ROS includes free radicals such as superoxide (O_2^-), peroxy radical (ROO^\cdot) as well as non-radical species such as hydrogen peroxide (H_2O_2). Excess accumulation of ROS also leads to several clinical syndromes associated with mtDNA mutations, the most common are NARP (neurogenic muscle weakness, ataxia and retinitis pigmentosa), MELAS (mitochondrial encephalomyopathy lactic acidosis and stroke like episodes), MERF (myoclonic epilepsy and ragged - red fibres), LHON (Leber hereditary optic neuropathy) and KSS (Kearns-Sayre syndrome) (Kirkinetzos and Moraes, 2001). In recent years there is an increasing interest in finding antioxidant phytochemicals to protect the human body from ROS related diseases.

Based on above apprehensions the present work was undertaken for phytochemical screening of *Adhatoda vasica* and *Vitex negundo* and evaluation of their antioxidant potency.

MATERIALS AND METHODS

Plant materials

The fresh tender leaves of *Vitex negundo* and *Adhatoda vasica* were collected from Ranchi ($23^\circ 21' 0''$ N LR, $85^\circ 20' 0''$ E L). 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 and Ground to fine powder (Jonani and Sondhi, 2002).

Phytochemical screening

Preliminary phytochemical screening were conducted on *Adhatoda vasica* and *Vitex negundo* leaf sample with

previously published standards (Trease and Evans, 1989; Harborne, 1984).

Antioxidant activity

The antioxidant properties of plant samples were determined by Spectrophotometric quantitation method (Prieto *et al.*, 1999). Various concentrations of samples (5 µg, 50 µg, 100 µg) were taken in a series of test tubes. The 1.9mL of reagent solution (0.6m Sulphuric acid, 28mm Sodium phosphate and 4mm 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 695nm against blank. Antioxidant capacities were expressed as equivalents of ascorbic acid. Butylated hydroxyl anisole (BHA) was used as reference standard.

Reducing power

Spectrophotometric quantitation (Ferreira *et al.*, 2007) 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 2.5mL of 1 % potassium ferricyanide (10 mg/mL). The mixture was incubated at 50°C for 20min and cooled down. Then 2.5mL of 10 % trichloroacetic added to the test tube and centrifuged at 6500 rpm for 10min. An aliquot (2.5mL) of supernatant was diluted with distilled water (2.5mL) and then ferric chloride (0.5mL, 0.1 %) was added and allowed to stand for 10min. The absorbance was recorded spectrophotometrically at 700 nm. Ascorbic acid was used as standard.

RESULTS AND DISCUSSION

Phytochemicals

The results of phytochemical analysis are presented as Fig. 1. The result reveals highest tannin content in both plants (61.3 ± 1.33 and 93.9 ± 2.33mg/g in *Adhatoda vasica* and *Vitex negundo* respectively) and lowest phenol content (1.3 ± 0.1 and 8.1 ± 0.5 mg/g in *Adhatoda vasica* and *Vitex negundo* respectively). The concentration of all studied phytochemicals was recorder higher in *Vitex negundo* than in *Adhatoda vasica* (Fig. 1) except alkaloids which as higher in *Adhatoda vasica* (11.3 ± 1.2mg/g) than *Vitex negundo* (8.6 ± 1.01 mg/g).

Medicinal properties of plants are due to the secondary metabolites (alkaloids, phenols, tannin etc.) present in different plant parts (Palombo, 2006). The phenols possess redox properties and thus impart antioxidant properties to the plants in which they are present. They act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Cook and Samman, 1996). Flavonoids and tannins are major group of compounds that act as primary antioxidants or free radical scavengers (Polterait, 1997).

Tanins, alkaloids, saponins, flavonoids and sterols have been found to be active against pathogenic bacteris (Kennedy and Wightman, 2011). Thus the leaf of both plants can be used as effective medicines owing to their phytochemical constituents.

Antioxidant activity

The results on antioxidant activity of the plants have been presented as Fig. 2. The total antioxidant activity of the *Vitex negundo* and *Adhatoda vasica* extracts expressed as the mg

of ascorbic acid/100mg, which showed that both samples have good antioxidant capacity. Comparatively higher antioxidant activity (Fig. 2) was recorded for *Vitex negundo* which underlines its suitability as antioxidant supplement. Tiwari and Tripathi (2007) 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 (Sakihama *et al.*, 2002; Puklido *et al.*, 2000). Antioxidant activity of methanolic and hexane extracts of *Cordia wallichii* were examined by Sheikh *et al.* (2009) and he concluded methanolic extract to be more effective (28.2%) than hexane extract (16.7%). 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 methanol was used for the present extraction. Reactive oxygen species (ROS) are involved in the cell growth, differentiation, progression and death. Low concentrations of ROS may be beneficial or even indispensable in processes such as intracellular signalling and defense against micro-organisms. Nevertheless, higher amounts of ROS play a role in the aging process as well as in a number of human disease states, including cancer, ischemia and failures in immunity

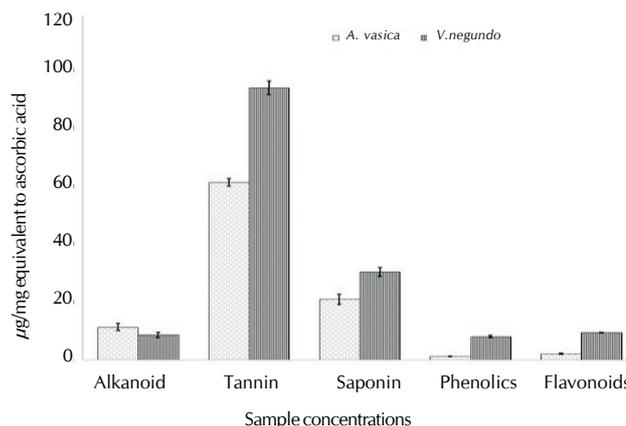


Figure 1: Quantitative analysis of phytochemicals in *Vitex negundo* and *Adhatoda vasica* (values in mg/g; n=3; M ± SD)

