

Phytochemical Screening for Secondary Metabolites in *Boswellia serrata* Roxb. and *Wrightia tinctoria* (Roxb.) R.Br.

Prattipati SUBHASHINI DEVI^{1*}, Botcha SATYANARAYANA¹,

Maradana TARAKESWARA NAIDU²

¹Andhra University, Department of Biochemistry, Visakhapatnam, India; devi.subashini@gmail.com (*corresponding author); satyabiochemau@gmail.com

²Andhra University, Department of Botany, Visakhapatnam, India; tarakeswaranaidu@gmail.com

Abstract

Boswellia serrata, an important source of oleo-gum resin commonly known as Indian olibanum and *Wrightia tinctoria* are well documented for their pharmaceutical properties. Indiscriminate removal, difficulty in vegetative propagation and poor germination accounts for the depletion of useful plant species. Plant tissue culture techniques are used as an alternative method for the production of specific metabolites and also for the propagation of plant species at a large scale. In the present study preliminary screening for the presence of secondary metabolites was reported in order to understand the levels of phytochemicals, so that *in vitro* production of secondary metabolites using cell cultures will be initiated in future studies. Both qualitative and quantitative analysis of the two plants show the presence of all the three major groups of secondary metabolites, like nitrogen containing alkaloids, phenolic compounds like flavonoids, tannins, terpenes like steroids, saponins etc. Significant levels of all three major secondary metabolites were present in both species. However, higher activities of phenols (277.0 ± 4.36), tannins (240.67 ± 5.21), alkaloids (963.3 ± 11.7) and flavonoids (150.0 ± 2.89) were observed in *B. serrata*. All the three major groups of secondary metabolites in both species demonstrate the rich content of many useful biochemicals like pharmaceuticals, flavors, fragrances, agricultural chemicals etc. However, higher quantities in *B. serrata* indicates its richness in medicinal properties over *W. tinctoria*.

Keywords: alkaloids, flavonoids, phytochemical screening, phenols, tannins

Introduction

Since ancient times, people have been exploring the nature for plants in the search of new drugs. This has resulted in the use of a large number of medicinal plants with curative properties that can treat various diseases. Nearly 80% of the world's population relies on traditional medicine for primary healthcare, most of which involve the use of plant extracts. In India, almost 90% of the prescriptions were plant based in the traditional systems like unani, ayurveda, homeopathy and siddha (Lee, 1999). A large number of phytochemicals belonging to several classes have been shown to have inhibitory effects on all types of microorganisms *in vitro* (Cowan, 1999). Plant products have been part of phytomedicine since immemorial time. Knowledge on the chemical constituents of plants is desirable because such information will be valuable for the synthesis of complex chemical substances. Such phytochemical screenings of various plants are reported by many workers (Siddiqui *et al.*, 2009; Kumar *et al.*, 2010; Chitravadivu *et al.*, 2009).

Boswellia serrata Roxb. (Burseraceae) is a perennial deciduous resin producing tree distributed in the tropical parts of Asia, Africa and Middle East (Anonymous, 1962; Gaofeng *et al.*, 2006). The gum resin obtained from its bark is

called 'Indian Olibanum' or 'Salai guggul' and is credited for its astringent, stimulant, expectorant, diuretic, antipyretic and antiseptic properties; it has also reported to be useful in ulcers, goiter, piles, diarrhoea etc. In recent years the gum resin has been used extensively in pharmaceutical formulations for relieving aches and pain, particularly associated with arthritis (Singh and Atal, 1986; Chikamai, 2002).

Wrightia tinctoria (Roxb.) R.Br. is a small deciduous tree of the family Apocynaceae distributed in Central India, Burma and Timor (Chary, 1980). This plant is extensively used in the Indian system of medicine. Fresh leaves are pungent and are chewed for toothache relief (Kirtikar and Basu, 1975; Anonymous, 1976). Bark and seeds are anti-dysenteric, carminative, astringent, aphrodisiac, diuretic, used in flatulence, stomach pain and bilious affections. The plant is very useful as stomachic, in the treatment of abdominal pain, skin diseases, anti-diarrhoeal and anti-haemorrhagic (Shah and Gopal, 1988). Oil emulsion of *W. tinctoria* pods is used to treat psoriasis and also have fungicidal activity against *Pityrosporum ovale* recovered from dandruff (Krishnamoorthy and Ranganathan, 2000; Reddy *et al.*, 2000).

