

# Minerals and Phytochemical Analysis of Bark of *Derris scandens* (Roxb.) Benth

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**Abstract:** *The Himalaya mountain Range is hub of medicinal plants. The Derris scandens (Roxb) Benth is a plant of fabaceae which has been used by indigenous people of Manipur, North-East India, for treatment of various purposes such as removal stones in kidney, loss of appetite, menstrual cycle in women, massage of body, solvent in laboratory, nourishment of child etc. It is commonly found in the forests of northern Oudh, in central and southern India, extending to Bengal, Assam, the Andaman and Nicobar Islands, Srilanka, Burma, southern China and North Australia (Duthie 1903–1929) The plant usually grows in the mountainous region of Manipur. The elemental analysis and phytochemical screening of the bark of the plant was carried out. Inductively coupled plasma -mass spectrometry and FAAS determined the presence of the elements Na (0.6µg/ml), K (10.9µg/ml), Fe (1.156µg/ml), Mg (2.2156µg/ml), Ca (13.0µg/ml), As (less than 5 ng/ml), V (less than 2 ng/ml), Cr (7 ng/ml), Mn (167 ng/ml), Ni (6 ng/ml), Co (less than 2ng/ml), Cu (31ng/ml), and Pb (12ng/ml). Phytochemical screening of the bark extracts of water, chloroform and petroleum ether confirmed that water extract contain maximum phytochemicals viz. glycosides, saponin, alkaloid, terpenoid, steroid, phenol and tannin as compounds. The present finding will definitely helpful in the preparation of further pharmaceutical formulations.*

**Keywords:** Fabaceae, ICP-MS, FAAS, minerals, Phytochemicals, nourishment

## 1. Introduction

The fabaceae or leguminous family also commonly known as pea or legume or bean family is one of the important flowering plant due to its medicinal value. It consists of varieties of trees and herb plants of perennial or annual and can be recognized by their fruits and stipulated leaves. Presently, it is considered as one of the third largest plant family after orchidaceae and asteraceae family based on its abundance and has about 730 genera and over 14000 species. Some of the species of this family are found in the mountainous region and the valley of Manipur and are used as food namely, glycine max (soybean), phaseolus (beans), pisumsativum (pea), cicerarietinum (chickpeas), medicago sativa (alfalfa), arachishypogaea (peanut), ceratoniasiliqua (carob), and glycyrrhizaglabraet [1]. Many of the plants of this family are known for their medicinal properties and used in the natural preventive treatments of common diseases like asthma, abscess, anthelmintic, astringent, cough and cold, fever, paralysis, piles, diarrhea, worm, heart disease, eczema, dad, whooping cough, ulcers, snake-bite, ring worm, diuretic, breast pain, bronchitis, dysentery, gonorrhoea, leprosy, burning sensation, kidney disease, blood pressure, malaria, syphilis, cholera, ophthalmia, psoriasis, sciatica etc.

Some of the fabaceae family plants that are used for treatment of diseases. They are *Derris scandens* (Roxb) Benth, *Acacia nilitica* (L.), *Albizialebbek* (L.), *Albrusprecaborious* L, *Albiziaprocera* (Roxb.) Benth, *Bauhinia acuminata* L., *Cajanuscajan* (L.), *Caesalpiniaabondus*L, *Mimosapudica* L, *Pisumsativum* L, *Tamarindusindica* L, etc. [1]

In literature, the *derris scandens* (Roxb) Benth, one of the class of plant within fabaceae family is used for 1)

pretreatment for cells before irradiation which synergistically sensitizes HT-29 cells to the radiation-induced cell death by apoptosis or by mitototic catastrophe. This plant extract are also used for treatment of silences pro-survival signaling [3]. 2) The compound isolated from *derris* plant is used as insecticide and act as a feeding deterrent against the pests for stored grains [2]. 3) The compound obtained from this plant have also the capability for cytokine secretion [4]. In this regard, our laboratory has focused on isolating the medicinally important plant extracts and structurally define the major components. Further validate the use of the extracts using common techniques.

Based on our hypothesis, we proposed to extract and characterize the commonly practiced plants extracts from *Derris scandens*. In Manipur, the product obtained from this plant are used as 1) removal of kidney stones. 2) against loss of appetite. 3) treatment of menstrual cycle in women. 4) massage of body. 5) solvent in laboratory. 6) nourishment of child etc. This information obtained through the survey of local experts who used this plant.

