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PRELIMINARY SCREENING OF PHYTOCHEMICAL AND MINERAL CONTENT OF CHLOROFORM AND METHANOL EXTRACTS OF *STRYCHNOS NUX VOMICA* L AND *OPHIORRHIZA RUGOSA* WALL

BHAVYA D. K* [1] AND KRISHNAMOORTHY M[2].

[1] P.G.Department of Biochemistry, St.Aloysius College (Autonomous), Mangalore-575003 India.
[2] Yenepoya Research Centre,Yenepoya University,Mangalore-575018,India.

ABSTRACT

Medicinal plants are the nature gifts to human to lead disease free healthy life. The examination of plants for bioactive secondary metabolites is an area which most scientist have recently focused. *Strychnous nux vomica* L(SNV) and *Ophiorrhiza rugosa* Wall(OR) are endangered plants of the family Loganiaceae and Rubiaceae respectively .In the present study they were analyzed for their phytochemical constituents and minerals. The study revealed the presence of alkaloids, phenols, flavonoids by qualitative analysis. The total phenolic content was 0.143mg and 0.3297mg/mg respectively in the methanol extract of *S.nux vomica* and chloroform extract of *O.rugosa*. Mineral analysis showed the presence of Fe, Na, Mg, Mn, Zn and Cu in both the plants and Cd and Pb are absent in both the plants.

KEY WORDS: Phytochemical, Mineral, Atomic absorption spectroscopy, *Ophiorrhiza rugosa* Wall, *Strychnous nux vomica* L.

BHAVYA D. K
P.G.Department of Biochemistry, St.Aloysius College (Autonomous), Mangalore-575003 India.

INTRODUCTION

The use of plants as medicine is an ancient practice common to all societies especially the Indian society. This practice continues to exist in the developing nations. It is on this basis that researchers keep on workingwith medicinal plants in order to produce the best medicines for physiological uses [1]. Minerals are inorganic substances that are found in soil and rocks. They are essential nutrients that the body needs to survive and carry out daily functions and processes. We receive minerals by eating plants that absorb them from the earth and by eating meat from animals, which graze on plants [2]. Minerals keep us healthy and have key roles in several body functions. We acquire these important nutrients from our daily diet. Some minerals such as calcium are needed in large quantities, while others, such as zinc are only needed in trace amounts. Zinc is an essential mineral that is important for keeping our immune system strong and helps our body fight infections, heal wounds and repair cells [3, 4]. Medicinal plants are the back bone of traditional remedy. Plants have been a rich source of drug because they produce a wide array of bioactive molecules. Ophiorrhiza is a predominantly herbaceous genus distributed from East India to the west of Pacific and south of China to the north of the Australia. Some species of Ophiorrhiza have been used in folk medicine as an antitussive, expectorant, analgesic, anti-helminthic in stomachache, and headache[5].*Ophiorrhiza rugosa* Wall (Rubiaceae) (OR)is a small herb, often rooting near the base, glabrous or more or less pubescent. The shape of the leaf is elliptical or elliptic lancelet, 10x5cm long. Its apex is obtuse or acute, usually base rounded, glabrous or with a few scattered hairs above. Lower surface of the leaf is whitish in color and pubescent on the nerves beneath. Petioles are up to 1cm long. Flowers are white, small, in short terminal cymes up to 2.5cm in diameters. Corolla tube is 3-5 mm long. Capsules are 6x2mm size, obreniform, compressed, glabrous or pubescent. These plants are commonly available on the banks of streams in shady places. These plants are most commonly available in rainy season. Flowering season is August- January[6]. *Strychnos nux-vomica* L (Loganiaceae) (SNV)is a medium sized tree, both wild and cultivated, throughout India. The plant is popularly known as Snake wood in English. In the Indian system of medicine, the medicinal attribution of this species has been known for a long time. As per the traditional claims, the root bark is used in cholera, leaves are used in chronic wounds and ulcers, and seeds are used as an appetizer, antiperiodic purgative, in asthma, diabetes, skin diseases etc,.

