

## Evaluation of the Chemical Composition *Rauwolfia serpentina* and *Ephedra vulgaris*

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**Abstract:** Indian medicinal plants (*Rauwolfia serpentina* and *Ephedra vulgaris*) were analyzed for their chemical composition, vitamins and minerals. The results revealed the presence of bioactive constituents comprising alkaloids (1.24 to 1.48 m g/100 g), saponins (1.46 to 1.72 m g/100 g), flavonoids (1.46 to 1.86 m g/100 g), phenols (0.06 m g/100 g) and tannins (0.04 to 0.5 m g/100 g). The medicinal plants contained ascorbic acid (26.42 to 44.03 m g/100 g), riboflavin (0.20 to 0.42 m g/100 g), thiamine (0.11 to 0.18 m g/100 g) and niacin (0.02 to 0.09 m g/100 g). These herbs are good sources of minerals such as Ca, P, K, Mg, Na, Fe and Zn. The importance of these chemical constituents is discussed with respect to the role of these herbs in ethnomedicine in India.

**Key words:** *Rauwolfia serpentina* • *Ephedra vulgaris* • Bioactive compounds • Ethnomedicine

### INTRODUCTION

In India, many indigenous plants are used in herbal medicine to cure diseases and heal injuries. Some important chemical substances found in plants are alkaloids, carbon compounds, hydrogen, nitrogen, glycosides, essential oils, fatty oils, resins, mucilage, tannins, gums and others [1]. Most of these are potent bioactive compounds found in medicinal plant parts that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs [2]. The active principles differ from plants to plant due to their biodiversity and they produce a definite physiological action on the human body. Calixto [3] reported that most of the cultivated medicinal and aromatic plants are exported as crude drugs. Ijeh *et al.* [4] noted the growing interest on the medicinal properties of a number of common plants. Edeoga *et al.* [5, 6] have elucidated the importance of these medicinal plants and their importance in the pharmaceutical industry.

These medicinal plants have been underutilized in orthodox medicine but have continued to be used in ethnomedicinal preparations. Today about 300 species of medicinal and aromatic plants are used world wide in the pharmaceutical, food, cosmetics and perfume industries [7]. Alkaloids are very important in medicine and constitute most of the valuable drugs. They have marked physiological effect on animals [8]. Alkaloids such as solasodine have been indicated as a starting

material in the manufacture of steroidal drugs [9]. Phenolic compounds are widely distributed in the plant kingdom and presence of phenols is considered to be potentially toxic to the growth and development of pathogen [10]. Saponins are glycoside of both triterpenes and sterols and have been detected in over seventy families of plants [11].

In medicine, it is used to some extent as an expectorant and emulsifying agent. Tannins are fairly frequently encountered in food products of plant vegetable origin such as tea and many fruits. The oxidation inhibiting activity of tannins have been known for a long time and it is assumed to be due to the presence of gallic and diagallic acids [12]. Flavonoids are 15-carbon compounds generally distributed throughout the plant kingdom [13]. Some isoflavones act as allelochemicals widely used in insecticides. They might also play a role in disease resistance [14].

Despite the use of this plant for such purposes, there is little information on the nutritional and chemical composition of *Rauwolfia serpentina* and *Ephedra vulgaris* leaves. This work is therefore aimed at documenting the nutrient and chemical compositions of *Rauwolfia serpentina* and *Ephedra vulgaris* leaves.

### MATERIALS AND METHOD

**Plant Materials:** The experimental leaves were collected from Kolli hills, Tamil Nadu, India. The plant materials (leaves) were identified and authenticated by

Dr S. Saravana Babu, Botany Department, C.N. College, Erode, India. The leaves were air-dried for 10 days and milled into powder with the aid of an electrical grinder and finally stored in airtight bottles before analysis.

**Chemical Analysis:** The major elements, comprising calcium, phosphorus, sodium, potassium, magnesium and trace elements (iron and zinc) were determined according to the method of Shahidi *et al.* [15]. The ground plant samples were sieved with a 2 mm rubber sieve and 2 g of each of the plant samples were weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO<sub>3</sub>/HCl/H<sub>2</sub>O (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through a Whatman No 42 filter paper and the volume was made to the mark with deionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer. A 10 cm-long cell was used and concentration of each element in the sample was calculated on percentage of dry matter. Phosphorus content of the digest was determined colorimetrically according to the method described by Nahapetian and Bassiri [16]. To 0.5 ml of the diluted digest, 4 ml of demineralised water, 3 ml of 0.75M H<sub>2</sub>SO<sub>4</sub>, 0.4 ml of 10% (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>.4H<sub>2</sub>O and 0.4 ml of 2% (w/v) ascorbic acid were added and mixed. The solution was allowed to stand for 20 min and absorbance readings were recorded at 660 nm. The content of phosphorus in the extract was determined.