The phytochemical test of this plant extracts in water, chloroform and petroleum ether found that water extract contained maximum of glycosides, saponin, alkaloid, terpenoid, steroid, phenol and tannin compound.

The determination of traces element in the plant *derris* plant were performed using inductively plasma coupled-mass spectrometry and FAAS. The plant powdered contained K and Ca in major amount and Na, Fe, Mg, As, V, Cr, Mn, Ni, Cu, and Pb. are present in smaller amount. The presences of above compounds such as glycosides, saponin, alkaloid, terpenoid, steroid, phenol and tannin etc and the traces elements have contribution to above uses. As far as my

literature survey is concerned no one has performed determination of elements in this plant.

## 2. Material and Method

### 2.1 Description of plant sample

*Derris scandens* plant is a tree climber usually grows near the river in mountainous region. It is a perennial and took some years to become mature plant. It flowers in May–June at the start of rainy and spring season. Stages of the plant sample are shown in **Fig1**, **Fig 2**.



**Figure 1:** Plant



**Figure 2:** Flower

### 2.2 Collection of Sample

About 20 kg the bark of the plant were collected from the mountainous region of Sapormeina, Kangpokpi District of Manipur in North East India, **Fig 3**. The collections of barks were done in January to February.

### 2.3 Processing of Sample

- Cleaning of sample:** The bark of plant was thoroughly washed with normal water to remove impurities and then again washed with distilled water. Finally, plant bark was washed with deionised water to remove impurities present in the bark.
- Drying of the extract:** The bark of plant was dried in shed and open air at room temperature so that not to affect chemical composition of the plant bark. The colour of the bark is brown outside and light yellow inside. The bark was shed air dried for 3 to 4 weeks until the colour changes into brown. The dried plant bark was stored in air tight container to avoid incorporation of unwanted impurity that might change chemical composition of the sample.
- Powdering of the extract:** The dried plant bark was powdered using electric grinder. The powder plant bark was stored in glass container for analysis, **Fig 4**, **Fig 5**.



**Figure 3:** Plant's bark Sample



**Figure 4:** Powdering process



**Figure 5:** Powdered sample

### 2.4 Elemental Analysis

- Sample preparation for analysis:** The plant bark powder was measured around 1 gm and dissolved in 10mL of nitric acid followed by the volume was made 100 mL by adding deionised water. This solution is preserved for ICP-MS and FAAS studies. The pellets of 1 cm diameter were prepared under hydraulic pressure and these were send for SEM- EDAX study.
- Characterization methods:** The determination of elements was done by Flame atomic absorption spectroscopy with model GBC 906AA AAS unit and deuterium-arc background correction was employed. The elements Na, K, Fe, Mg, and Ca were determined up to ppm ( $\mu\text{g/ml}$ ). Nano-pure water (18.3 Mega ohms) as diluent in this estimation. And the air-acetylene flame was employed. The determination of elements was also done by inductively coupled plasma mass spectrometer with model VG PQ ExCell, VG Elemental, UK. The elements such as Cr, Mn, Co, Cu, Pb, As, V were determined and reported up to ppb ( $\text{ng/ml}$ ), The Relative standard deviation was measured by using the following formula  $\text{Relative standard deviation (RSD)} = (\text{SD} \div \text{Mean}) \times 100$ , where x is provided in ppm or ppb as mean. The Relative standard deviation values for Flame atomic

absorption spectroscopy and inductively coupled plasma mass spectroscopy techniques are calculated to be 2-5 % and 5-10%, respectively.

## 2.5 Phytochemical Screening

### a) Preparation of different extracts

- The bark powdered of plant, *Derris scandens* (Roxb.) Benth was soaked in water for 5 days and the water soluble extracts was removed through filtration. The water soluble extract was concentrated by Rotavapor and dried it at room temperature to get solid form.
- The soluble extract obtained through filtration was then mixed with petroleum ether in separating funnel and two layers were formed. The aqueous layer was separated, concentrated and then dried at room temperature to obtained solid form.
- The organic layer after removal of aqueous layer was mixed with chloroform in separating funnel and two layers were formed. The chloroform extract was then separated, concentrated and dried at room temperature to obtained solid form.