MATERIALS AND METHODS

Plant material

OR and SNV were collected from the Western Ghats of Karnataka, authenticated by Dr. Gopalakrishna Bhat K., a botanist.

Sample preparation

The leaves of *O.rugosa*(OR) and seeds of *S.nux vomica* (SNV) collected from ripened fruits, were dried at 37°C (shade dried) .The dried materials were then ground to a powder using mortar and pestle, the powder is stored in airtight closed bottles. The powdered (50g) samples of each plant were subjected for successive extraction with chloroform and methanol for 48 hrs at room temperature with continuous stirring. After extraction supernatants were collected by filtration and the solvents were removed by rotary evaporator for obtaining the extracted compounds,that were used for phytochemical analysis.

Preliminary phytochemical analysis

Phytochemical examinations were carried out for all the plant extracts as per the standard methods[7] to identify the useful constituents like alkaloids, flavonoids, saponins,tannins, phenols and terpenoids.

Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
Wagner's test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of a brown/reddish precipitate indicates the presence of alkaloids.
Dragendroff's test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of a red precipitate indicates the presence of alkaloids.
Hager's test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates
Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.
Molisch test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube followed by sulphuric acid treatment. Formation of the violet ring at the junction indicates the presence of carbohydrates.
Benedict'sTest: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Detection of saponins
Froth test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of froth indicates the presence of saponins.
Foam test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes, then it indicates the presence of saponins.

Detection of phenols
Ferric chloride test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of flavonoids
Lead acetate test: Extracts were treated with a few drops of lead acetate solution. Formation of yellow coloured precipitate indicates the presence of flavonoids.

Determination of total phenolic content
Total phenols were estimated in the methanol and chloroform extract of both the plants by the method proposed by Barreira *et al* [8]. Phenols react with phosphomolybdic acid in Folin-Ciocalteu reagent in alkaline medium to produce a blue-coloured complex (molybdenum blue) which can be determined spectrophotometrically at 725 nm. Gallic acid is used as the standard.

Mineral analysis
One gram of powdered seeds of SNV and leaves of OR respectively were subjected for acid digestion (Conc. HNO_3) .After complete evaporation of brown fumes , 10 ml of acid mixture (HNO_3 and $HClO_4$;4:1 ratio) was added and digested to evaporate white fumes for 2-3 hrs. After complete evaporation of white fumes, residue was made up to 25ml with 10% HNO_3. Blank was prepared by the same procedure without sample. The mineral contents were detected and quantified using Atomic absorption spectroscopy[9] (GBC Scientific Eq. Pvt. Ltd- SAVANT-AA).

Statistical Analysis
The data were subjected to statistical analysis. All the assays were recorded in triplicates and the values were expressed as mean ± S.D.

RESULTS AND DISCUSSION

The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities.

Table 1
Qualitative analysis of phytochemical constituents.

Phytochemicals	SNV(Methanol)	SNV(chloroform)	OR(Methanol)	OR(Chloroform)
Detection of alkaloids				
Mayer's test	+	+	+	+
Wagner's test	+	+	+	+
Dragendroff's test	+	+	+	+
Hager's test	+	+	+	+
Detection of Carbohydrates				
Molisch test	+	+	+	+
Benedict's test	+	+	+	+
Detection of Saponins				
Froth test	-	-	-	-
Foam test	-	-	-	-
Detection of phenols				
Ferric chloride test	+	-	+	+
Detection of flavonoids				
Lead acetate test	+	-	+	+

SNV=Strychnos nux vomica, OR= Ophiorrhiza rugosa.

Table 1 shows the phytochemical analysis of chloroform and methanol extracts of SNV and OR. Phytochemical screening of the crude extracts revealed the presence of alkaloids, carbohydrates and phenolics in all the extracts where as flavonoids were present in all the three extracts except chloroform extracts of SNV. Saponins were absent in all the four extracts.

