

**PHARMACOGNOSTIC STUDIES ON MEDICINAL PLANTS:
*JUSTICIA ADHATODA.***

Alka Sharma¹ and Ashwani Kumar^{2*}

¹Department of Botany University of Rajasthan, Jaipur 302004.

²Alexander Von Humboldt Fellow and Former Head of Department of Botany, University of Rajasthan, Jaipur 302004.

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***Corresponding Author**

Ashwani Kumar

Alexander Von Humboldt
Fellow and Former Head
of Department of Botany,
University of Rajasthan,
Jaipur 302004.

ABSTRACT

The nature has provided a complete storehouse of remedies to cure ailments of mankind. About 80 per cent of the world's population depends partially or wholly on traditional medicine for its primary health care needs. Herbal medicines as the major remedy in traditional medical system have been used in medical practice for thousands of years and have made a great contribution to maintain human health. The global interest in study and practice of crude drugs has therefore, considerably increased during the last two decades because of growing awareness about the toxicity and side effects of synthetic drugs, their limitations in many areas of therapy, comparatively high cost and often drug beyond the reach of common man. Worldwide interest on herbal

medicine has gained momentum therefore, the standardization of herbal drugs is the most desirable at this time. Globally, a large population utilizes traditional medicines. Therefore, herbal medicines are rapidly increasing in economic importance. Thus pharmacognosy appears to be of great value in identification of commercial samples of the market to find their authenticity and establishing identity of adulterant or substituent. Besides lack of standardization, unscrupulous commercial practice of adulterating and substituting the genuine herbal drugs are posing great hurdle in popularizing the time-tested herbal-based traditional medicines. The number of medicinal formulations developed by Vaidyas has a positive correlation with number of diseases to be tested and *Justicia adhatoda* is used in treating diverse ailments.

KEYWORDS: crude drugs, traditional medicine, herbal medicine.

1. INTRODUCTION

The term pharmacognosy is derived from two Greek words 'Pharmacon' meaning drug or medicine and 'gnosis' meaning knowledge. This term was first coined by C.A. Seydler in his dissertation entitled '*Analectapharmacognosia*' in 1895. Herbal medicines as the major remedy in traditional medical system have been used in medical practice for thousands of years and have made a great contribution to maintain human health (Kunwar and Adhikari, 2005; Emeka and Elizabeth, 2009).

It is estimated that roughly 1500 plant species in Ayurveda, 1200 plant species in Siddha have been used for drug preparation (Jain, 1987). Though the Indian traditional systems of medicine are time-tested and practised successfully from times immemorial, there is lack of standardization with regard to identification of crude drugs, methods of preparation and quality of finished products. Recently pharmacognostical studies has been carried out on large number of plants *viz.* *Astercantha longifolia* (Wahi *et al.*, 1974), *Andrographis paniculata* (Trivedi and Rawal, 2001), *Butea monosperma* (Srivastava *et al.*, 2002), *Catheranthus roseus* (Heijden *et al.*, 2004), *Pedaliium mures* (Shukla and Khanuja, 2004), *Ageratum conyzoids* (Moka *et al.*, 2005), *Barleria prionitis* (Chen *et al.*, 2006), *Phyllanthus* spp. (Khatoon *et al.*, 2006), *Pogostemon* (Deguerry *et al.*, 2006), *Salvia* (Kamatou *et al.*, 2006, 2008), *Mentha* spp. (Gulluce *et al.*, 2007), *Thymus* spp. (Rota *et al.*, 2008), *Hibiscus sabdaritta* (Suhad and Viorica, 2008), *Rosmarinus* spp. (Bousbia *et al.*, 2009), *Ocimum* spp. (Hussain *et al.*, 2008, 2010), *Vasakhanda kushmandaka* (Ghosh *et al.*, 2012), *Adhatoda vasica* (Siddiqui and Hussain, 1993; Paliwa *et al.*, 2000; Shrivastava *et al.*, 2001; Choudhary *et al.*, 2009; Dhankhad *et al.*, 2011; Rashmi *et al.*, 2012; Kumar *et al.*, 2013a, 2013b, 2013c and Gangwar and Ghose, 2014).

The leaves of *Adhatoda vasica* and its adulterant *Ailanthus excelsa* Roxb. were distinguished on the basis of palisade ratio and anatomical characters of leaf and petiole by Satakopan and Thomas (1970). *Mucunacochin chinensis* is the adulterant of the Unani drug 'Karanj' (Genuine: *Pongamia pinnata* L. Pierre). and it was established with the use of fluorescence analysis and other pharmacognostical parameters.

2. Identification of crude drugs.

Metcalf and Chalk (1957) stated that microscopical methods are often necessary to establish the botanical identification of commercial samples of medicinal plants, timbers, fibres etc.

and may play an important part in checking adulteration and substitution. It involves examining longitudinal and transverse sectional views.

Mehrotra and Sharma (1984) analyzed the various market samples of the Ayurvedic drug 'Sappan' and compare with its genuine drug *Caesalpinia sappan* L. using morphological and anatomical parameters, they established the genuineness of the drug. Yamaji *et al.* (1993) after studying the anatomical characters of flower stalk and xylem vessels of rhizome established that the drug 'Spang-RtziDo-Do' is evolved from *Pterocarpus hookeri*. Park *et al.* (1995) studied the market samples of 'Man Byung Cho' based on morphological and anatomical characters of leaf midrib and leaf lamina, concluded that they belong to the leaves of *Rhododendron brachycarpum* var. *roseum*.