The phytochemical test was also conducted for these three solid extracts as below.

### b) Standard Protocol for Phytochemical Test

#### 1) Alkaloid test:

- Alkaloids Mayer's test: A 2ml of plant extract was taken in test tube and two drops of Mayer's reagent was added to the test tube. The formation of a white creamy precipitate shows the presence alkaloid.
- Dragendroff's test: A few drops of Dragendroff's reagent was added into the 2ml of extract resulting in formation of red precipitate. This shows the presence of alkaloids in the sample.

#### 2) Saponins test:

- A 20 ml solution was made by dissolving 50 mg of extract in distilled water. The mixture was shaken for 15 minutes in the graduated cylinder. The final solution forms a 2 cm thick foam which shows the presence of saponins.
- The extract was mixed with 5.0 ml of distilled water in a test tube and it was mixed vigorously. To the frothing a few drops of olive oil was mixed vigorously. The appearance of foam showed the presence of saponins.

#### 3) Flavonoids test:

- Alkaline reagent test: The 2ml of the extract was treated with 2ml of 2.0 percent of sodium hydroxide. This intense yellow colour solution was observed to become colourless on addition 2 drops of dilute acid. This experiment shows the presence of flavonoids.

- Lead acetate test: A few drops of lead acetate solution are treated with the extract. The yellow colour solution formation shows the presence of flavonoids.

#### 4) Detection of phenols

- To the plant extract a few drops of ferric chloride solution was added. The formation of bluish black colour shows the presence of phenols.

#### 5) Tannins test:

- The extract is mixed with a few drops of 1 % gelatin solution containing sodium chloride. The white precipitate formation shows the presence of tannins.
- The 10 ml of bromine water was mixed with 0.5 g aqueous extract. The decolouration of bromine water indicates the presence of tannins.

#### 6) Tests for Glycosides

- Liebermann's Test: 2.0 ml of acetic acid and 2ml of chloroform was added to the whole aqueous plant crude extract. The mixture was then cooled and H<sub>2</sub>SO<sub>4</sub> (conc.) was slowly added to the aqueous extract solution. Formation of green colour showed the entity of a glycone, steroidal part of glycosides.
- Keller-Kiliani Test: A solution of glacial acetic acid (4.0 ml) with one drop of 2 % FeCl<sub>3</sub> solution was mixed with the 10 ml aqueous solution of plant extract and 1 ml H<sub>2</sub>SO<sub>4</sub> (conc.). Brown ring formed between the layers which showed the presence of cardiac steroidal glycosides.
- Salkowski's Test: 2 ml concentrated H<sub>2</sub>SO<sub>4</sub> was added to the whole aqueous plant crude extract. A reddish brown colour formed which indicated the presence of steroidal a glycone part of the glycoside.

#### 7) Test for Steroids.

- 2ml of chloroform and concentrated H<sub>2</sub>SO<sub>4</sub> were added to the 5 ml aqueous plant crude extract. Appearance of red colour in the lower chloroform layer indicated the presence of steroids.

#### 8) Test for Terpenoids.

- 2.0 ml of chloroform was added to the 5 ml aqueous plant extract and evaporated on the water bath and then gradually heated after adding with 3ml of H<sub>2</sub>SO<sub>4</sub> (conc). A grey colour formed which showed the entity of terpenoids [5] [6].

## 3. Result and Discussion

FAAS technique provides the presence of elements such as Na, K, Fe, Mg, Ca in ppm level, whereas ICP-MS technique provide the presence of elements such as Cr, Mn, Cu, Pb in ppb level.

**Table 2:** Result of elements by FAAS expressed in ( µg/ml)

Elements	Na (µg/ml)	K (µg/ml)	Fe (µg/ml)	Mg (µg/ml)	Ca (µg/ml)	As (ng/ml)	V (ng/ml)
composition	0.6	10.9	1.15	2.2	13.0	Less than 5	Greater than 2

**Table 3:** Result of elements by ICPMS expressed in ng/ml

Elements	Cr (ng/ml)	Mn (ng/ml)	Ni (ng/ml)	Co (ng/ml)	Cu (ng/ml)	Pb (ng/ml)
Comp.	7	167	6	Less than 2	31	12

**Copper:**

The concentration of copper in the bark of Derris plant is 31 ng/ml shown in table 2 and 3. In 1984, FAO/WHO set 3.00 ppm as the permissible limit in edible plants for copper but for medicinal plants permissible level was not set. In 2005, WHO set permissible level in China and Singapore were 20 ppm and 150 ppm, respectively.

