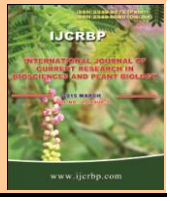
EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 Volume 2 Number 3 (March-2015) pp. 67-72

www.ijcrbp.com



Original Research Article

Phytochemical and Mineral Analysis of Root of *Loeseneriella arnottiana* Wight

P.S. Prajna[1]* and P. Rama Bhat[2]

[1]Research and Development Centre, Bharathiar University, Coimbatore - 641 046, Tamil Nadu, India
[2]Post Graduate Department of Biotechnology, Alva's College, Moodbidri - 574 227, Karnataka, India

**Corresponding author.*

Abstract

A preliminary investigation on phytochemical analysis of root extracts of *Loeseneriella arnottiana* (Syn.: *Hippocratea arnottiana*) revealed the presence of tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. Quantitative analysis showed the presence of highest protein (80%) and phenolic (9.2%) contents in methanolic extract and highest flavonoid content (1.91%) in chloroform extract. Different elemental constituents at trace levels in the plants play an effective role in the type of medicines prepared. Root extract of *L. arnottiana* was analysed for some essential elements like Fe^{+2}, Mn^{+2} , K^{+}, Mg^{+2}, Na^{+}, Ca^{+2}, Zn^{+2} and ash values which confirmed the presence of mineral matter in the root. The results also revealed the presence of medicinally important bioactive constituents in the root.

Keywords

Ash analysis
Medicinal plant
Minerals
Phytochemicals

Introduction

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Medicinal plants have formed the basis of health care throughout the world. It is useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents that produce a definite physiological action on the human body. Phytochemicals are naturally occurring in the plants that have defense mechanism and protect from various diseases. Medicinal plants come into preparation of various drugs singly or in combination or even are used as the source of raw material for the other medicines (Mohanta et al., 2003).

Active constituent of medicinal plants are metabolic products of plant cells and a number of trace elements play an important role in the metabolism (Rajurkar and Damame, 1997). They also play an important roles in chemical, biological, biochemical, metabolic, catabolic and enzymatic reactions in the living organism which will lead to the formation of active organic constituents (Serfor-Armah et al., 2002). More than 40 elements have been considered essential to life systems for the survival of both mammals and plants. An element is considered essential when reduction of its exposure below a certain limit results consistently in a reduction in a physiologically important function, or when the element is an integral part of an organic structure performing a vital function in that organism

(Armah et al., 2001). Ashes give us an idea of the mineral matter contained in a plant because mineral matter may be the cause of a pharmacological effect (Sunggyu, 2005).

The genus *Hippocratea* belongs to the family Celastraceae or staff vine or bittersweet family. The family has about 90-100 genera and 1,300 species of vines, shrubs and small trees. Most of the representatives of this family are shrubs and some are climbers by their branchlets, twisting round their supports. *Loeseneriella arnottiana* (Syn.: *Hippocratea arnottiana*) is a member of this family. It is a climbing shrub distributed in West Coast and Western Ghats from South Canara to Malabar and Travancore and is found to be used by folklore practitioners. Members of Hippocrataceae are found to have various pharmacological properties. In the present study an attempt was made to explore the medicinal value of *Loeseneriella arnottiana* by determining the phytochemical and the mineral elements present in the root.

Materials and methods

Collection of plant material

The roots of *Loeseneriella arnottiana* plant were collected from Bantamale reserve forest, Sullia Taluk, Karnataka, India and authenticated.

Extraction

The roots were shade dried, powdered and extracted using water and different solvents *viz.,* ethanol, methanol, chloroform, acetone and petroleum ether by Soxhlet method.

Preliminary phytochemical screening

Freshly prepared extracts of the roots of *L. arnottiana* were subjected to preliminary phytochemical screening as per the method of Harbone (1984).

Steroids/terpenoids: Two mg of dry extract was shaken with chloroform. A red colour after the addition of concentrated sulphuric acid along the sides of the test tube indicated the presence of steroids.

Alkaloids: Dragendroff's test was used for the detection of alkaloids. Dragendroff's reagent was added to a little of the extract dissolved in respective solvent. Alkaloids gave orange red precipitate.

Saponins: Formation of froth which lasts for a long time when the sample was mixed with water and shaken well was taken as an indication of saponin.

Flavonoids: A few drops of 1% ammonia solution were added to the aqueous extract of the powdered root sample in a test tube. A yellow coloration observed would mean it contains flavonoids.