Shah and Khanna (1961) distinguished the fruits of *Embelia ribes* with its grayish black colour and warty surfaces with that of *E. robusta*, which has reddish, longitudinally wrinkled surface and more prominent calyx with five sepals. Chakraborti *et al.* (1988) studied the stem barks of *Strychnosnux-vomica* Linn. and *S. potatorum* Linn. and distinguished the authenticity of 'Nux-vomica' bark from other barks.

2.1. Microscopic identification

Microscopy of the powder is another parameter used to identify and distinguish the drug from its substitutes and adulterants. The *Saraca asoka* (Roxb.) De Wilde. bark from its adulterants is distinguished by the analysis of the powder and put forth a key for the identification of the 'Ashoka' bark powder. Srivastava and Srivastava (1988) identified the adulterants of *Catharanthus roseus* Linn. by the analysis of powdered drug.

3. Estimation of alkaloids

Percentage of active constituent of a medicinal plant is influenced by geographical and climatic factors. Liu *et al.* (1993) estimated the alkaloid content of three species of *Phellodendron* and distinguished with one another based on quantity of alkaloid, texture and colour of the herbs. Dadun and Jain (1992) concluded that the percentage of alkaloid contents of *Ephedra sinica* Stapf. is dependent upon the season and geographical locality in which the plant is grown. the oil yield of *Olearia phlogopappa* (Labill) DC. is higher in summer months than in autumn. Essential oil yield in *Mentha spicata* was more in summer than plants grown in winter (Hussain *et al.*, 2008). Plants are used by tribal communities for various ailments in different parts of India (Katewa and Arora, 1997; Katewa and Guria, 1997 and Katewa *et al.*,

2004). However, a proper documentation of medicinal plants is lacking and many times adulterants are passed as genuine drugs.

4. Therapeutic Applications of *Justicia adhatoda*

The plant *Adhatoda vasica* has been used in the indigenous system of medicine in India for more than 2000 years (Atal, 1980). It is an official drug and mentioned in the pharmacopoeia of India. The plant has been included in WHO manual "The use of traditional medicine in primary health care" to profit health workers. There are hundreds and thousands of constituent that all work together against the diseases unlike allopathic system.

Saponins present antiprotozoal, antifungal and antiviral activities, as well as cytostatic effects on various cancer cells, lower serum cholesterol, stimulation of cell-mediated immune system and enhancement of antibody production, as demonstrated in experimental animals (Francis *et al.*, 2002). Tannins have biological activities related to their capacity of protein precipitation and astringent propriety, which has led to their use as antidiarrhoeal, antiseptic and wound sealant, as well as marked antimicrobial, antifungal and antiviral activities (Monteiro *et al.*, 2005). On the other hand, different coumarins showed antioxidant, anticoagulant, anti-inflammatory and analgesic activities, among many other biological effects (Blanco, 2011). The presence of these compounds therefore, suggests good pharmacological potential for *Justicia* species.

4.1 Expectorant, bronchodilator and liquefying sputum activity

The drug obtained from this plant is used in cough, cold and pulmonary infection as expectorant and bronchodilator. It liquefies sputum and provide relief (Dimock *et al.*, 1890; Amin and Mehta, 1959; Lahiri and Pradhan, 1964; Kirtikar and Basu, 1975; Nadkarni, 1976; Atal, 1980; Lal and Yadav, 1983; Sharma *et al.*, 1992; Pushpangadan *et al.*, 1995; Mahrotra, 1996; Dhule, 1999; Ahmad and Javed, 2007; Kumar *et al.*, 2010; Thokchom *et al.*, 2011; Yadav *et al.*, 2011 and Jha *et al.*, 2012).

4.2 Anti-allergic and anti-asthmatic activity

Vasicine and Vasicinone are two alkaloids from the drug of this plant that exhibit anti-allergic and anti-asthmatic activity (Shah and Joshi, 1971; Jain and Verma, 1981; Wagner, 1989; Muller *et al.*, 1993; Mahrotra, 1996; Paliwal *et al.*, 2000; Ahmad *et al.*, 2007; Khan and Yadav, 2010 and Thokchom *et al.*, 2011).

Asthma is caused by inflammation caused by over expression of pro-inflammatory immune response, predominantly by eosinophils and lymphocytes. Rayees *et al.* (2014) worked on anti-asthmatic activity of azepino (2.1-b) quinazolones, the synthetic analogues of vasicine, an alkaloid from *Adhatoda vasica* and 10 azepino [2,1-b] quinazolone derivatives (R1-R10) were synthesized and evaluated for their anti-asthmatic activity using murine model of asthma. The compounds R₂, R₄, R₆, R₇ and R₈ caused a notable decrease. Th 2 cytokine secretion and eosinophilia in asthma-induced animals. However, decrease was highly significant in case of R₈-treated animals. The pharmacokinetics of R₈ was carried out in mice after oral and intravenous administrations.

4.3 Antitubercular Activity

Adhatoda has diverse medicinal activity including antitubercular activity (Jain and Puri, 1984; Barry *et al.*, 1955; Sunita and Singh, 2010; Kumar *et al.*, 2010 and Victoria, 2010). Activity of bromhexine and ambroxol, semi-synthetic derivatives of vasicine from the Indian shrub *Adhatoda vasica*, against *Mycobacterium tuberculosis in vitro* has been reported by Grange and Snell (1996); Jha *et al.* (2012) reported that the enzyme b-ketoacyl-acyl-carrier protein synthase III that catalyses the initial step of fatty acid biosynthesis (FabH) via a type II fatty acid synthase has unique structural features and universal occurrence in *Mycobacterium tuberculosis*. Thus it was considered as a target for designing of anti-tuberculosis compounds. The combination of docking/scoring provided interesting insights into the binding of different inhibitors and their activity. These results will be useful for designing inhibitors for *M. tuberculosis* and will be a good starting point for natural plant-based pharmaceutical chemistry and can be a good arsenal to arrest infection at their initial stage. Shobana *et al.* (2014) reported that crude and step gradient extracts of *A. vasica* showed significant activity against *M. tuberculosis*.