Table 2
Total phenolic content of methanol and chloroform extract of OR and SNV.

Plant extracts	Total phenolic content (mg/mg of extracts)
SNV(Methanol)	0.143±0.0012
SNV(chloroform)	0.0345±0.0013
OR(Methanol)	0.1795±0.0052
OR(Chloroform)	0.3297±0.0054

The total phenolic content present in the methanolic extract of SNV and OR were 0.143±0.0012 and 0.1795±0.0052 (mg/mg of extracts) respectively. Chloroform extracts of SNV and OR contains 0.0345±0.0013 and 0.3297± 0.0054(mg/mg of extracts) respectively. Highest phenol content was observed in chloroform extract of OR.

Figure1
Standard Graph of Gallic acid

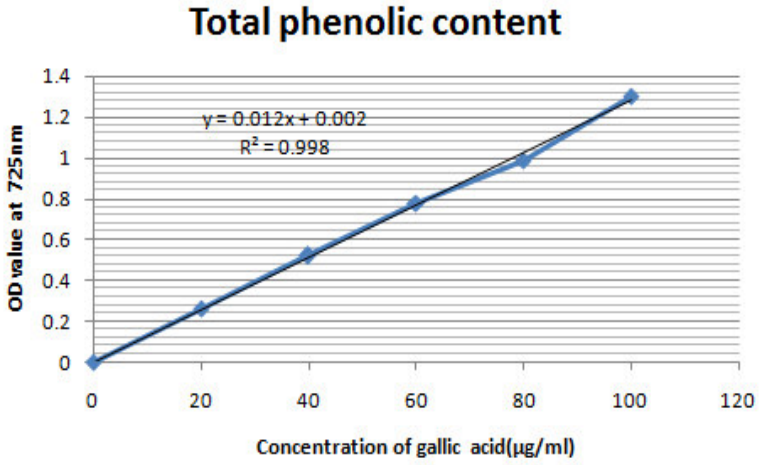

Table 3
Operating parameters for working element.

Elements/minerals	Wavelength -nm	Lamp intensity- mA	Slit width- nm	Replicates
Fe	372	7	0.20	3
Na	589	5	0.5	3
Mg	202.6	8	1.0	3
Mn	279.5	5	0.2	3
Pb	283.30	5	0.5	3
Cd	228.80	3	0.5	3
Cu	324.70	3	0.5	3
Zn	213.90	5	0.5	3

Table 4
Mineral contents (µg/g of sample) in SNV and OR

Minerals	SNV	OR
Fe	22.6±0.531	831±0.595
Na	67.5±0.547	287±0.592
Mg	621±0.664	3217±1.31
Mn	31.1±0.225	82.6±0.547

Table 5
Heavy metal contents (µg/g of sample) in SNV and OR

Minerals	SNV	OR
Zn	0.233±0.0153	32.5±0.509
Cu	4.43±0.121	9.51±0.341
Cd	ND	ND
Pb	ND	ND

ND=Not detectable.

The mean concentration level of mineral and heavy metal found in OR and SNV is summarized in Table 4 and 5. The analysis of various elements in both the plants indicated that Fe,Na,Mg,Mn,Zn, Cu were present and Cd, Pb were absent in both the plants..These elements are very essential for the synthesis of secondary metabolites. Mg content was more in SNV and OR than the other mineral. Although sodium is often maligned as a cause of high blood pressure, it also plays several essential roles in the body. Sodium helps control blood pressure and regulates the function of muscles and nerves, that is why sodium concentrations are carefully controlled by the body [10, 11]. However, most people consume far more sodium than their bodies need. Iron is an essential element for human being and animals and is an essential component of heamoglobin. The iron content is present in SNV and OR respectively 22.6±0.531 and 831±0.595 µg/gm. Plants are used to relieve various diseases in Ayurveda. Present study showed that methanol and chloroform extracts of leaves of OR and seeds of SNV can be used as potential source for drug preparation due to presence of alkaloids, phenols and flavonoids.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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