**Preparation of Fat Free Sample:** 2 g of the sample were defatted with 100 ml of diethyl ether using a Soxhlet apparatus for 2 h.

**Alkaloid Determination:** 5 g of the sample were weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed [17, 18].

**Tannin Determination:** 500 mg of the sample was weighed into 100 ml plastic bottle. 50 ml of distilled water

was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out into a tube and mixed with 3 ml of 0.1 M FeCl<sub>3</sub> in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120 nm wavelength, within 10 min. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured [19].

**Determination of Total Phenols:** For the extraction of the phenolic component, the fat free sample was boiled with 50 ml of ether for 15 min. 5 ml of the extract was pipette into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelengths [17, 18].

**Saponin Determination:** The samples were ground. 20g of each plant samples were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated in percentage [18].

**Flavonoid Determination:** 10 g of the plant samples were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed [20].

**Determination of Riboflavin:** 5 g of the sample was extracted with 100 ml of 50% ethanol solution and shaken for 1h. This was filtered into a 100 ml flask; 10 ml of the

extract was pipette into 50 ml volumetric flask. 10 ml of 5% potassium permanganate and 10 ml of 30% H<sub>2</sub>O<sub>2</sub> were added and allowed to stand over a hot water bath for about 30 min. 2 ml of 40% sodium sulphate was added. This was made up to 50 ml mark and the absorbance measured at 510 nm in a spectrophotometer.

**Determination of Thiamin:** 5 g of the sample were homogenized with ethanolic sodium hydroxide (50 ml). It was filtered into a 100 ml flask. 10 ml of the filtrate was pipette and the colour developed by addition of 10 ml of potassium dichromate and read at 360 nm. A blank sample was prepared and the colour also developed and read at the same wavelength.

**Determination of Niacin:** 5 g of the sample was treated with 50 ml of 1 N sulphuric acid and shaken for 30 min. 3 drops of ammonia solution were added to the sample and filtered. 10 ml of the filtrate was pipette into a 50 ml volumetric flask and 5 ml potassium cyanide was added. This was acidified with 5 ml of 0.02 N H<sub>2</sub>SO<sub>4</sub> and absorbance measured in the spectrophotometer at 470 nm wavelengths.

**Determination of Ascorbic Acid (Vitamin C):** 5 g of the sample was weighed into an extraction tube and 100 ml of EDTA/TCA (2:1) extracting solution were mixed and the mixture shaken for 30 min. This was transferred into a centrifuge tube and centrifuged at 3000 rpm for about 20 min. It was transferred into a 100 ml volumetric flask and made up to 100 ml mark with the extracting solution. 20 ml of the extract was pipette into a volumetric flask and 1% starch indicator was added. These were added and titrated with 20% CuSO<sub>4</sub> solution to get a dark end point [21].

## RESULTS AND DISCUSSION

The quantitative determination of phytochemical constituents of *Rauwolfia serpentina* and *Ephedra Vulgaris* were summarized in Table 1. High quantity of flavonoids, saponins and alkaloids were found on *Rauwolfia serpentina* and *Ephedra vulgaris*. The flavonoid content was more on *Rauwolfia serpentina* (1.86 m g/100 g) than *Ephedra vulgaris*, which contains 1.46 m g/100 g flavonoid. The values of phenolic compounds and tannins were very trace on both plants.

The mineral contents of both plants are shown in Table 2. Calcium was the most abundant macro element present ranging from 1.04 m g/100 g in *Ephedra Vulgaris*

Table 1: Phytochemical composition of *Rauwolfia serpentina* and *Ephedra Vulgaris* expressed as mg/100 g dry weight

Phytochemicals	<i>Ephedra vulgaris</i>	<i>Rauwolfia serpentina</i>
Alkaloids	1.24±0.11	1.48±0.02
Flavonoids	1.48±0.20	1.72±0.11
Phenols	1.46±0.02	1.86±0.11
Tannins	0.04±0.10	0.51±0.20

Results are mean of triplicate determinations on a dry weight basis ± standard deviation

Table 2: Mineral composition of *Rauwolfia serpentina* and *Ephedra Vulgaris* on m g/100 g dry weight

Minerals	<i>Ephedra vulgaris</i>	<i>Rauwolfia serpentina</i>
Macroelements		
Magnesium	0.12±0.11	0.10±0.20
Calcium	1.04±0.30	0.32±0.10
Potassium	0.03±0.10	0.04 V 0.11
Phosphorus	0.32±0.20	0.18±0.22
Sodium	0.02±0.11	0.02±0.10
Microelements		
Iron	3.78±0.30	1.85±0.20
Zinc	5.68±0.10	5.38±0.11