4.4 Abortifacient and easy child birth delivery

Justicia adhatoda has abortifacient and uterotonic activity, making it useful for inducing abortion and for stimulating uterine contractions for speedy childbirth (Kirtikar and Basu, 1975; Pahwa *et al.*, 1987; Nath *et al.*, 1992; Claeson *et al.*, 2000 and Hussain and Hore, 2007).

4.5 Radioprotective Activity

Pretreatment with *Adhatoda vasica* significantly prevented radiation induced chromosomal damage in bone marrow cells of swiss albino mice, suggests that *Adhatoda* plant extract has

significant radio protective effects (Kumar *et al.*, 2005, 2007 and Sharma *et al.*, 2009). Chincholkar *et al.* (2012) fed mice with 500mg/kg and 1000 mg/kg body weight of ethanolic extract of *A. vasica* prior to 9 gray g-radiation exposure. They observed significant decrease in the frequency of chromosomal aberrations as well as the micronucleus possessing cells in the groups pretreated with 1000 mg/kg body weight *A. vasica* extract as compared to mice which were not administered extract prior to exposure.

Kaur *et al.* (2013) worked on RBC, WBC and Hb counts and enzymatic SOD activity in Albino mice. They observed that there was decrease in RBC, WBC, Hb counts and SOD enzymatic SOD activity, in case of groups of mice administered with the dose of extract of *A. vasica* before gamma radiation than those which were exposed without administration of the dose. These results substantiate the medicinal effect on blood count and enzymatic activity.

4.6 Hepatoprotective Activity

Bhattacharya *et al.* (2003 and 2005) have suggested hepatoprotective activity of *Adhatoda vasica* aqueous leaf extract on d-galactosamine-induced liver damage in rats. Pandit *et al.* (2014) have evaluated the antioxidant effect of *Adhatoda vasica* in carbon tetrachloride (CCl₄) induced hepatotoxicity in rats and suggested that hepatoprotective effect might be at least partly due to its antioxidant effect.

4.7 Insecticidal Activity

Dymock *et al.* (1890), Gamble (1922) and Driberg (1935) reported that extract of leaves of *Adhatoda vasica* is antifeedant and toxic to cell forms of lower life, white ants, flies and mosquitoes suggesting its insecticidal activity. Sadak (2003), studied the effect of crude methanolic extract on *Spodoptera littoralis* larvae. The mortality rate of larvae was 100 per cent when fed on fresh leaves after 26 days of unsubstantial growth. Al-shaibani *et al.* (2008) reported ovicidal and larvicidal properties of *Adhatoda vasica* extract against gastrointestinal nematodes of sheep *in vitro*.

4.8 Wound healing Activity

Several studies show wound healing property of *Adhatoda vasica* leaves extract (Dimock *et al.*, 1890; Bhargava *et al.*, 1988 and Vinothapooshan and Sundar, 2010).

4.9 Anti-ulcer Activity

Anti-ulcer activity of *Adhatoda vasica* is reported by Shrivastava *et al.* (2006) and Vinothapooshan and Sunder (2011a). *Adhatoda vasica* leaf powder showed a considerable degree, around 80 per cent, of anti-ulcer activity in rats by ethanol extract in comparison to pylorus and aspirin.

4.9 Malarial fever Relieving Activity

Leaf juice of *Adhatoda vasica* is prescribed for relief in malarial fever (John, 1984 and Manandhar, 1991).

4.10 Sucrose Inhibitory Activity or Antidiabetic Activity

The methanolic extract from the leaves of *Adhatoda vasica* showed excellent sucrose inhibitory activity with sucrose as a substrate. The alkaloids, vasicine and vasicinol, inhibited sucrose activity, with an IC_{50} value of 125 mM and 250 mM respectively. Thus *A. vasica* can be explored as a natural antidiabetic agent (Hong *et al.*, 2008).

4.11 Antiinflammatory Activity

Vasicine, one of the alkaloids of *Adhatoda vasica*, showed anti-inflammatory activity against lung damage in rats (Chakraborty and Bratner, 2001 and Shrinivasarao *et al.*, 2006).

4.12 Allelopathic Activity

Mitra and Prasad (2010) studied allelopathic activity of *Adhatoda vasica*. Its aqueous leaf and flower extracts had stimulating effect on seed weight of turnip.

4.13 Antimutagenic Activity

Prophylactic pretreatment of *Adhatoda vasica* extract in cadmium intoxicated mice showed marked ($p < 0.001$) inhibition of lipid peroxidation (LPO) and Xanthin oxidase (XO) activity (Jahangir *et al.*, 2006).

4.14 Antibacterial Activity

The leaves of *Adhatoda vasica* possess moderate antibacterial activity (Panthi and Chakraborty, 2006; Ilango *et al.*, 2009; Karthikayan *et al.*, 2010; Kumar *et al.* (2011); Thokchom *et al.*, 2011; Josephin and Mohan, 2012 and Sheeba and Mohan, 2012). Sarker *et al.* (2009) tested its antibacterial activity against *Bacillus subtilis* and *Vibrio cholera* while Karthikayan *et al.* (2010) tested against *Staphylococcus aureus*, *S. epidermidis*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*,

Klebsiella pneumonia and *Candida albicans*. Kaur *et al.* (2012) investigated the *in vitro* antimicrobial activity of *A. vasica*. *In vitro* screening showed a strong activity of Adhatoda's alkaloids against the bacteria *Staphylococcus aureus* and significant antimicrobial activity against bacterial strains *Streptococcus pneumonia*, *Escherichia coli* and *Klebsiella pneumonia*.