In the present study qualitative and quantitative analysis for preliminary as well as secondary metabolites in *Boswellia serrata* and *Wrightia tinctoria* were reported.

Materials and methods

Collection of plant material

Fresh leaf material of *Boswellia serrata* and root material of *Wrightia tinctoria* were collected during August month from Pydi Bhimavaram forest area, near Rajahmundry, Andhra Pradesh, India. Taxonomic identification of the plants was carried out with the herbarium present in the Department of Botany, Andhra University, Visakhapatnam.

Extraction of plant material

The fresh leaf material of *Boswellia serrata* and root material of *Wrightia tinctoria* collected were washed thoroughly with running tap water and air dried under shade. After complete shade drying the plant material was grinded and the powder was kept in small plastic bags with paper labelling. The grinded leaf and root material of 5 gm crushed in 25 ml of sterile water, boiled at 50-60 °C for 30 minutes in water bath and it was filtered through Whatman No.1 filter paper. Then filtrate was centrifuged at 2500 rpm for 15 minutes and the filtrate was stored in sterile bottles at 5 °C for further use. The filtrate was used for the phytochemical screening.

The extraction was done by using soxhlation extraction method with analytical grade methanol as refluxing solvent. At the completion of extraction process, the plant extract was recovered from the mixture by distillation and stored at 4 °C until further use. The percentage of plant extract content was calculated by using standard formula. Methanolic extract was used for the quantitative estimation of phenols, flavonoids, tannins and alkaloids.

Phytochemical screening

Preliminary qualitative phytochemical screening was carried out with the following methods.

Steroids: 1 ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids (Gibbs, 1974).

Terpenoids: 2 ml of extract were added to 2 ml of acetic anhydride and concentrated H₂SO₄. Formation of blue/green rings indicates the presence of terpenoids (Ayoola, 2008).

Fatty Acids: 0.5 ml of extract was mixed with 5 ml of ether. This extract was allowed for evaporation on filter paper and dried. The appearance of transparency on filter paper indicates the presence of fatty acids (Ayoola, 2008).

Tannins: 2 ml of extract were added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins (Treare and Evans, 1985).

Saponins: 5 ml of extract were mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins (Kumar et al., 2009).

Anthocyanins: 2 ml of aqueous extract were added to 2 ml of 2N HCl and ammonia. The appearance of blue-violet color indicates the presence of anthocyanins (Paris and Moysé, 1969).

Leucoanthocyanins: 5 ml of aqueous extract were added

to 5 ml of isoamyl alcohol. If upper layer appears red in colour it indicates for presence of Leucoanthocyanins (Paris and Moysé, 1969).

Coumarins: 3 ml of 10% NaOH were added to 2 ml of aqueous extract; formation of yellow colour indicates the presence of coumarins (Rizk, 1982).

Emodins: 2 ml of NH₄OH and 3 ml of Benzene were added to the extract. Appearance of red colour indicates the presence of emodins (Rizk, 1982).

Alkaloids: Aqueous extract was mixed with 2 ml of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Phenols: Aqueous extract was mixed with 2 ml of 2% solution of FeCl₃. A blue-green or black coloration indicates the presence of phenols.

Flavonoids: Aqueous extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes, which indicated the presence of flavonoids.

Quantitative analysis

Total phenolic content

The total phenolic content was determined spectrophotometrically by the method described by Sadasivam and Manickam (1996). To 2 ml of phenol extract, 1.0 ml of Folin Ceocalteau reagent was added. After 3 minutes, 13 ml of distilled water were added. Next, 2 ml of sodium carbonate (7.5%) solution were added and the volume was adjusted to 20 ml. The above mixture was kept for 1 hour for colour development and absorbance was recorded at 630 nm. The concentration of total phenolic content in plant extracts was calculated from the calibration curve of gallic acid and it was expressed as gallic acid equivalents/gram fresh weight. Each experiment was repeated three times.

Total flavonoid content

Total flavonoid content was measured by aluminum chloride colorimetric assay described by Marinova et al. (2005). A calibration curve was constructed by using standard Catechin (250 µg/ml) solution. 1 ml of plant extract was added to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5% NaNO₂ was added. After 5 minutes, 0.3 ml of 10% AlCl₃ were added. At 6th min, 2 ml of 1 M NaOH were added and the volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Each experiment was repeated three times.

