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Quantitative analysis of flavonoids in *Adhatoda vasica* Nees extracts

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ABSTRACT

The quantitative determination of flavonoid (flavonols) contents calculated in terms of quercetin equivalent in different Adhatoda vasica extracts. Aluminium chloride colorimetric method was used for flavonoids determination. Quercetin was used to explore the calibration curve. The flavonoid contents of the extracts in terms of quercetin equivalent ($y = 0.002x + 0.0318$, $R^2 = 0.9989$) were found between 30.05±0.03 to 61.66±0 .05 mg/g and it was found maximum (61.66±0.05) in petroleum ether extracts.

Keywords: *Adhatoda vasica*, Flavonoid , Quercetin, Aluminium chloride, Extracts.

INTRODUCTION

Adhatoda vasica Nees *(family Acanthaceae)*, commonly known as Vasaka or Arusha, is a shrub 1–2.5 m high with opposite ascending branches . The leaves are simple, opposite, 7–19 cm long and 4–7 cm wide. The flowers are white, pink or purple. The plant grows throughout the Indian peninsula up to an altitude of 1300 m [10]. The drug contains stem, leaf, flower, fruit and seeds [2]. The Vasaka plant perennial, evergreen shurb and bitter in taste. The plant lives for multiple seasons and retains its leaves throughout the year [14,16].

Adhatoda vasica is an Ayurvedic medicinal plant which is a home remedy for several diseases and human requirements. It is mentioned in Vedas as a herbal remedy for treating cold, cough, whooping cough and chronic bronchitis and asthma, as sedative expectorant, antispasmodic and anthelmintic. It is an official drug and is mentioned in the India Pharmacopoeia [6]. The drug is employed in different forms such as fresh juice, decoction, infusion and powder; also given as alcoholic extract and liquid extract or syrup. The leaf juice is stated to cure diarrhoea, dysentery and glandular tumor and the plant is an emmenagogue. The powder is reported to be used as poultice on rheumatic joints as counter-irritant on inflammatory swelling, on fresh wounds, urticaria and in neuralgia [7].

Vasaka is a bitter quinazoline alkaloid, the major alkaloids are vasicine and vasicinone which is present in all parts of the plant [1]. The leaves, roots and young plants of *Adhatoda vasica* contain the quinazoline alkaloids vasicine, 7-hydroxyvasicine, vasicinolone, 3-deoxyvasicine, vasicol, vasicoline, vasicolinone, adhatodine and anisotine as main compounds betaine, steroids carbohydrate and alkanes [8]. In the flowers triterpines (a-amirine), flavonoids (Apigenin, astragalin, kaempferol, quercetin, vitexin) have been found [3]. polyphenols comprise basically of phenolic acids including benzoate and hydroxycinnamate derivatives, and flavonoids. Polyphenolic substances are naturally present in essentially all plants and are prominent in vegetables and fruits [15].

Flavonoids are naturally-occurring polyphenolic compounds with a C6-C3-C6 backbone. This group of plant pigments which are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine can be chemically subdivided into six structural categories:

Flavones, flavonols, flavanones, flavanonols, flavan-3-ols (catechins), and anthocyanidins. These compounds (aglycones) are commonly glycosylated (at one or more sites with a variety of sugars) and may also be alkoxylated or esterified [9,17]

MATERIAL AND METHODS

Collection of plant material

Leaves of Adhatoda vasica nees , a member of family Acantheace, was used for the experiments. It was collected from medicinal garden of Faculty of Pharmacy, Integral University, Lucknow.

Authentification of plant material

Sample of plant material was given to NBRI Lucknow, India for identification and taxonomic authentification. The test report from CIF, NBRI, Lucknow, conformed the taxonomic authentification of plant material sample. Specification No.: NBRI-SOP-202, Receipt no. 19/76 & date:, 27-2-2009

Chemicals and Instruments

Aluminium chloride, potassium acetate and methanol, petroleum ether, chloroform, alcohol, Standard substance: Quercetin. UV spectrophotometer (Shimadzu), soxhlet apparatus.

Preparation of the extracts

Plant extracts were prepared using four different extracting solvents:

(a) Ether extract: The leaves of *Adhatoda vasica* was dried under shade and then crushed in to powder with a mechanical pulveriser. The powdered plant material (20 gm of *Adhatoda vasica* leaves) was extracted with 250 ml of petroleum ether for 12 hours, reflux at 20^0C. After filtering and evaporating to dryness, the crude extracts were obtained.

(b) Hydro-alcohol extract: The leaves of *Adhatoda vasica* was dried under shade and then crushed in to powder with a mechanical pulveriser. The powdered plant material (20 gm of *Adhatoda vasica* leaves) was extracted with 250 ml of ethanol: water (50: 50) for 18 hours, reflux at 50^0C. After filtering and evaporating to dryness, the crude extracts were obtained.

(c) Chloroform extract: The leaves of *Adhatoda vasica* was dried under shade and then crushed in to powder with a mechanical pulveriser. The powdered plant material (20 gm of *Adhatoda vasica* leaves) was extracted with 250 ml of chloroform for 10 hours, reflux at 60^0C. After filtering and evaporating to dryness, the crude extracts were obtained.