4.15 Anthelmintic Activity

Extract of roots of *Adhatoda vasica* (Ahmad and Javed, 2007), and juice made from its bark and leaves show anthelmintic activity (Rahman *et al.*, 2008).

4.16 Modulatory Activity

Shinawie (2002) and Vinothapooshan and Sunder (2011b) have suggested modulatory activity of *A. vasica* extract.

4.17 Gonorrhoea

Extract of fresh flowers are used for treatment of gonorrhoea (Manandhar, 1991 and Hussain and Hore, 2007).

4.18 Rheumatism

Paste prepared from leaves of *A. vasica* relieves pain of rheumatism (Rao and Jamir, 1982 and Kumar *et al.*, 2010).

4.19 HIV-Protease inhibitor Activity

Singh *et al.* (2010) evaluated the efficacy of crude drug of *A. vasica in vitro*. Pepsin Assay as a substitute of HIV-protease was used for screening HIV-protease inhibition. The crude drug of *A. vasica* exhibited potent inhibitory activity of enzyme Pepsin. They concluded that it might be a potent inhibitor of HIV-protease.

4.20 Anti-typhoid Activity

Kumar *et al.* (2013a) have reported the loss of antioxidant system during infection of *Salmonella typhi*. The leaf extract of *A. vasica* and *Vitex neugundo* showed considerable antioxidant activity. Besides antioxidant activity, the leaves of both the plant inhibit growth of *S. typhi*. The present study shows that leaf extracts of *A. vasica* and *V. neugundo* confer anti-typhoid activity. Sawant *et al.* (2013), Ullah *et al.* (2013), and Shobana *et al.* (2014) also confer anti-typhoid activity of *A. vasica*.

4.21 Renal Protective Activity.

According to Kumar *et al.* (2013b) Gentamycin is a potent broad spectrum antibiotic used in a diverse infective condition. In renal infection, when prescribed, causes acute renal damage. The beneficial effect of *A. zeylanica* against gentamycin nephrotoxicity, possibly depends on its ability to scavenge the gentamycin induced free radicals. So the plant has the potential to ameliorate gentamycin nephrotoxicity.

4.22 Informant consensus Factor (ICF) and Relative Importance of Plant Use (UV).

For data analysis, informant Consensus Factor (ICF) (Heinrich *et al.*, 1998 and Gazzaneo *et al.*, 2005) was employed to indicate how homogenous the information is. All citation were placed into ailment categories for which the plant were chosen randomly, or if informants do not exchange information about their use. Values will be high (near 1) if there is a well defined selection criterion in the community and/or if information is exchanged between informants.

The ICF was calculated as in the following formula.

$$ICF = \frac{Nur - Nt}{Nur - 1}$$

Where

Nur = Number of used citation in each category,

Nt = Number of species used.

5. RESULTS

Informant consensus Factor of *Adhatoda vasica* Nees. (Synonym : *Justicia adhatoda*, *Adhatoda zeylanica*) of different ailments is given in Table 4.1. During the present survey, ninety informants were consulted for 22 diseases. The information is mainly available for *Adhatoda vasica* Nees. species and informant consensus factor is near 1.0 for all the ailments surveyed.

The use value (UV), a quantitative method that demonstrates the relative importance of species known locally, was also calculated according to the following formula:

$$UV = \frac{\sum U}{N}$$

Where

UV = the use value of a species,

SU = The number of citation per species,

N = The number of informants.

$$= \frac{\text{The No. of citations / species}}{\text{The No. of informants}}$$

$$= \frac{77}{232} = 0.33$$

The use value of *Justicia adhatoda* Nees. is 0.33.

During the present investigation *Justicia adhatoda* has been selected for its pharmacognostical studies due to its medicinal importance.

Vasicine molecule, responsible for multiple therapeutic activity, was first isolated in 1924 by Sen and Ghose (Sen and Ghose, 1924). The plant *Justicia adhatoda* (Acanthaceae) is the main source of this molecule. It is a quinazoline type of alkaloid. In addition to vasicine, the plant also contains alkaloids 1-vasacinone deoxyvasicine, maiontone, vasicinolone and vasicinol (Rachana *et al.*, 2011 and Gangwar and Ghosh, 2014). Kumar *et al.* (2013c) reported that *A. vasica* is a rich source of phenolic compounds, flavonoids which are responsible for strong anti-oxidant properties that help in prevention and therapy of various oxidative stress related diseases. They also evaluated physicochemical and inflorescence analysis (Table 1).

Table-1: Informant Consensus Factor of *Justicia adhatoda* Nees. categorized by medicinal use for corporal ailments.

S.No.	Ailments and Activities	No. of species used	No. of citations used	informant consensus
1.	Expectorant bronchodilator	1	16	1.0
2.	Anti-allergic anti-asthmatic	1	10	1.0
3.	Antitubercular	1	8	1.0
4.	Abortifacient and easy childbirth	1	5	1.0
5.	Radioprotective	1	5	1.0
6.	Anti-bacterial	1	10	1.0
7.	Insecticidal	1	6	1.0
8.	Wound healing	1	4	1.0
9.	Hepatoprotective	1	3	1.0
10.	Anti-ulcer	1	2	1.0
11.	Malarial fever	1	2	1.0
12.	Anti-diabetic	1	1	1.0

13.	Anthelmintic activity	1	2	1.0
14.	Modulatory activity	1	2	1.0
15.	Gonorrhoea	1	2	1.0
16.	Rheumatism	1	2	1.0
17.	Renal protective	1	1	1.0
18.	HIV-protease inhibitor	1	1	1.0
19.	Allelopathic	1	1	1.0
20.	Anti-mutagenic	1	1	1.0
21.	Anti-inflammatory	1	2	1.0
22.	Anti-typhoid	1	4	1.0

Adhatoda vasica Nees. with multitude of uses carries great potential to be developed as a drug by the pharmaceutical industry (Gangwar and Ghosh, 2014).