Glycosides: Glycosides were detected by Borntrager's test. The extract was hydrolyzed with dilute hydrochloric acid for few hours in water bath. The hydroxylate was extracted with chloroform and equal quantity of dilute ammonia was added to the chloroform layer. Pink colour showed the presence of glycosides.

Tannins: To 5g of powdered root sample in a beaker was added 50ml of distilled water and boiled for 3 min on a hot plate and then filtered. To aliquot of the filtrate was added 10% ferric chloride solution. A blue or green colour would indicate the presence of tannins.

Resins: Resins were identified by the appearance of turbidity after dissolving the extract in acetone and pouring into distilled water.

Quantitative phytochemical analysis

Protein: Protein content was estimated by (Sadasivam and Manickam, 2009). Protein reacts with phosphomolybdic-phosphotungstic components in the Folin- Ciocalteau reagent and produce blue coloured complex which is measured at 660 nm.

Phenolics: Phenolic content was analysed using Folin-Ciocalteau colorimetric method (Sadasivam and Manickam, 2009). Phenols react with phosphomolybdic acid in the Folin- Ciocalteau reagent in alkaline medium to produce blue coloured complex which is measured at 650 nm.

Flavonoids: Aluminium chloride colorimetric method was used for flavonoids determination. 0.5 ml of extract in methanol were separately 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. It remained at room temperature for 30 minute

the absorbance of the reaction mixture was measured at 415 nm.

Physicochemical evaluation

Ash: Ash is the inorganic residue remaining after water and organic matter have been removed by heating, which provides a measure of total amount of minerals within the drug.

Determination of total ash value: Accurately weighed about 1g of air dried powdered drug was taken in a crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air-dried drug.

Determination of acid insoluble ash value: The ash obtained as directed under total ash was boiled with 25 ml of 2N hydrochloric acid for 5 min. The insoluble matter was collected on an ash less filter paper, washed with hot water, dried the filter paper, ignited and weighed. Then calculate the percentage of acid insoluble ash with reference to the air-dried drug.

Determination of water soluble ash value: The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 min at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

Elemental analysis: About 0.1g of root sample is digested in 10ml of 3:1 (nitric acid: perchloric acid) acid. Digested sample is filtered and volume is made up to 25ml in distilled water and analysed by atomic absorption spectroscopy.

Results and discussion

Highest protein content (80%) was found in methanolic extract followed by ethanolic extract (72.3%) whereas, lowest in petroleum ether extract (9%) (Fig. 1). Phenolic content was found highest in methanolic extract (9.2%) and ethanol extract (8.96%) and lowest in chloroform extract (0.48%) (Fig. 2). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh et al., 2007). They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han et al., 2007).

Fig.1: Estimation of proteins in the roots of *Loeseneriella arnottiana* extracted in different solvents (Error bars indicate ± SD).