Results are mean of triplicate determinations on a dry weight basis ± standard deviation

Table 3: Vitamin composition of *Rauwolfia serpentina* and *Ephedra Vulgaris* on m g/100 g dry weight

Vitamin	<i>Ephedra vulgaris</i>	<i>Rauwolfia serpentina</i>
Ascorbic acid	26.42±0.10	44.03±0.20
Riboflavin	0.20±0.01	0.42±0.10
Thiamine	0.11±0.20	0.18±0.02
Niacin	0.09±0.11	0.02±0.10

Results are mean of triplicate determinations on a dry weight basis±Standard deviation

to 0.32 m g/100 g in *Rauwolfia serpentina*. This is followed closely by phosphorus, which was present from 0.32 mg/100 g in *Ephedra vulgaris* to 0.18 mg/100 g in *Rauwolfia serpentina*. Zinc was present at 5.68 mg/100 g in *Ephedra vulgaris*, while *Rauwolfia serpentina* contains 5.38 m g/100 g of zinc. Iron content was 3.78 m g/100 g in *Ephedra Vulgaris* and 1.85 m g/100 g in *Rauwolfia serpentina*. Results of analysis of *Rauwolfia serpentina* and *Ephedra Vulgaris* showed that the plants are rich in vitamins (Table 3). Ascorbic acid (vitamin C) was found to be 44.03 m g/100 g in *Rauwolfia serpentina* and 26.42; m g/100 g in *Ephedra Vulgaris* Riboflavin, thiamine and niacin were also detected in both plants. The presence of phenolic compounds in the plants indicates that these plants may be anti-microbial agent.

The high saponin content of *Ephedra Vulgaris* and *Rauwolfia serpentina* justifies the use of the extracts from these plants to stop bleeding and in treating wounds. Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of Saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [22, 23]. These properties bestow high medicinal activities on the extracts from *Rauwolfia serpentina* and *Ephedra vulgaris*. Apart from saponins, other secondary metabolite constituents of *Rauwolfia serpentina* and *Ephedra Vulgaris* detected include the alkaloids and flavonoids. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects [24, 25].

They exhibit marked physiological activity when administered to animals. Flavonoids, on the other hand are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity [26, 27, 23]. Flavonoids in intestinal tract lower the risk of heart disease. As antioxidants, flavonoids from these plants provide anti-inflammatory activity [23]. This may be the reason *Rauwolfia serpentina* and *Ephedra Vulgaris* have been used for the treatment of diseases in herbal medicine. Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes. These perhaps, explain why traditional medicine healers in Southeastern India often use *Ephedra Vulgaris* and *Rauwolfia serpentina* in treating many disorders [28]. Calcium was the most abundant macro element in the plants. Normal extra cellular calcium concentrations are necessary for blood coagulation and for the integrity, intracellular cement substances [29].

Thus, the potentials of *Ephedra Vulgaris* to stop bleeding and its use in treating wounds could be as a result of its high calcium content. The lower sodium content of *Ephedra Vulgaris* and *Rauwolfia serpentina* might be an added advantage due to the direct relationship of sodium intake with hypertension on human [30]. The presence of zinc in the plants could mean that the plants can play valuable roles in the management of diabetes, which result from insulin malfunction [29]. These plants are good sources of ascorbic acids, riboflavin, thiamin and niacin (Table 3).

Natural ascorbic acid is vital for the body performance [23]. Lack of ascorbic acid impairs the normal formation of intercellular substances throughout the body, including collagen, bone matrix and tooth dentine.

A striking pathological change resulting from this defect is the weakening of the endothelial wall of the capillaries due to a reduction in the amount of intercellular substances [31, 23]. Therefore, the clinical manifestations of scurvy hemorrhage from mucous membrane of the mouth and gastrointestinal tract, anemia, pains in the joints can be related to the association of ascorbic acid and normal connective tissue metabolism [31, 23]. This function of ascorbic acid also accounts for its requirement for normal wound healing. As a result of the availability of ascorbic acid in *Rauwolfia serpentina* and *Ephedra Vulgaris* these plants are used in herbal medicine for the treatment of many diseases [32, 23].

This study, therefore, has provided some biochemical basis for the ethnomedical use of extracts from *Rauwolfia serpentina* and *Ephedra Vulgaris* in the treatment and prevention of infections. As rich source of phytochemicals, minerals and vitamins *Rauwolfia serpentina* and *Ephedra Vulgaris* can be a potential source of useful drugs.

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