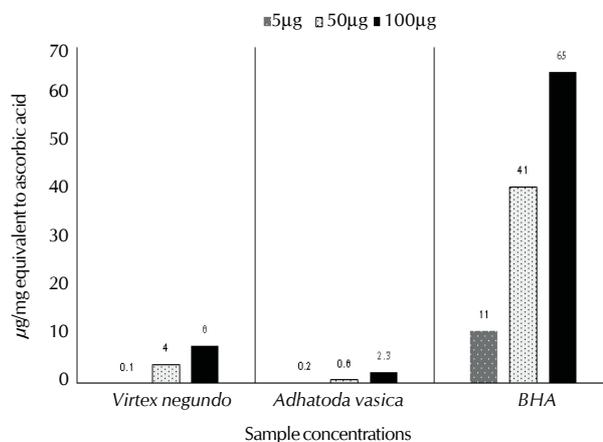


Figure 2: Antioxidant activity expressed in µg/mg equivalent to ascorbic acid

and endocrine functions. As a safeguard against the accumulation of ROS, several non-enzymatic and enzymatic antioxidant activities exist. Therefore, when oxidative stress arises as a consequence of a pathologic event, a defence system promotes the regulation and expression of these enzymes (Mates *et al.*, 1999). Accumulation of ROS results in oxidation of essential proteins which is associated with a number of age-related diseases, including Alzheimer's disease, rheumatoid arthritis, Amyotrophic lateral sclerosis, cataractogenesis, Parkinson's disease, systemic amyloidosis etc. (Halliwell and Gutteridge, 1999; Stadtman, 2004).

Mshvildadze *et al.* (2004) reported that antioxidant activities are directly related to the saponin content. Whereas Rodrigues *et al.* (2005) reported that the beneficial effects of saponin on serum lipids were related to a direct antioxidant activity of saponins. Elekofehinti *et al.* (2012) concluded that *Solanum anguivis* saponins were capable of improving the antioxidant defense in rats. The antioxidant activity of Malaysian *D. grandis* is mainly due to the saponin (18.9mg/g) content of the plant. Satayanshu *et al.* (2013) reported phenolic content in *Vitex trifolia* (74.5 GAE g⁻¹), *T. chebula* (531.5 74.5 GAE g⁻¹), *T. bellerica* (362.5 74.5 GAE g⁻¹), *E. officinalis* (221.6 74.5 GAE g⁻¹), *A racemosus* (10.0 74.5 GAE g⁻¹) and found a linear relation between antioxidant activity and phenolic contents of plants. Melo *et al.* (2010) 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. Van *et al.* (1996) concluded that flavonoids can be used as cardioprotective agents in doxorubicin-induced cardiotoxicity, which is caused by the formation of free oxygen radicals. Benabdesselam *et al.* (2007) concluded that antioxidant activity of *Fumariacar peolata* and *fumaria bastardii* are due to their alkaloid content.

Reducing power

The results of reducing power ability of *Adhatoda vasica* and *Vitex negundo* showed strong reducing power (Fig. 3).

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity of the plants (Oktay *et al.*, 2003). The compounds having reducing power ability act as electron donor and reduce the oxidized intermediates of lipid peroxidation processes (Chanda and Dave, 2009). In the present test the Fe³⁺/ferricyanide complex is converted to ferrous form. By measuring the pearl's Prussian blue at 700nm it was possible to determine the concentration of Fe³⁺ and subsequently the reducing power.

Reducing ability of plant extracts are directly related to the phytochemical content of the extract (Cai *et al.*, 2004). Tannic acid has high reducing power than ascorbic acid and trolox (Pulido *et al.*, 2000). Norhaiza *et al.* (2009) reported positive correlation between reducing capacities and individual antioxidant phytochemicals in the order α – Carotene > flavonoids > Ascorbic acid > Total anthocyanins phenolics. Padma *et al.* (2013) concluded that reducing power of *Imperata cylindrical* may be due to present of tannins (12.53 ± 0.56 mg/g) and phenols (7.09 ± 0.14 mg/g).

Since *Adhatoda vasica* and *Vitex negundo* contains high

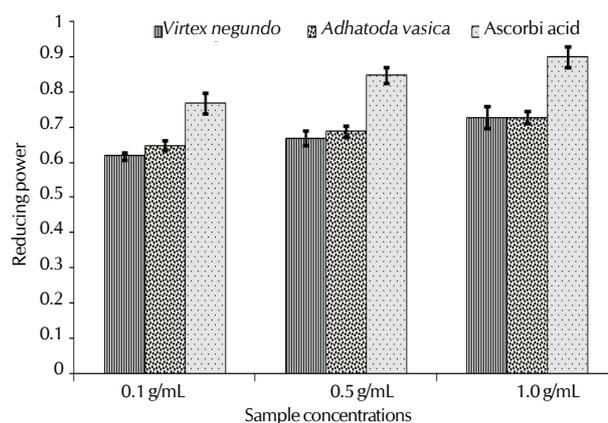


Figure 3: Reducing power of *Vitex negundo* and *Adhatoda vasica* in comparison with ascorbic acid

amount antioxidant phytochemicals (Fig. 1) such as tannins and saponins and sufficient amount of alkaloids and phenols and flavonoids and the plants show strong total antioxidant activity and reducing power (which is likely to be due to their phytochemical contents), therefore the leaf samples of *Adhatoda vasica* and *Vitex negundo* can be used as antioxidant supplements

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