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Quantitative Analysis of Total Phenolic Content in Adhatoda vasica Nees Extracts

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Abstract : Adhatoda vasica leaves were analyzed for total phenolic contents. This phenolic component is responsible for antioxidant activity. The amount of total phenols were analyzed with the Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalents (standard curve equation: $y = 0.0061x + 0.0396$, $R^2 = 0.9991$). The total phenol varied from 63.95±2.1 to 92.4±0.14 mg/g in the extracts. The maximum phenolic content was found in the aqueous extract (92.4±0.14 mg/g).
Keywords: *Adhatoda vasica, total phenols, Folin-Ciocalteu reagent, Gallic acid.*

Introduction

Adhatoda vasica Nees *(family Acanthaceae)*, commonly known as Vasaka or Arusha. The Vasaka plant perennial, evergreen and highly branched with unpleasant smell and bitter taste, the plant lives for multiple seasons and retains its leaves throughout the year. It is a shrub 1.0 m to 2.5 m in height, with opposite ascending branches. The drug contains stem, leaf, flower, fruit and seeds[2,14]. The plant grows throughout the India, where it is found in sub-Himalayan track up to an altitude of 1000 meters above sea level, and in Maharashtra especially, in Konkan resion. Beside India, it is found in Myanmar, Sri Lanka, Burma and Malaysia[1]. Vasaka is a bitter quinazoline alkaloid. The major alkaloids are vasicine and vasicinone which are present in all parts of the plant. The leaves contain several alkaloids (vasicinone, vasicinol, adhatodine, adhatonine, adhavasinone, anisotine and peganine), betaine, steroids carbohydrate and alkanes . In the flowers triterpines (a-amirine), flavonoids (Apigenin, astragalin, kaempferol, quercetin, vitexin) have been found[1-3].

This plant is source of vitamin C and has medicinal uses, mainly antispasmodic, fever reducer, anti-inflammatory, anti bleeding, branchodilator, antidibetic, disinfectant, anti-jaundice, oxytocic and expectorant[1]. Most of these attributes fall mainly in to respiratory therapy category for cold, asthma, bronchitis and tuberculosis. Antioxidant activity of plants might be due to their phenolic compounds[9]. Synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. These synthetic antioxidants also show low solubility and moderate antioxidant activity[10-11].

Material and methods

Authentification of plant material

The leaves of ***Adhatoda vasica nees*** were collected from the medicinal garden of faculty of pharmacy, Integral University, lucknow. Sample of plant material was given to NBRI Lucknow (U.P.) 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. & date : 19/76, 27-2-2009

Preparation of the extracts

Plant extracts were prepared using three different extracting solvents:

A. Ether extract:

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 using soxhlet extractor. After filtering and evaporating to dryness, the crude extracts were obtained.

B. Hydro-alcohol extract:

The powdered plant material (20 gm of adhatoda vasica leaves) was extracted with 250 ml of ethanol: water (50: 50) for 8 hours, reflux at 50^0C. After filtering and evaporating to dryness, the crude extracts were obtained.

C. Aqueous extract:

The powdered plant material (20 gm of adhatoda vasica leaves) was extracted with 250 ml of water for 18 hours, reflux at 70^0C. After filtering and evaporating to dryness, the crude extracts were obtained[8].

Chemicals and Instruments

Folin-Ciocalteu's phenol reagent, gallic acid, anhydrous sodium carbonate and methanol. UV spectrophotometer (Shimadzu).

Preparation of Folin-Ciocalteu's Phenol reagent

100 gm of sodium tungstate and 25gm of sodium molibedate were dissolve in 800 ml of water in a 1500 ml flask then 50ml of phosphoric acid and 100 ml of HCl were added and refluxed for 10 hours. After cooling, add 150 gm of lithium sulphate, 50 ml of water and 4 to 6 drops of bromine water were added and allowed to stand for 2 hours. The solution was boiled for 15 minutes and cooled before filtration. The reagent should have no greenish tint[7].