Tannin content

The tannin content was determined by the method given by Sadasivam and Manickam (1996). To 1 ml of plant extract 3.5 ml of distilled water and 0.5 ml of Folin-Denis reagent were added. Contents were allowed to mix then 1 ml of saturated sodium carbonate solution was added. The final volume was made up to 10 ml with distilled water. The solution was mixed well at room temperature and the absorbance was measured against prepared reagent blank at 760 nm. Tannin content in plant extracts was calculated from the calibration curve of tannic acid (10-100 µg) and it was expressed as tannic acid equivalents/gram weight. Each experiment was repeated three times.

Alkaloid content

Total alkaloid content in the extract was estimated by spectrophotometric method based on using Dragendorff's reagent. The amount of bismuth present was estimated after precipitating the alkaloids with Dragendorff's reagent (Sreevidya and Mehrotra, 2003). Each experiment was repeated three times.

Statistical analysis

Each experiment was repeated three times; the data were subjected to one way ANOVA using Minitab version 15. A significance level of 0.05 was used for all statistical tests.

Results and discussion

The percentage of extract after soxhlation was found to be 13.3% for *Boswellia serrata* and 1.33% for *Wrightia tinctoria* respectively. The phytochemical screening and analysis of the two medicinally important species showed that leaf and roots were rich in steroids, terpenoids, fatty acids, tannins, saponins, coumarins and emodins. Flavonoids, phenols and alkaloids were also present (Tab. 1).

Quantitative analysis of *B. serrata* reveals the phenolic content of (277.0±4.36), tannins (240.67±5.21), alkaloids (963.3±11.7) and flavonoids (150.0±2.89). Similarly, the

Tab. 1. Preliminary screening of secondary metabolites in *Boswellia serrata* and *Wrightia tinctoria*

Name of the phytochemicals	<i>Boswellia serrata</i>	<i>Wrightia tinctoria</i>
Steroids	+ve	+ve
Terpenoids	+ve	+ve
Fatty acids	+ve	+ve
Tannins	+ve	+ve
Saponins	+ve	+ve
Anthocyanins	-ve	-ve
Leucoanthocyanins	-ve	-ve
Coumarins	+ve	+ve
Emodins	+ve	+ve
Flavonoids	+ve	+ve
Phenols	+ve	+ve
Alkaloids	+ve	+ve

root extract of *W. tinctoria* also showed the phenolic content of (67.0±2.65), tannins (97.67±1.20), alkaloids (155.67±4.70) and flavonoids (13.83±0.72) respectively (Tab. 2).

For the past two decades most of the research efforts were focused on the production of secondary metabolites using cell suspension cultures, hairy root cultures (Chandra et al., 2006, 2008; Karuppusamy, 2009). Phytochemical screening of the two species *B. serrata* and *W. tinctoria* provided information about the richness of several secondary metabolites, which can be helpful for *in vitro* production of secondary metabolites on a large scale, which is not explored so far for these two species.

Medicinal plants are the richest bio-resources for traditional and modern medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities of synthetic drugs (Ncube et al., 2008). Phytochemical screening of plant extracts showed the presence of steroids, terpenoids, fatty acids, tannins, saponins, coumarins, emodins, flavonoids, phenols and alkaloids.

Steroids are important sources of sex hormones (Santhi et al., 2011) and also possess anti-bacterial activities (Raquel, 2007). Presence of steroidal compounds in several plants was reported by Savithamma et al. (2011). Terpenoids and tannins possess analgesic and anti-inflammatory activities (Nand et al., 2012). Tannins have stringent property, hasten the healing of wounds, inflamed mucous membrane (Okwu and Josiah, 2006) and bind to proline rich proteins and block the protein synthesis (Yadav and Agarwala, 2011). They also inhibit the growth of many fungi, yeasts, bacteria and virus (Chung et al., 1998). Coumarins are potential antioxidant and their antioxidant activity is due to their stability to scavenge free radicals and to chelate metal ions (Tseng, 1991). The presence of above compounds in *B. serrata* and *W. tinctoria* showed their effective medicinal properties.