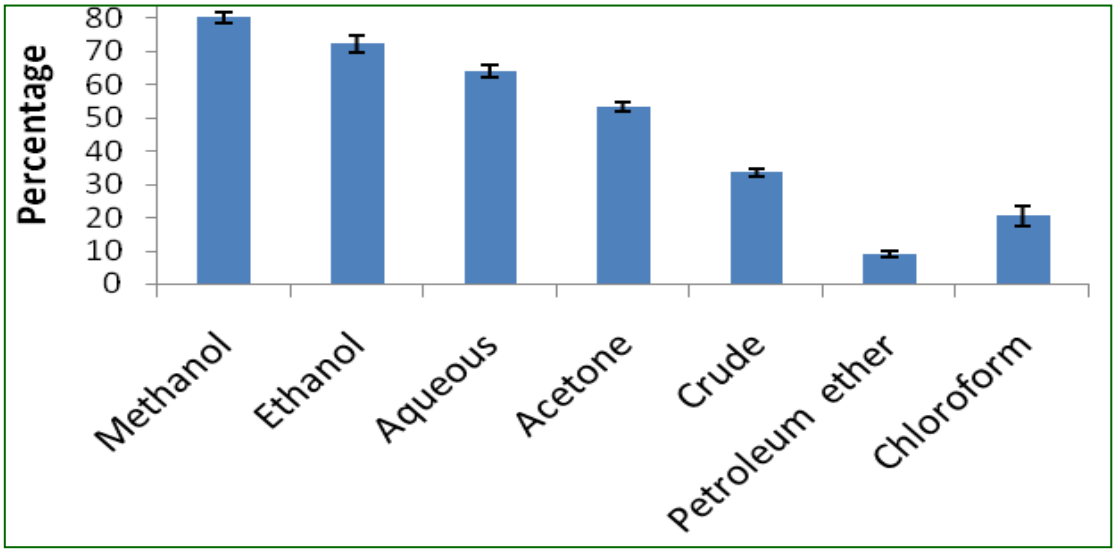
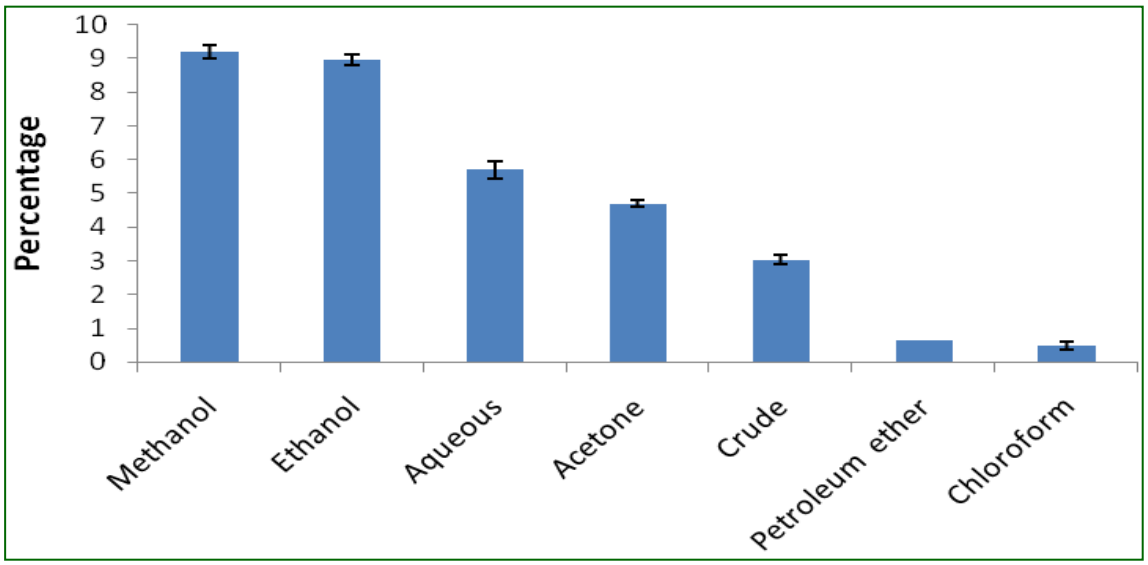


Fig. 2: Variations in total phenolics in roots of *Loeseneriella arnottiana* extracted in different solvents (Error bars indicate ± SD).



Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols, etc. Preliminary phytochemical analysis of the plant extracts is shown in Table 1 and flavonoid contents of roots are given in Fig. 3. In the present study the phytochemicals such as tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids were observed. According to Marjorie (1996) and Ali et al. (2008) the flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and will be involved in antimicrobial activities against wide array of microorganisms *in vitro*. These substances also effective antioxidant and showed strong anticancer activities as reported by Salah et al. (1995). Highest flavonoid content was found in chloroform extract (1.91%) followed by acetone extract (1.53%) and lowest in petroleum ether extract (0.12%). Astringent action of tannins which makes the plants useful in the treatment of diabetes and rickets. The plant extracts were also containing traces of saponins which were known to produce inhibitory effect on inflammation (Just et al., 1998).

Saponins have the property of precipitating and coagulating red blood cells (Sodipo et al., 2000). Steroids have been reported to have antibacterial properties (Raquel, 2007) and they are very important compounds especially due to their relationship with compounds such as sex hormones. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity. Glycosides are known to lower the blood pressure according to many reports (Nyarko et al., 1990). The results obtained in this study further strengthen the medicinal value and its applications.

Table 1. Qualitative phytochemical analysis of roots of *Loeseneriella arnottiana*

Phytochemicals	Present (+) / Absent (-)
Terpenoids	+
Alkaloids	+
Saponins	+
Flavonoids	+
Glycosides	+
Tannins	+
Resins	-

Table 2. Ash values of roots of *Loeseneriella arnottiana*

Ash contents	Percentage
Total ash	7.565
Acid insoluble ash	4.54
Water soluble ash	25.84

The ash values in the roots of *L. arnottiana* are given in Table 2. The total ash value of root was 7.56% which implies that the root has normal complexes of inorganic and organic component (British Pharmacopoeia, 1980). The value of the acid insoluble ash is 4% which implies that the normal acid insoluble ash has a portion of the ash contents which was acid soluble and hence may be physiologically important as