Procedure for determination of total phenolic contents

The amount of total phenolics in extracts was determined with the Folin- Ciocalteu reagent. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE)[4]. Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentration of 0.1 and 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced into test tubes and mixed with 2.5ml of a 10 fold dilute Folin- Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was at read at 760 nm spectrometrically. All determination was performed in triplicate. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction. This blue colour is measured spectrophotometrically. Thus total phenolic content can be determined[5-6].

Table 1: Absorbance of Standard Compound (Gallic Acid)

Concentration (µg/ml)	Absorbance (Mean) λ_{max}=760 nm
0.8	0.0456
1.6	0.0505
3.12	0.0572
6.25	0.0786
12.5	0.1133
25	0.1937

Table 2: Total Phenolic Content of Adhatoda Vasica in Different Plant Extracts

Sample	Concentration(µg/ml)	Mean±SD
Petroleum Ether	1000	63.95±2.1
Hydro-alcohol	1000	81.51±2.7
Aqueous	1000	92.4±0.14

Each value in the table was obtained by calculating the average of three experiments ± standard deviation

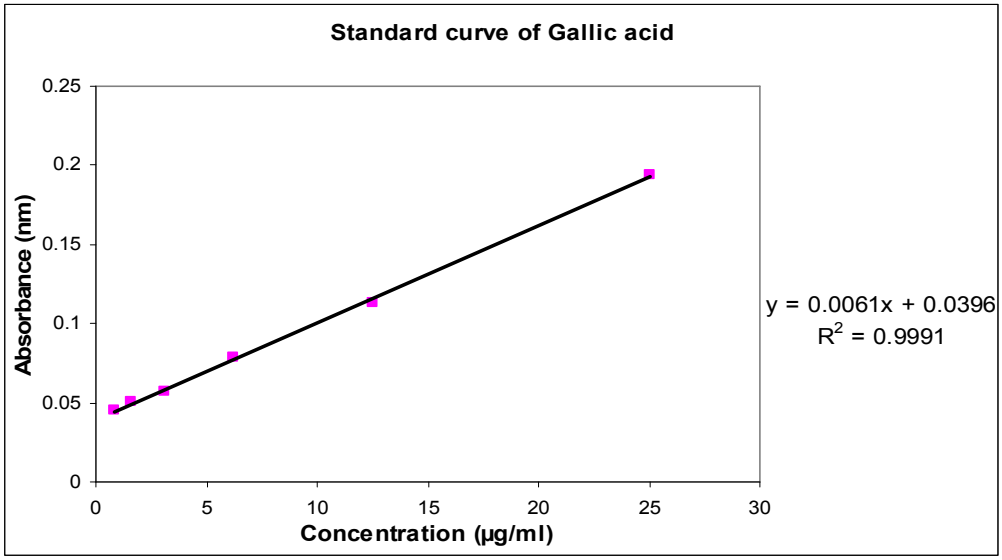

Figure 1: Standard curve of Gallic acid

Results and Discussion

The amount of total phenol was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: $y = 0.0061x + 0.0396$, $R^2 = 0.9991$, Where y is absorbance at 760 nm and x is total phenolic content in the different extracts of adhatoda vasica expressed in mg/gm. Phenolic compounds are a class of antioxidant agents which acts as free radical terminators[12]. Table.1 shows the variation of mean absorbance with concentration of Gallic acid. Table.2 shows the contents of total phenols that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent. The total phenol varied from 63.95±2.1 to 92.4±0.14 mg/g in the extracts. The maximum phenolic content was found in the aqueous extract (92.4±0.14 mg/g) of adhatoda vasica Nees.

Conclusion

The amount of total phenols were determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalents. The maximum phenolic content was found in the aqueous extract (92.4±0.14 mg/g). The result of the present study showed that the extract of Adhatoda vasica, which contain highest amount of phenolic compounds which exhibited the greatest antioxidant activity. The high scavenging property of Adhatoda vasica may be due to hydroxyl groups existing in the phenolic compounds. Free radicals are often generated as by products of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases[13-14].

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