The presence of flavonoids, phenolics and alkaloids in higher quantities in *B. serrata* and *W. tinctoria* showed their effective role in several biological processes such as antiapoptosis, anticarcinogen, antiatherosclerosis, cardiovascular protection,

Tab. 2. Quantitative screening of phytochemicals (phenols, tannins, alkaloids, flavonoids) in methanolic leaf extract of *B. serrata* and root extract of *W. tinctoria*

S.No	Phytochemical	<i>Boswellia serrata</i> Roxb.*	<i>Wrightia tinctoria</i> (Roxb.) R.Br.*
1	Phenols (mg/gm)	277.0±4.36	67.0±2.65
2	Tannins (mg/gm)	240.67±5.21	97.67±1.20
3	Alkaloids (mg/gm)	963.3±11.7	155.67±4.70
4	Flavonoids (mg/gm)	150.0±2.89	13.83±0.72

*Each value represents mean±SD of three independent experiments

inhibition of angiogenesis and cell proliferation activities (Han et al., 2007). Similar results were reported in leaf extracts of *Bryophyllum pinnatum* and *Ocimum gratissimum* (Ofokansi et al., 2005). Several authors reported that alkaloids have analgesic (Antherden, 1969), antispasmodic and anti-bacterial properties (Okwu and Okwu, 2004).

Conclusions

The traditional system of medicine like ayurvedic, homeopathy, naturopathy etc. use medicinal plants as raw materials. *B. serrata* and *W. tinctoria* are such medicinal plants, having wide applications. *B. serrata* as a source of oleogum resin is rich in phenolics, tannins, alkaloids and flavonoids etc. These phytochemicals are credited for their astringent, stimulant, expectorant, diuretic and antipyretic properties. Similarly *W. tinctoria* is also known for its medicinal properties like in the treatment of psoriasis, stomach pains, toothache, dysentery etc. due to the presence of all the three major secondary metabolites. Since about 30% of worldwide sale of drugs is based on natural products and knowledge about traditional indigenous medicine is important, exploitation of pharmacological properties of the above two species is needed and will be continued in further investigations.

References

Anonymous (1962). The wealth of India, A dictionary of Indian raw materials and Industrial products, SIR Publication, New Delhi India, p. 208-210.