(d) Aqueous extract: The leaves of *Adhatoda vasica* was dried under shade and then crushed in to powder with a mechanical pulveriser. The powdered plant material (20 gm of *Adhatoda vasica* leaves) was extracted with 250 ml of water for 16 hours, reflux at 70^0C. After filtering and evaporating to dryness, the crude extracts were obtained [5].

Procedure for determination of total flavonoids

Aluminium chloride colorimetric method was used for flavonoids determination [11]. Quercetin was used to perform the calibration curve (standard solutions of 10, 20, 30, 40, 50 and 100.0 µg/ml in methanol. Sample extracts (20g plant material in 250 ml different solvent extracts) were all evaporated to dryness and dissolved in methanol to be ready for the analytical test. Each plant extracts (0.5 ml of 100:1000 µg /ml) in methanol were separately introduced into test tubes and mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The tubes were covered with parafilm and it remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm. The flavonoid content was also expressed as quercetin equivalent. All determination was performed in triplicate [4].

RESULTS AND DISCUSSION

To perform the calculations of flavonoids in *Adhatoda vasica*, the chang et al method was used, a standard curve is needed which is obtained from a series of different quercetin concentrations. (Table 1)

Table 1: Absorbance of Standard Compound (Quercetin)

Concentration (µg/ml)	Absorbance (Mean) λ_{max}=415 nm
2.5	0.0365
5	0.0417
10	0.0521
20	0.0735
30	0.0906
40	0.1127

In Figure.1. the flavonoid content of the extracts was calculated in terms of quercetin equivalent using the standard curve equation ($y = 0.002x + 0.0318$, $R^2 = 0.9989$) were found between 30.05±0.03 to 61.66±0.05 mg/g and it was found maximum (61.66±0.05) in petroleum ether extracts (Table 2), Where y is absorbance at 415 nm and x is flavonoid content in the different extracts of *Adhatoda vasica* expressed in mg/gm. Each value in the table was obtained by calculating the average of three experiments ± standard deviation

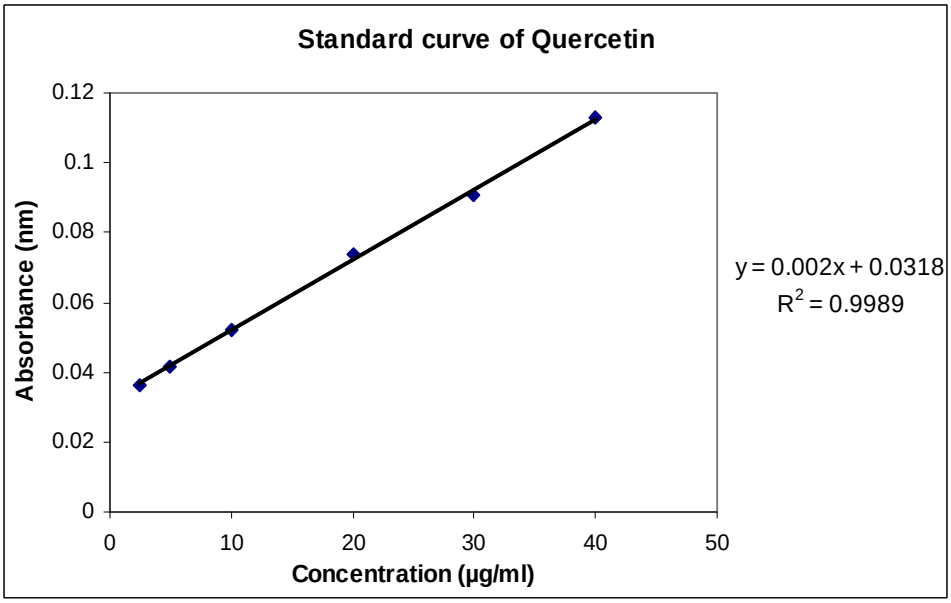

Figure 1: Standard curve of Quercetin

Table 2: Flavonoids of *Adhatoda vasica* in Different Plant Extracts

Sample	Conc. (µg/ml)	Absorbance (Mean) λ_{max}=415 nm	Flavonoid (mg/g) Mean ± SD
Petroleum Ether	1000	0.1213	61.66±0.05
Hydro-alcohol	1000	0.1451	46.49±0.05
Chloroform	1000	0.1154	40.58±0.06
Aqueous	1000	0.2012	30.05±0.03

CONCLUSION

The extract of *A. vasica*, which contain highest amount of flavonoid, exhibited the greatest antioxidant activity [12]. The high scavenging property of *Adhatoda vasica* may be due to hydroxyl groups existing in the phenolic compound's chemical structure that can provide the necessary component as a radical scavenger [13]. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes, anti-inflammatory action [14]. The flavonoid contents of the extracts in terms of quercetin equivalent ($y = 0.002x + 0.0318$, $R^2 = 0.9989$) were found between 30.05±0.03 to 61.66±0.05 mg/g and it was found maximum (61.66±0.05) in petroleum ether extracts.

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