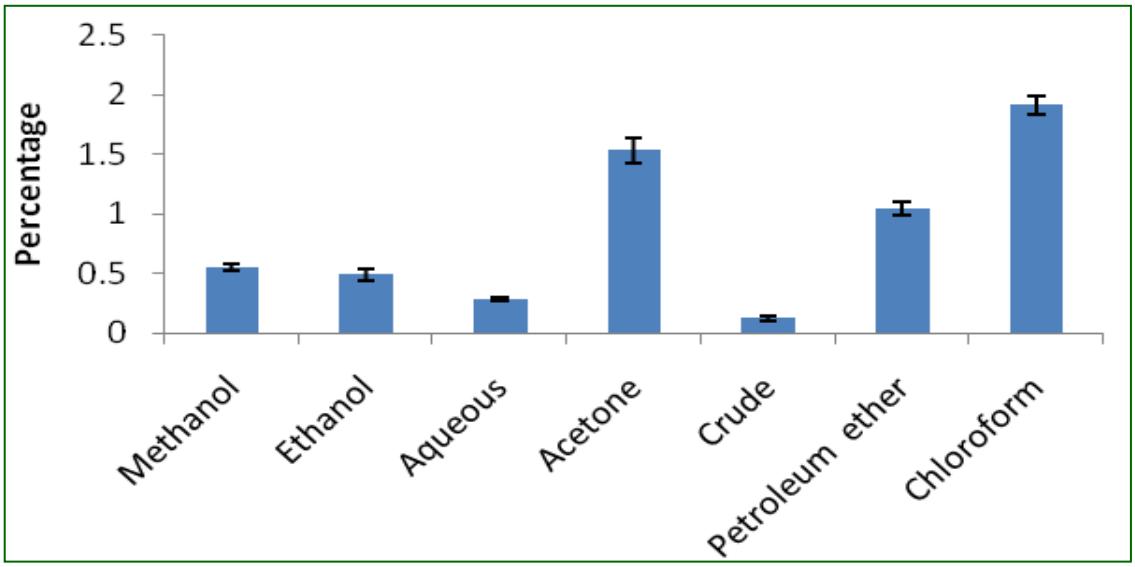
salts in the body when consumed. It is also indicative of high digestibility of the plant (Ibrahim et al., 2010).

In the present study the root extract of *L. arnottiana* exhibited some essential elements like Fe^{+2}, Mn^{+2}, K^{+}, Mg^{+2}, Na^{+}, Ca^{+2} and Zn^{+2} at different concentrations (Table 3). The concentrations of potassium and calcium were found to be highest of 6776 ppm and 2660 ppm respectively. Potassium participates actively in the maintenance of the cardiac rhythm (Martin et al., 1985) and in constipation. Ca is the main constituent of the skeleton and is important for regulating many vital cellular activities such as nerve and muscle function, hormonal actions, blood clotting and cellular mortality. Concentrations of magnesium, sodium and iron were found to be 530ppm, 205ppm and 136ppm respectively. Iron is an essential element for human beings and animals and is an essential component of hemoglobin. It facilitates the oxidation of carbohydrates, protein and fat to control body weight, which is very important factor in diabetes. Mg has important roles in the metabolism of cholesterol as well as heart diseases. Sodium is essential for regulation of osmotic pressure of the body and helps to maintain acid-base and water balance of the body. Its deficiency causes loss of body weight and nerves disorder.

Table 3. Elemental analysis in the roots of *Loeseneriella arnottiana*

Element	Concentration (ppm)
Iron (Fe)	136.00
Manganese (Mn)	1.25
Magnesium (Mg)	530.00
Sodium (Na)	205.00
Potassium (K)	6776.75
Calcium (Ca)	2660.00
Zinc (Zn)	9.00

Fig. 3: Estimation of flavonoids in roots of *Loeseneriella arnottiana* extracted in different solvents (Error bars indicate ± SD).



Zinc is the component of more than 270 enzymes and its deficiency in the organism is accompanied by multisystem dysfunction (Zinpro, 2000). Zinc and manganese was found to be lesser in concentration of about 9ppm and 1.25ppm respectively. Mn in human causes myocardial infarction, diabetes and other cardiovascular diseases (Barceloux, 1999). Among the various elements detected can be used in the treatment of different diseases. The knowledge of element concentrations in the plant gives a new insight into their potential use in therapeutics. The active constituents of medicinal plant or the metabolic products of plant cells and number of trace elements are closely linked to human growth and general health. The variations in concentration of the elements are attributed to the nature of the plant as well as its surroundings (Rajurkar and Damame, 1997).

