**RESEARCH ARTICLE**

# Antioxidant and antimicrobial properties of *Adhatoda vasica* L. Nees

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**ABSTRACT**

Plant *Adhatoda vasica* L. Nees (Acanthaceae) commonly known as Malabar nut is an evergreen two – three m tall shrub, sometime used as hedge, branches opposite and stem yellowish. Leaves simple, 10-20 cm long and 3 to 7.5 cm or sometime much more broad, elliptical, ovate-lanceolate, and tapering towards apex. Inflorescence terminal or sub terminal spikes, flowers white bilabiate and fruits two-valve capsule, which dehisces when mature, or dry. The plant leaves, bark and root known for traditional medicinal use in Ayurveda. The plant parts generally bitter and useful in cough, bronchitis, asthma, skin disease, eczema and scabies. The leaves extensively employed in preparations indicated in respiratory ointments and particularly in cough syrups. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfer electrons from a substance to oxidizing agent oxidation reaction can produce free radicals, which start chain reaction that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and other oxidation reaction by being oxidize themselves. In present investigation preliminary antioxidants evaluated from the experimental plant and antimicrobial sensitivity test carried out against some human pathogenic microbial strains to support out the potential compounds of pharmacognostic interest.

**Keywords:**Antioxidants, antimicrobial sensitivity, pharmacology

## INTRODUCTION

Antioxidants remarkably occur in plants having potential to protect the plant from severe damage. More danger of free

radicals, plants produces more antioxidant. Antioxidant are nutraceuticals whose deficiency states are associated with variety of dreaded conditions, viz. cardiovascular diseases, diabetic, cataracts, rheumatoid arthritis, Alzheimer's disease and others. The medicinal properties of plants have been investigated in the recent scientific development, throughout the world, due to their antioxidant activities, no side effect and economic viability (Anndy *et al.,* 2003). Flavonoids and phenolic compounds widely distributed in plants which have been reported to excess multiple biological effect, including antioxidant, free radicals scavenging abilities, anti-inflammatory, anti carcinogenic etc. (Miller,1996). They are also suggested to be iron chelator i.e. the novel natural's antioxidant and radicals scavenging properties (Boyer *et al*., 1988: Havsteen 1983) Basic clinical and epidemiological research has suggested a potential protective effect of antioxidant nutrients such as (Vitamin C) Ascorbic acid, Anthocyanin, β carotene, Lycopene, chlorophyll etc. on the risk of cancer cardiovascular diseases and aging (Aviram, 2000). Ascorbic acid is an important antioxidant essential for the normal regulation of the colloidal condition of connective tissue, osteaid tissue, dentine and the intercellular cement substance of the capillaries. It is concerned in the hydroxylation of proline and hydroxyproline as an important constituent of collages. Severe ascorbic acid deficiency produces scurvy. Lycopene is a powerful antioxidant that categorically retards damage caused to DNA and protein. Lycopene offers distinctly and appreciable much better skin protection against the UV-light than beta-carotene. It especially accumulated in the various segments of human body viz. skin, adrenal gland, prostrate glands, testes etc. It also renders adequate protein against cancer. Lycopene noticeably arrest the insulin like growth factor-1 stimulation of cancerous growth (Xianquan *et al*., 2005). In recent year, numerous studies have shown that anthocyanin displays a wide range of biological activities including antioxidant, anti-inflammatory, anti-microbial and anticarcino-genic activities, improvement of vision, induction of apoptosis and neuroprotective effects. In addition, anthocyanin displays a variety of effects on blood vessels and platelets that may reduced the risk of coronary heart diseases (Mazza*,* 2007). Chlorophyll is nowadays a solution of many problems like vitamin deficiency, pollution after effect, countering heart and diabetic diseases. It prescribed in the form of leaf juices, liquid chlorophyll, chlorophyll tablets and leafy pastes. This all due to biochemical nature of chlorophyll which on dissociation and substitution reaction gives rise to various essential organic compound like hemoglobin and vitamin B12 (Ursula *et. al.,* 2005). Phytol tail of chlorophyll dissociates into two beta- carotene molecule which is a precursor of vitamin A. Hence, plant pigments especially of medicinal plants acts as sources of antioxidant (Hsu *et al*., 2013). *Adhatoda vasica* reported to prevent oxidative damage of carbon tetrachloride induced hepatotoxic effect in rats (Pandit *et al*., 2004). Maurya and Singh, (2010) accounted highest amount of phenolic compounds which scavenging the free radicals and exhibits greatest antioxidant activity. The positive effect of gamma irradiation on the natural antioxidants of *Justica Adhatoda* showed release of phenolic compounds (Rajurkar *et al.*, 2012). The ethanolic extract of *A. vasica* showed high antioxidant activity with cytoprotective potential in cell culture (Mamta *et al.*,2013). Interestingly, the methanolic and aqueous extract of *A. vasica* has potential phytochemical composition of flavonoids, phenols with antioxidant and cytotoxic effect (Rao *et al.*, 2013). Recently, (Kumar *et al.*, 2014) evaluated pharmacological screening of leaf extract of *A. vasica* against dysentery and diarrhea due to presence of chemical compounds tannins, alkaloids, saponins and flavonoids.