- Anonymous (1976). The wealth of India: Raw materials, New Delhi: CSIR Publications 10:588-590.
- Antherden LM (1969). Textbook of pharmaceutical chemistry. 8thEd, Oxford University Press, London, p. 813-814.
- Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obawe K, Ezennia EC, Atangbayila TO (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South Western Nigeria. Trop J Pharm Res 7(3):1019-1024.
- Chandra S, Chandra R, Sharma HP, Jha S (2006). Production of hairy root biomass of *Spilanthes paniculata* (DC.) Jansen. The Indian Pharmacists 5:69-73.
- Chandra S, Chandra R, Sharma HP, Jha S (2008). *Agrobacterium rhizogenes* mediated genetic transformations of *Spilanthes paniculata* (DC.) Jansen. Int J of Plant Sci 3:471-476.
- Chary STR (1980). Floristic study of Achampet Taluk Mahabubnagar of Andhra Pradesh. Hyderabad, Osmania University, PhD. Thesis, p. 285.
- Chikamai BN (2002). Review and synthesis on the state of knowledge of *Boswellia* species and commercialization of Frankincense in dry lands of Eastern Africa FAO/EU/FORNESSA AFREA (Forestry Research Network for Sub-Saharan Africa), Nairobi, Kenya.
- Chitravadivu C, Manian S, Kalaihelvi K (2009). Qualitative analysis of selected medicinal plants, Tamilnadu, India. Middle-East J Sci Res 4(3):144-146.
- Chung KT, Wong TY, Wei CL, Huang YW, Lin Y (1998). Tannins and human health: a review, Criti Rev Food Sci Nutr 38(6):421-464.
- Cowan MM (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews 12(4):564-582.
- Gaofeng Y, Mark LW, Guoqing H, Min Y, Duo L (2006). Natural products and anti-inflammatory activity. Asia Pac J Clin Nutr 15(2):143-152.
- Gibbs RD (1974). Chemotaxonomy of flowering plants. Vol.1, McGill Queen's University Press, Montreal and London.
- Han X, Shen T, Lou H (2007). Dietary polyphenols and their biological significance. Int J Mol Sci 8:950-988.
- Karuppusamy S (2009). A review on trends in production of secondary metabolites from higher plants by *in vitro* tissue, cell cultures. J of Med Plant Res 3:1222-1239.
- Kirtikar KR, Basu BD (1975). Indian medicinal plants, Delhi, Ed. Jayyed Press 2:1581.
- Krishnamoorthy JR, Ranganathan S (2000). Antipityrosporium ovale activity of a herbal drug combination of *Wrightia tinctoria* and *Hibiscus rosasinensis*. Ind J Dermatol 45(3):125-26.
- Kumar A, Ilavarasn R, Jayachandran T, Decaraman M, Aravindhnan P, Padmanaban N, Krishnan MRV (2009). Phytochemical investigation on a tropical plant. Pak J Nutri 8:83-85.
- Kumar AP, Rajkumar, Kanimozhi M (2010). Phytochemical screening and antimicrobial activity from five Indian medicinal plants against human pathogens. Middle- East J Sci Res 5(3):157-162.
- Lee KH (1999). Anticancer drug design based on plant-derived natural products. J Biomed Sci 6(4):236-250.
- Marinova D, Ribarova F, Atanasoval M (2005). Total phenolics and flavonoids in Bulgarians vegetables. J Univ Chem Tech Metal 40:255.
- Nand P, Drabu S, Gupta RK (2012). Phytochemical and antimicrobial screening of medicinal plants for the treatment of acne. Indian J Nat Prod Res 3(1):28-32.
- Ncube NS, Afolayan AJ, Okoh AI (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African J Biotechnol 7(12):1797-1806.
- Ofokansi KC, Esimone CO, Anele CR (2005). Evaluation of the *in vitro* combined antibacterial effect of the leaf extracts of *Bryophyllum pinnatum* (Fam: Crassulaceae) and *Ocimum gratissimum* (Fam: Labiatae). Plant Prod Res J 9:23-27.
- Okwu DE, Josiah C (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. Afri J Biotech 5(4):357-361.
- Okwu DE, Okwu ME (2004). Chemical composition of *Spondias mombin* linn. plant parts. J Sustain Agric Environ 6(2):140-147.
- Paris R, Moyses H (1969). Precis de matiere medicinale. Paris: Masson.
- Raquel FE (2007). Bacterial lipid composition and antimicrobial efficacy of cationic steroid compounds. Biochemica et Biophysica Acta. 2500-2509.
- Reddy YSR, Venkatesh S, Ravichandran T, Murugan V, Suresh B (2000). Antinociceptive activity of *Wrightia tinctoria* bark. Fitoterapia 73:421-423.
- Rizk AM (1982). Constituents of plants growing in Qatar. Fitoterapia 52:35-42.
- Sadasivam S, Manickam A (1996). Biochemical methods, Second edition, New Age International (P) Ltd Publishers, New Delhi, p. 108-110.
- Santhi R, Lakshmi G, Priyadarshini AM, Anandaraj L (2011). Phytochemical screening of *Nerium oleander* leaves and *Momordica charantia* leaves. Inter Res J Pharm 2(1):131-135.
- Savithamma N, Linga Rao M, Sushrutha D (2011). Screening of medicinal plants for secondary metabolites. Middle-East J Sci Res 8(3):579-584.
- Shah GL, Gopal GV (1988). Ethnomedical notes from the tribal inhabitants of the north Gujarat (India). J Eco Tox Bot 6:193-221.
- Siddiqui S, Verma A, Rather AA, Jabeen F, Meghvansi MK (2009). Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Advan Biol Res 3(5-6):188-195.
- Singh GB, Atal CK (1986). Pharmacology of an extract salai guggal ex- *Boswellia serrata*, a new non-steroidal anti-inflammatory agent. Agents Actions. 18(3):647-652.
- Sreevidya N, Mehrotra S (2003). Spectrophotometric method for estimation of alkaloids precipitates with Dragendorff's reagent in plant materials. J AOAC Inter 86(6):1124-1127.
- Treare GE, Evans WC (1985). Pharmacognosy 17 edition, Bahive Tinal, London, p. 149.
- Tseng A (1991). Chemoprevention of tumors in MTV-H ras transgenic mice with coumarins. Proc Am Assoc Cancer Res, 32:2257.
- Yadav RNS, Agarwala M (2011). Phytochemical analysis of some medicinal plants. J Phytol 3(12):10-14.