Being toxic, the quantity of copper in plants should be very high though copper plays a vital role in various metabolic processes. It forms many essential components in enzyme systems such as cytochrome oxidase, lysyl oxidase and an iron-oxidizing enzyme in the blood. The deficiency of copper caused anaemia because of its role in facilitating iron absorption and in the incorporation of iron in haemoglobin but its deficiency is found to be rare [7]

**Manganese:**

The concentration of manganese is around 2.2 µg/ml in the Bark of Derris plant as shown in table 2 and 3. In 1984, FAO/WHO set 2.00 ppm as the permissible limit in edible plants for manganese but for medicinal plants permissible level was not set. Its concentration is little higher than set by FAO/WHO

The element manganese is required in the metabolism of food incorporated into bone and biochemical process [8]. It is stored in kidney and liver. It plays important roles in the normal bone structure, reproduction and normal functioning of the central nervous system. The deficiency of manganese causes reproductive failure in both male and female [9]. It also helps in prevention and treatment of diabetes mellitus [10].

**Chromium:**

The concentration of chromium is 7 ng/ml as shown in table 2 and 3 that is less than the permissible level 2 ppm set by (FAO/WHO, 1984) in edible plants. The element chromium plays important roles in the regulation of carbohydrate, nucleic acid, and lipoprotein metabolism and it potentiates insulin action [11]. It may result in liver, kidney and lung damage on chronic exposure to Cr [12]. It also acts as an activator of several enzymes. The deficiency of chromium causes following abnormality in the human body. They are 1) It decreases the efficiency of insulin and increases sugar and cholesterol in the blood. 2) It causes an insulin resistance, impair in glucose tolerance and may be a risk factor for atherosclerotic disease

**Nickel:**

The concentration of nickel is 6 ng/ml below 1.63 ppm which is the permissible limit for nickel set by WHO in edible plants but the permissible limits for medicinal plants have yet not been set. The element nickel is accumulated usually in the leaves [13] but it also present in the roots, stems and seeds. The toxicity of nickel is less common in human being because of its less capacity to absorb in the body [14].

**Iron:**

The Iron is one of important elements for the human beings and animals as it is an essential component of haemoglobin. It plays important role of oxidation of carbohydrates, protein and fat to control body weight if not it is likely to have

diabetes. The scientific analysis suggests that the intake of Iron in higher percentage effects health [15].

The lower percentage intake of Fe causes gastrointestinal infection, nose bleeding, and myocardial infarction as its role is associated with haemoglobin and the transfer of oxygen from lungs to the tissue cells. The element Iron deficiency is common nutritional deficiency in humans

And it is usually occurred due to insufficient dietary intake, excessive menstrual flow or multiple births. Its deficiency result to anaemia [16] [17]. The element iron is important to make tendons and ligaments, for maintaining a healthy immune system and certain chemicals in our brain are controlled by the presence or absence of iron.

The concentration of iron is 1.15 µg/ml in the bark of Derris scandens and it is less than the permissible level set by WHO for iron 20 ppm [18].

**Lead:**

The element Lead has no important role in biochemical or physiological processes and it is hazardous to human being. It usually causes blood pressure, kidney damage, miscarriages and subtle abortion, brain damage, decline fertility of men through sperm damage, diminishing abilities of children and disruption of nervous systems [19]. The permissible limit set by WHO in 1992 is 0.43 ppm for edible plants but for medicinal plants the limit was 10 ppm set by China, Malaysia, Thailand and WHO [20]. The bark of Derris scandens contained 12 ng /ml which below permissible limit.

**Potassium:**

The element potassium is an important macro-element for a human. It plays roles in muscle contraction, in lipids metabolism, in proteins synthesis, maintaining the fluid and electrolyte balance in the body and in nerve impulses sending [21]. The bark of this plant contained 10.9 µg/ml.