## MATERIALS AND METHODS

Fresh leaves, stem and root are metabolically active parts of the plant and site of synthesis for many chemical compounds hence chosen for analysis. Plant materials collected from Melghat

forest areas brought laboratory cleaned and preserved. Estimation of antioxidants like (Vit-C) Ascorbic acid, Anthocyanin, Lycopene, Chlorophyll from plant materials carried out as per the protocols of Thimmaiah (1999).

The plant parts cut in small pieces, cleaned carefully and washed under tap water to remove impurities followed by shade drying. Dried plant parts crushed in blender, powdered and preserved in airtight bottles. Soxhlet extraction process followed in petroleum ether ethanol, methanol, and acetone and different solvent fractions obtained. Dried extracts were stored in labeled sterile wide mouthed screw capped bottles at $4^0$C and used for further study (Parekh and Chanda, 2008). The standard pathogenic bacterial and fungal strain obtained from Microbial Type Culture Collection and Gene Bank (IMTECH), Chandigarh, India. The bacteria rejuvenated in Nutrient broth (Hi-media laboratories, Mumbai, India) at $37^0$C for 18 hrs and then stored at $4^0$C on Nutrient agar. The fungal organisms were sub cultured on Sabaroud's dextrose agar. Four bacterial strains like gram-negative *Proteus vulgaris* (MTCC-744), *Shigella flexneri* (MTCC-1457), *Salmonella typhimurium* (MTCC-98), gram-positive *Staphylococcus aureus* (MTCC-96) and one fungal pathogen *Aspergillus niger* (MTCC-28) were selected. Disc diffusion method was used for the antibacterial sensitivity test by following the standard methods (NCCLS, 1990). The results were compared with the standard bacterial antibiotics like (10 µg/ml) Tetracycline and Nystatin for fungi.

## RESULTS AND DISCUSSIOIN

### Determination of antioxidants

Antioxidants mostly known to protect our body from the formation of free radicals. Ascorbic acid is not synthesized in human being and dietary or oral consumption only provide this vitamin. The high quality of ascorbic acid was found in fresh leaves of *Adhatoda vasica* showed 1200 µgm of ascorbic acid content (Table- 1). The normal human body when fully saturated contains about 5000 mg of vitamin C, at which 30mg found in adrenal glands, 200mg in extra cellular fluids & really distributed in varying concentrations throughout the cells at the body. (Danne, 1990). Lycopene is one of the over 600 or more carotenoids pigments. Some studies reported that lycopene could inhibit the growth of cancer and endometric cancers (Rao and Agarwal 2000). The moderate lycopene value of 0.84 µgm found in *Adhatoda vasica* (Table -1). The moderate quantity of anthocyanin was found in fresh leaves of *Adathoda vasica* i.e. 62.25 µgm. The antioxidant activity (Scavenging free radicals metal chelation; protein binding) of anthocyanin including the protection of LDL against oxidation has been demonstrated in a number of *In vitro* systems. (Aviram, 2000) The total chlorophyll content in *Adhatoda vasica* found 0.60 µmg from the fresh leaves of the plant (Table-2). Chlorophyll has anti inflammatory, antioxidant and wound healing properties. It is efficient delivery of magnesium helps the blood to carry oxygen to cell and tissues. Chlorophyll also removes carbon dioxide and carbon monoxide, and has been found to

**Table 1: Observations for *Adhatoda vasica***

| Sr. No | Name of the compound | Plant part taken for analysis | Weight of plant part | Vol. of extract | Vol. of extract taken for analysis | Absorbance (nm) | Value found in µgm |
|---|---|---|---|---|---|---|---|
| 1. | Ascorbic acid | Leaves | 1 g | 10 ml | 1ml | 0.193 | 1250 µgm |
| 2. | Lycopene | Leaves | 1 g | 10 ml | 1ml | 0.028 | 0.87 µgm |
| 3 | Anthocyanin | Leaves | 2g | 10 ml | 1ml | 0.249 | 62.25 µgm |
| 4. | Chlorophyll | Leaves | 1g | Total chlorophyll | | 0.545 | 0.555 µmg |