Conclusion

The results of present research work revealed the presence of medicinally important bioactive constituents in the root of *L. arnottiana*. Several studies have confirmed the presence of these phytochemicals and minerals which contribute to medicinal as well as physiological properties in the treatment of different ailments. Therefore, the root extracts could be a good source for drugs.

References

Ali, S.S., Kasoju, N., Luthra, A., Singh, A., Sharanabasava, H., Sahuand, A., Bora, U., 2008. Indian medicinal herbs as source of antioxidants. Food Res. Int. 41, 1-15.

Armah, Y.S., Nyarko, B.J.B., Akaho, E.H.K., Kyere, A.W.K., Osae, S., Boachie, K.O., Osae, E.K., 2001. Activation analysis of some essential elements in five medicinal plants used in Ghana. J. Radioanalyt. Nucl. Chem. 250(1), 173-176.

Barceloux, G. D., 1999. Manganese, Nickel. Clin. Toxicol. 37, 239-258, 293-309.

British Pharmacopoeia, 1980. Ash value, Acid Insoluble Ash, Water Soluble Extractive and Alkohol Soluble Extractive, Vol. II, Appendix xii, Majesty's Stationary Office, London. pp. 1276– 1277.

Han, X., Shen, T., Lou, H., 2007. Dietry polyphenols and their biological significance. Int. J. Mol. Sci.8(9), 950-988.

Harbone., 1984. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 2nd Edn. Chapman and Hall, London.

Ibrahim, J., Ajaegbu, V.C., and Egharevba, H.O., 2010. Pharmacognostic and phytochemical analysis of *Commelina benghalensis* L. J. Ethnobot. Leaflet. 14, 610–615.

Just, M.J., Recio, M.C., Giner, R.M., Cueller, M.U., Manez, S., Billia, A.R., Rios, J.L., 1998. Antiinflammatory activity of unusual lupine saponins from *Bupleurum fruticescens*. 64, 404-407.

Marjorie, C., 1996. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12, 564-582.

Martin Jr., D. W., Mayers, P. A., Rodwell, V. W., Granner, D. K., 1985. Harper's Review of Biochemistry, 20th Edn. Lange Medical Publications, California. pp. 651-660.

Mohanta, B., Chakraborty, A., Sudarshan, M., Dutta R.K., Baruah, M., 2003. Elemental profile in some common medicinal plants of India. Its correlation with traditional therapeutic usage. J. Radioanalyt. Nucl. Chem. 258(1), 175-179.

Nyarko, A.A., Addy, M.E., 1990. Effects of aqueous extract of *Adenia cissampeloides* on blood pressure and serum analyte of hypertensive patients. Phytother. Res. 4(1), 25-28.

Rajurkar, N.S., Damame, M.M., 1997. Elemental analysis of some herbal plants used in the treatment of cardiovascular diseases by NAA and AAS. J. Radioanalyt. Nucl. Chem. 219(1), 77-80.

Raquel, F.E., 2007. Bacterial lipid composition and antimicrobial efficacy of cationic steroid coppounds. Biochem. Biophys. Acta 1768(10), 2500-2509.

Sadasivam, S., Manickam, A., 2008. Biochemical Methods. New Age International (P) Limited, New Delhi.

Salah, N., Miller, N.J., Pagange, G., Tijburg, L., Bolwell, G.P, Rice, E., Evans, C., 1995. Polyphenolic flavonoids as scavenger of aqueous phase radicals as chai breaking antioxidant. Arc. Biochem. Broph. 2, 339-346.

Serfor-Armah, Y., Nyarko, B.J.B., Akaho, E.H.K., Kyere, A.W.K., Osae, S., Oppong Boachie, K., 2002. Multielemental analysis of some traditional plant medicines used in Ghana. J. Trace Microprobe Tech. 20, 419-427.

Singh, R., Singh, S.K., Arora, S., 2007. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. Food Chem. Toxicol. 45, 1216-1223.

Sodipo, O.A., Akiniyi, J.A., Ogunbamosu, J.U., 2000. Studies on certain characteristics of extracts of bark of *Pansinystalia macruceras* (K. Schemp) Picrre Exbeille. Global J. Pure Appl. Sci. 6, 83-87.

Sunggyu L., 2005. Encyclopedia of Chemical Processing. 3rd Edn. Vol. I, CRC Press. pp. 31-33.

Zinpro Corporation, 2000. Epithelial tissue: body's first line of defense depends upon trace minerals. Trace Miner. Focus 6, 1-8.