Developing new dosage forms without disturbing the basic principle of *Justicia adhatoda*, is a present day need. Vasa Candy is a new more stable, palatable dosage prepared from *A. vasica* and *Piper longum* (Yadav *et al.*, 2014). Adusa cough syrup, Avaleha, Vasaka from Himalaya and Vasaka from WOPL and Adusa tablets are some of the important drugs prepared from *A. vasica*.

In Ayurvedic preparations, *Vasaka* leaf juice (*Vasa swarasa*) is incorporated in more than 20 formulations including *Vasarishtha*, *Mahatiktaka ghrita*, *Triphala ghrita*, *Vasavaleha*, *Vasakasava*, *Mahatriphalaghrita*, *Panchatiktaghritaguggulu* and *Panchatikta ghrita*.

Adhatoda vasica is widely used in traditional medicine all over the world. Recently various workers in India as well as abroad have been carried out pharmacognostical studies on *A. vasica* (Rachana *et al.*, 2011; Kumar *et al.*, 2013c; Gangwar and Ghose, 2014 and Yadav *et al.*, 2014).

6. MATERIALS AND METHODS

Plant description was collected from the standard floras and samples were compared with specimens kept in University of Rajasthan Herbarium.

7. Morphology

A dense shrub 1.2-2.4 m., sometimes arborescent; leaves : elliptic, acute at both ends, entire, minutely pubescent, 0.2×7.5 cms; Spike: 2.5 to 7.5 cm long, dense; bracts: 2-7.5 cm, elliptic, ovate, subacute, puberulus or glabrous; bracteoles : 0.25×0.37 cm, oblong; calyx: 0.8-1.25 cm. deeply 5 lobed, lanceolate; corolla: corolla tube 0.8 to 1.25 cm, 3 cm broad, long lip white, palate transversely rare barred; stamens : glabrous, anther cells acuminate at the base

sometimes minutely white tailed; carpel : ovary and style base minute hairy; capsule : 2 cm, long, clavate pubescent, 4 seeded; seeds: 0.5 cm. in diam., glabrous, tuberculate, verrucose.

8. Macroscopic Characters

8.1 Stem.

Cylindrical excepting at the nodes where it is somewhat flattened and swollen and angular. The outer surface of the stem in the lower region is smooth excepting a few scattered lenticels, but in the upper region, it is minutely puberulent. Externally the bark has a grayish white colour, which become light brown in older stems and internally it has a light yellow colour. The internodes vary, from 2-8 cm in length particularly in the upper half part of the stem. At the nodes, the leaves are arranged opposite and decussate and the branches are ascending. Odour is characteristic and tastes somewhat bitter.

8.2 Leaves

The leaves are simple, petiolate and exstipulated. Young leaves are about 5-12 cm long and 1.5 to 5 cm broad with petiole 1.5 to 2.0 cm in length. They are lanceolate, light green in colour and puberulous. The mature leaves are however, 11-20 cm long and 6-10 cm broad. Somewhat glabrous and mostly elliptic lanceolate to ovate-lanceolate. They have slightly crenate or entire margin, a tapering base and an acuminate apex. Dorsal surface is green in colour but the ventral one is slightly pale. There are 8-12 pairs of bilateral veins which are reticulate.

8.3 Flowers

Flowers are found in short, dense, axillary, peduncles; peduncles being 3 to 9 cm long, stout and arranged almost towards the branches. Flowers are subsessile each accompanied by one big bract and two small bracteoles. The bracts attached to the lowermost flowers are ovate, subacute, 1.5 to 4.5 cm by 1.0 to 3.0 cm., 5-7 nerved which are reticulated and the bracteoles are 0.5 to 1 cm by 0.3 to 0.6 cm ovate- lanceolate, acute, one nerved, puberulent and ciliolate hairs. The lower most flowers numbering 2 to 4 are open on the spike while the rest are small and in the bud condition. Accordingly the size of the bracts and bracteoles accompanying the upper flower is smaller. Calyx is 6-12 mm long, campanulate, 5-fid, slightly pubescent, oblong-lanceolate, acute 3-nerved.

Corolla is 2.0 to 3.0 cm long with white pink or purple stripes on the throat. Corolla tube is short, 6-12 mm long with its lower half cylindrical, 3-4 mm in diameter and upper half

laterally inflated and the limb is bilabiate, the posterior being erect and anterior, broad and recurved. Filament attached at the base, long and stout each with 2 large diverging anther cells one much higher than the other, the lower one being apiculate at the base. Ovary and style base are pubescent. Capsules 1.5-2.4 cm by 0.5-0.7 cm, clavate and minutely hairy and 4 seeded. Seeds are 4-6 mm by 4-5 mm, glabrous, sub-orbicular, tubercular and verrucose.

8.4 Microscopical Characters

8.4.1 Stem

A transverse section shows the presence of both glandular and non-glandular trichomes; the former being present in small depressions of the epidermis. Following the epidermis is a band of collenchyma of 8-10 layers of cells and a wide zone of parenchyma consisting of 12-15 layers of cells. The stem possesses a siphonostele which encloses a wide central pith. Starch grains, calcium oxalate crystals of acicular and prismatic types and cystoliths are found abundantly in the cortical and pith region of the stem (Figure-4.3).