**Table 2: Preliminary antimicrobial sensitivity test of *Adhatoda vasica***

| Sr. No. | Solvent Extract | Zone of Inhibition [mm] | | | | |
|---|---|---|---|---|---|---|
| | | ***Proteus vulgaris* [MTCC-744]** | ***Shigella flexneri* [MTCC-1457]** | ***Staphylococcus aureus* [MTCC -96]** | ***Salmonella typhimurium* [MTCC -98]** | ***Aspergillus niger* [MTCC-281]** |
| 1. | Petroleum Ether | 08 | 06 | 09 | 09 | 15 |
| 2. | Ethanol | 12 | 11 | 10 | 15 | 17 |
| 3. | Methanol | 09 | 08 | 10 | 11 | 13 |
| 4. | Acetone | 09 | 13 | 07 | 11 | 12 |
| 5. | Tetracyclin [control] | 27 | 29 | 34 | 30 | - |
| 6. | Nystatin [control] | - | - | - | - | 31 |

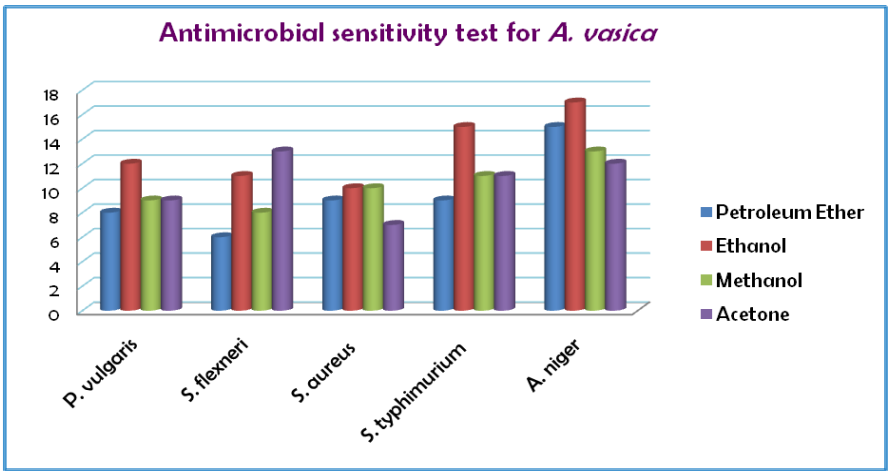


**Fig. 1: Analysis of antimicrobial sensitivity test**

reduce fecal, urinary, and body odor. Chlorophyll may reduce the binding of carcinogens to DNA in the liver and other organs (Hsu *et al.*, 2013).

**Antimicrobial sensitivity of *Adhatoda vasica***

The various types of extract showed consistently positive result against maximum microbial pathogens (Table 2, Fig. 1). The petroleum ether extract of the plant exhibited significant interaction with fungi *Aspergillus niger* with 15mm zone and less with *S. flexneri* pathogens. Subsequently, the ethanol extract found much sensitive with positive results against microorganisms like *P. vulgaris, S. aureus, S. typhimurium and A. niger* with maximum zone of 17 mm. The greenish black coloured extract of methanol was found sensitive to various pathogens with positive interaction like *P. vulgaris, S. aureus, S. typhimurium* and *A. niger* with 13mm zone but less reactive to *S. flexneri* with 8mm zone (Rao *et al.*, 2013) The acetonic extract of the plant showed least response against microorganisms *P. vulgaris* and *S. aureus,* and moderate active against *S. flexneri S. typhimurium* while greatest against fungi *A. niger* with zone 12mm (Table-2, Fig -1). The ethanol and methanol extract of the plant found more sensitive to all the pathogens as compared to the, petroleum ether and acetone extract. As compare to bacterial strains the fungal strain *A. niger* showed highest and remarkable antifungal sensitivity (Mamta *et al.*, 2013).

**CONCLUSION**

From the analysis and results it revealed that *Adhatoda vasica* is important medicinal plant with rich antioxidant potential and antimicrobial

sensitivity against pathogenic microorganisms. Its noteworthy that the conventional drugs more sensitive to gram positive bacteria (*S. aureus*) but in present investigation the extracts were more sensitive to gram negative bacteria (*P. vulgaris, S. typhimurium,* and *S. flexneri*). Hence, besides conventional drug practice more advance exploration needed for pharmacognostic uses.

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