**Sodium:**

Sodium composition in this plant is 0.6 µg/ml. It is important for all living organisms. It is an important electrolyte in the blood. It plays role in dehydration. The excess percentage of Na causes cell break down [22]. The element sodium is essential for many regulation systems in the body. Sodium is required is 2.4 g daily [23].

**Calcium:**

The element calcium plays role in bones, teeth, muscular system and heart functions. Calcium is essential for absorption of dietary Vitamin B, for the synthesis of the neurotransmitter acetylcholine and for the activation of enzyme pancreatic lipase [24]. For the coagulation of blood, the proper functioning of the heart, nervous system and the normal contraction of muscles the element calcium is required.

The concentration of Ca in the bark of Derris scandens is 13.0 µg/ml as shown in figure 1 and 2. By regulating endo-exo-enzyme and blood pressure, Ca play role in the function of membrane and muscles [25].

Magnesium:

The bark of this plant contains 2.2 µg/ml. The element Magnesium is essential in plasma and extracellular fluid to maintain osmotic pressure, in many enzyme catalyzed reaction usually in those nucleotides participate reaction in which reactive species is Mg salt, e.g. MgATP. In many enzymes involved in proteins, lipids, carbohydrate metabolism and chlorophylls, Mg is present.

The deficiency of Mg causes abnormal irritability of muscle and convulsions and excess Mg with depression of the central nervous system [26].

The lack of magnesium in humans caused muscle spasms and has been associated with a high blood pressure, many cardiovascular diseases, diabetes, and osteoporosis. 350 mg/day for men and 300 mg/day for women is required daily in day [21]. The lack of Mg in intracellular is correlated with the impaired function of many enzymes utilizing high energy phosphate bonds, as in the case of glucose metabolism [27].

**Table 4:** Result of Phytochemical Screening of the Extracts

Name of extract	Alkaloid	Saponin	Flavonoid	phenol	Tannin	Glycoside	Terpenoid
Water	+	+	+	+	+	+	+
CHCl <sub>3</sub>	+	+	+	+	+	+	+
Petroleum Ether	-	+	-	+	-	-	+

Note: + indicates present, - indicates absent

The experimental result shown in table 4 shows that the above secondary compounds are contained in the bark of Derris plant. The bark of plant show maximum concentration to water extract and chloroform extract. Among this again more to water extract. The flavonoids exhibits antioxidant, anticancer, anti-inflammatory activities. It is shown to be effective in lower intestinal tracts, heart diseases and also has the properties of preventing oxidative damage and carcinogenesis. Also, the phenol and its derivatives have shown the abilities to blocked enzymes that cause inflammation; antioxidant, immune enhancers, anti-clotting and hormone modulators. Saponins have the roles on growth, feed intake, protein digestion, immune system, cell membranes, antioxidant effects and also antimicrobial roles [28]. The compound alkaloids act by blocking or intensifying the actions of neurotransmitters, chemicals released by nerve cells in response to an electrical impulse known as neural signal. Neurotransmitters diffuse into neighboring cells where they produce an appropriate response, such as an electrical impulse, in another nerve cell or contraction in a muscle cell.

#### 4. Conclusion

Out of three extracts bark of Derris scandens (Roxb) Benth screen for phytochemical examinations, water extract was found the presence of maximum phytoconstituents such as glycosides, saponin, alkaloid, terpenoid, steroid, phenol and tannins. Petroleum ether extract was found minimum phytoconstituents which is shown in the **Table 4**. In addition to that the bark powder of the plant sample observed the presence of the following elements such as Na (0.6 µg/ml), K (10.9 µg/ml), Fe (1.156 µg/ml), Mg (2.2156 µg/ml), Ca (13.0 µg/ml), As (less than 5 ng/ml), V (less than 2 ng/ml), Cr (7 ng/ml), Mn (167 ng/ml), Ni (6 ng/ml), Co (less than 2 ng/ml), Cu (31 ng/ml), and Pb (12 ng/ml). The concentration of traces elements like Ni, Cu, As, Cr, Pb and Mn are within the permissible limit set by WHO/FAO. The bark of Derris scandens (Roxb) Benth. can be used as food ingredient and also as medicine in the treatment of many human abnormalities.

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