The petiole consists of three vascular bundles- the central one being the largest. The arrangement of different structures and tissues follow the same pattern as in the case of the stem (Figure-4.4).

8.4.2 Leaf.

The lamina represents a structure of a dorsiventral type of a leaf with 2 layers of palisade on the upper side and 4-6 layers of spongy cells on the lower side. The lamina are more or less of uniform size, but extremely sinuous in outline, while those of the lower surface are somewhat wavy but show great variations in their dimensions. Both surfaces of the leaf show the presence of caryophyllaceous type of stomata but they are more numerous on the lower surface than on the upper one. They are distributed more or less uniformly on the lower surface mainly in the vicinity of the veins on the dorsal surface (Figure-4.5).

Glandular and non-glandular hairs are presents on both the surfaces of the leaf. The upper surface shows a greater distribution of covering trichomes and lesser number of glandular hairs as compared to the lower surface per unit area. The covering hairs, occur in far greater numbers on the petiole and neural regions of the leaf, they are uniseriate, 1-3 celled in the interneural regions of the leaf but, 4-celled towards the basal region and up to 5-celled on the petiole of the leaf. The glandular hair possesses a unicellular but distinct stalk and a glandular head of four cells arranged in a cruciate manner. Palisade ratio ranges from 4.5-8.5 and vein-

islet number is 6.5-7.5. Oil globules and cystoliths elongated and cigar shaped are commonly present in the palisade and spongy mesophyll. Acicular and prismatic forms of calcium oxalate crystals are present in mesophyll tissue.

8.4.2 Cell Contents.

Cystoliths, elongated and cigar shaped, are of wide occurrence and are found in the cortical and the pith regions of the stems, in the cortex of the petiole and the midrib and the spongy and palisade mesophyll of the leaf. Starch in the form of both simple and compound grains is found more or less in the same part of the stem and the leaf as the cystolith. Calcium oxalate occurs as circular and prismatic crystals in the parenchyma of a cortex and the pith of stem, parenchyma of the petiole and the midrib, while oil globules of volatile nature are present mainly in the palisade and spongy mesophyll of the leaf and to a small extent in the parenchyma of the stem, petiole and midrib.

8.4.3. Chemical composition.

Sen and Ghose (1924) isolated alkaloid called vasicine ($C_{11}H_{12}N_2O$) from *Adhatoda vasica* Nees.

It has been found that the leaves contain about 0.25% of the alkaloid vasicine which has been reported to occur in combination with adhatodic acid. Varying quantity of the alkaloid contents in the leaves ranging from 0.2 to 2.0% have been reported. Total phenol content and flavonoid contents were highest in the leaves of plant growing in soil with cow dung in the order cow dung > verimicompost > FYM compost (Upadhyaya *et al.*, 2010).

Four new quinazoline alkaloids-vasicoline, adhatodine, vasicolinone and anisotine from leaves and one each i.e. vasicinone and vasicol have been isolated from inflorescence and leaves respectively. Another alkaloid vasicinolone, has been isolated from roots. A new quinazoline alkaloid has been isolated from leaves and characterised as 1,2,3, 9-tetrahydro-5-methoxypyrrolo [2,1-b] quinazolin-3-ol (1-from). Adhavasine has been isolated and characterised. Adhavasine has been isolated from leaves.

Vasicinol, Vasicinone, deoxyvasicinone, deoxyvasicine (minor alkaloids) and vasicine isolated from leaves showed seasonal variation in the percentage of minor alkaloids and total alkaloids. Leaves collected in March-April showed a higher percentage of minor alkaloids, whereas those collected in June-Sept. had higher content of vasicine.

The base visicine or vasicine is monobasic and occurs as white needle-shaped crystals and has a melting point of 182°C. It is easily soluble in alcohol, is slightly soluble in cold water but more so in hot after with an alkaline reaction. A 2.0% solution in chloroform is optically inactive. It forms crystalline salts with mineral acids; oxidation product with KMnO_4 m.p. 213°-214°. Vasicine behaves as a tertiary base.

Visicine hydrochloride occurs in light, cream-coloured crystals, has a melting point of 180°C and is very soluble in water. Visicinetartrate was also prepared and is a soluble salt. The molecular weight of visicine was determined and found to be 188 which agree with the empirical formula $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}$ found by analysis.

9. Carbohydrate Estimation

Carbohydrates are among the most widely distributed compounds in both plant and animal kingdoms. Carbohydrate is an important structural component. They are also called hydrates of carbon and represented by the molecular formula $\text{C}_n\text{H}_{2n}\text{O}_n$. They contain Carbon, Hydrogen and Oxygen in the ratio of 1:2:1.

Carbohydrates are aldehyde and ketone derivatives of polyhydroxy alcohols. Each carbohydrate therefore, contains an aldehyde or a ketone group and is known as an aldose or a ketose. They are usually classified as monosaccharides, oligosaccharides and polysaccharides. Oligosaccharides and polysaccharides yield monosaccharide upon acid hydrolysis while monosaccharides are stable to acid hydrolysis. There is no sharp distinction between oligosaccharides and polysaccharides, both being made up of monosaccharide units. The oligosaccharides, however, contain fewer monosaccharide residue than polysaccharides. The term polysaccharide is usually employed for polymers containing at least ten monosaccharide units.

The most important monosaccharide is D-glucose which also forms the structural unit of the important polysaccharides. Wood with its high percentage of carbohydrates remained the raw material for framework of houses and furniture.

Free sugars are translocated from the shoot system to the heterotrophic region of the plant where they supply the organic building blocks required in biosynthesis while storage carbohydrates represent a means of conserving energy, as the simple sugars released upon their hydrolysis can be utilized for cellular respiration. Starch of higher plants consists of two

components amylase which ranges from 10-30% and amylopectin which ranges from 70-90%. Starch occurrence in leaves is transitory, being deposited during the day and translocated to other regions during the night.

10. MATERIALS AND METHODS:

Carbohydrate estimation was done by the anthrone reaction method (McCreedy *et al.*, 1950). Twenty mg of dried and finely powdered material of leaves was taken and washed repeatedly with 80% ethanol to remove all traces of soluble sugars, finally washed with water and placed in a mixture of 5 ml of water and 6.5 ml of perchloric acid at 0°C for 20 minutes in order to extract the starch. The mixture was centrifuged at 1200 r.p.m. for 30 minutes with the residual material; samples were centrifuged again and the supernatant of the sample was pooled together and the total extract was diluted to 100 ml and filtered. Anthrone reagent was prepared by dissolving 0.2g of anthrone in 100 ml of cold 95% H₂SO₄. 10 ml of anthronesulphuric acid was added to 5 ml of starch extract in an ice-bath. The reaction mixture was heated at 100°C for 7.5 minutes on a water bath and cooled rapidly to 25°C. Optical density was measured at 630 nm in a spectrophotometer (Carlzeiss). Standard curve was made by using glucose as a standard in 0.1 mg/g to 0.7 mg/g in different dilutions and treated with anthronesulphuric acid reagent in the same way.

11. RESULTS

Results of carbohydrate estimation are depicted in table-4.2.

Table-4.2: Carbohydrate contents in *Justicia adhatoda* Nees.

S.No.	Name of the plant	Carbohydrate contents
1.	<i>Justicia adhatoda</i> Nees.	3.45 mg/g

3.45 mg/g carbohydrate was recorded in *Justicia adhatoda* leaves.

12. Protein Estimation

Proteins are linear polymers composed of amino acids. All proteins contain Carbon, Hydrogen, Oxygen and Nitrogen, the presence of Nitrogen distinguishing them from carbohydrates and fats. Proteins constitute a large part of the structure of cells and are present in all tissues. Proteins are among the most complex and most important organic compounds found in plants.

On an average protein contains 16% Nitrogen. Some proteins also have sulphur in addition, and in a few proteins phosphorus and other elements may be present. The molecular weight

of proteins varies from about 12,000 daltons to several million. Their molecular size, shape, physicochemical properties and biological role vary considerably from one protein to another. They have varying content of Nitrogen and have special physiological functions. Much of early work on plant proteins was carried out by (Osborne, 1962). He classified proteins according to their properties of solubility, i.e., simple proteins and conjugated proteins.

13. MATERIALS AND METHODS

Total protein contents were estimated by using the method of (Lowery *et al.*, 1951). 100 mg of the dried and finely powdered leaves was placed in a reaction vessel. To the sample was added 10 ml of 10% cold trichloroacetic acid. Then sample was centrifuged and the supernatant discarded. The pellet was re-suspended in 10 ml of cold 5% trichloroacetic acid and heated at 80°C for 30 minutes. This was again centrifuged for 10 minutes at 1200 r.p.m. The pellet was washed with distilled water and centrifuged. The pellet obtained after centrifugation was dissolved in 10ml of 1.0 N sodium hydroxide and kept overnight at room temperature. The supernatant was used for determining the proteins.

The 1.0 ml of the test solution was added to 5 ml of Lowery's reagent. The reagent was prepared on the day of use by mixing the following.

50 ml of 2% sodium carbonate in 0.1 N sodium hydroxide.

0.5 ml of 0.5% copper sulphate.

0.5 ml of 1% sodium potassium nitrate and 1.0 ml of phenol reagent (commercial phenol reagent was diluted with an equal volume of distilled water on the day of use).

Optical density was measured at 750 nm spekol spectrophotometer. A blank was prepared similarly as the above procedure except the addition of plant material.

Standard curve was made by using bovine albumin as a standard in 0.1 mg/ml to 4.0 mg/ml in different dilutions and treated with Lowery's reagent and phenol reagent in the same way. The protein content in sample was calculated by comparing their optical densities against the standard curve. For each species three replications were analyzed and average computed.

14. RESULTS

Results of protein estimation are given in table-2.

Table-2: Protein contents in *Justicia adhatoda* Nees.

S.No.	Name of the plant	Protein contents
1.	<i>Justicia adhatoda</i> Nees.	5.2 mg/g

Protein contents were 5.2 mg/g in *Justicia adhatoda* leaves.

15. Determination of Physical Constants of crude drug

Determination of physical constants like ash and extractive values of leaves of different species throw light on botanical identity.

(i) Ash Values

The ash values are useful to determine the quality and purity of the crude drug. Ash contains inorganic radicals like phosphates, carbonates and silicates of Sodium, Potassium, Magnesium, Calcium, etc. Such variables are then removed by treating with acid.

16. METHOD AND RESULTS

During the present investigation crude drug was prepared by grinding the shade dried leaves of *J. adhatoda*.

Different ash values like total ash, water soluble ash, acid insoluble ash and sulphated ash of *J. adhatoda* were determined as per standard procedure mentioned in WHO Library.

Two gm. powder of *J. adhatoda* was taken in a previously ignited and weighed silica crucible. Then it was incinerated at a low temperature with gradual increased temperature (500-550°) and allowed to remain for more than 4 h. until no carbonized substance was left out in the ash. Later, each crucible was cooled in the desiccator, weighed to a constant value and the total ash contents were calculated (%).

For acid insoluble ash value total ash obtained was heated with addition of 25 ml of dilute HCl for 10 minutes. It was filtered and the residue was ignited in the furnace to get a constant weight. The weight of insoluble matter was then calculated.

17. RESULTS

Results are shown in table-3.

Table 3. Total ash, water soluble ash, acid insoluble ash and sulphated ash values of crude drug of *Justicia adhatoda* Nees.

S.No.	Nature of ash (%w/w)	Ash values (% w/w)
1.	Total ash	15.2
2.	Water soluble ash	3.9
3.	Acid insoluble ash	1.1
4.	Sulphated Ash	1.8

Total ash, 15.2; water soluble ash, 3.9; acid insoluble ash, 1.1 and sulphated ash, 1.8 (% w/w) were recorded in crude drug of *Justicia adhatoda* Nees.

(ii) Extractive Values

Extractive values are useful for evaluation of crude drugs as it gives an idea about the nature of the chemical constituents present in the crude drug.

18. METHOD AND RESULTS

Different extractive values like water soluble, methanol soluble, ethanol soluble and petroleum ether soluble, were determined as per standard procedure mentioned in WHO Library.

19. RESULTS

Results are given in Table-4.5 and Figure-4.7.

Water soluble extractives, 20.1, methanol soluble extractives, 6.3; ethanol soluble extractives, 5.9 and petroleum ether soluble extractives, 0.7 (% w/w) were recorded in crude drug of *J. adhatoda* Nees.

Table 4.5: Extractive values of crude drug of *Justicia adhatoda* Nees.

S.No.	Nature of extract (% w/w)	Colour	Extractive values
1.	Water soluble	Brown	20.1
2.	Methanol soluble	Green	6.3
3.	Ethanol soluble	Yellow green	5.9
4.	Petroleum ether soluble	Light yellow	0.7

(iii) Florescence Analysis

20. METHOD AND RESULTS

The crude drug was screened for florescence characteristic under day light and ultra-violet short (252 nm) and long (366 nm) light and observations were recorded.

21. RESULTS

Results are given in Table-4.6. The drug appeared olive coloured in day light and green and olive dark in UV short and UV long light respectively. It appeared dark brown in day light and grass green and dark green in UV short and UV long light respectively in its aqueous form.

Table-4.6: Fluorescence analysis of crude drug of *Justicia adhatoda* Nees.

S.No.	Solvent treatment	Day light	Short UV (252 nm)	Long UV (366 nm)
1.	Drug as such	Olive	Green	Olive dark
2.	Drug + water	Dark brown	Grass green	Dark green

(iv) Foreign Organic Matter

22. METHOD AND RESULTS

Foreign organic matter was determined from the weight of the crude drug in terms of per cent w/w as per standard procedure mentioned in WHO Library.

23. RESULTS

Results are recorded in Table-4. . Foreign organic matter was 1.1 (% w/w) in the crude drug of *Justicia adhatoda* Nees.

Table 4: foreign organic matter in the crude drug of *Justicia adhatoda* Nees.

Drug sample	Foreign organic matter (%w/w)
<i>Justicia adhatoda</i> Nees.	1:1

24. DISCUSSION

The global interest in study and practice of crude drugs has therefore, considerably increased during the last two decades because of growing awareness about the toxicity and side effects of synthetic drugs, their limitations in many areas of therapy, comparatively high cost and often drug beyond the reach of common man. Pharmacognosy is a study of drugs having their origin in plant and animal kingdoms. The subject pharmacognosy can also be expressed as an applied science that deals with biological, biochemical, therapeutic and economic features of natural drugs and their constituents. Tyler *et al.* (1981) defined that in a broad sense, pharmacognosy embraces knowledge of the history, distribution, cultivation, collection, selection, preparation, commerce, identification, evaluation, preservation and use of drugs and economic substances that affect the health of men and other animals.

The pharmacognosy deals with a number of scientific descriptions for solving problems related to identity, quality, purity and preservation of the drugs from plants. It is closely related to both botany and plant chemistry and it is regarded as the parent of both (Winton *et al.*, 1939). Variations exist in literature on traditional medicine, constituents of a drug, methods of preparation and the names of medicinal plant. Multitude of vernacular name of medicinal plants found in the literature pose problems in identifying the correct botanical name of medicinal plants. And, it is the worst confounded with the use of different vernacular names, for the same plant, in different localities in the country. Vernacular name of some medicinal plants find place in standard formularies and pharmacopoeia, although their botanical identity are unknown or ambiguous, for example: 'Avilthol' and 'Killiyooral' have no botanical identity and for the Sanskrit name 'Purnarnava' two plants *Trianthema portulacastrum*, and *Boerhavia repens* are mentioned (Mukelji, 1953).

A large variety of medicinal plants are growing in India but the trade of crude drugs always remained in the hands of unqualified and unskilled persons and causes the collection of the premature or incorrect drug and thus often lead to adulteration or substitution. Thus pharmacognosy appears to be of great value in identification of commercial samples of the market to find their authenticity and establishing identity of adulterant or substituent.

Pharmacognosy is basically divided into conventional and modern pharmacognosy. Conventional pharma-cognostical study is based on macroscopic, microscopic and quantitative microscopy. Macroscopic characters include shape, size, colour and texture of the drug in crude or powdered form while microscopic characters include the anatomical details of drug producing plant, maceration study and the size measurement of various type of cells. The quantitative microscopy includes the vein islet number, palisade ratio, stomatal number and stomatal indices and so restricted to leaf drug only. The modern pharmacognosy utilizes characteristics of analytical, phytochemical and certain physical constant values over the traditional science of taxonomy in plant systematics (Wallis, 1967). Most of the physical, botanical, chemical and microbial techniques employed in pharmacognosy are applicable to the analysis of drugs and used by public analysts, forensic scientists and quality control chemists associated with industries.

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