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COMPARATIVE STUDIES ON METABOLITE COMPOSITION OF *BOSWELLIA SERRATA* AND *BOSWELLIA OVALIFOLIOLATA*

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ABSTRACT:

Boswellia serrata Roxb. ex Colebr. and Boswellia ovalifoliolata Balakr. & Henry belongs to family Burseraceae and possesses huge medicinal properties. The later one is an endemic plant of Seshachalam hills in the southern part of India. Apart from medicinal properties, both the plants are widely used for other commercial purposes. In the present investigation, we compared the metabolite composition in both the plants to know the molecular insights particularly biochemical metabolism of the two systems. Results indicated the similarities and dissimilarities in certain metabolites in both the plants. Present work may be useful to know the biochemical composition in details for both the plants.

KEYWORDS: *Boswellia, Serrata, Ovalifoliolata, LC-MS/MS, Metabolites.*

INTRODUCTION

The man of today is no longer exists with food and shelter as his only need. He desires other commodities as well and plants raw materials that can be converted into useful products which contribute to his enjoyment of life (Rangacharyulu et al., 1991). Hence plants have an enormous role in human life and have been widely used as a source of medicine, nutraceutical, wood and various other purposes apart from food since the evolution began. Specifically, plants are extensively used as the components of phytomedicine for many years and mankind has the advantage of obtaining various chemicals from them (Dey, 1994; Pullaiah et al., 2001). Moreover, primitive people of all ages had some knowledge of medicinal plants, derived as a result of trial and error. At present all over the world, an estimate of thousands of plant species are known to have been used as drugs. Quantitative and qualitative studies with total metabolites are helpful in identifying the causal factors controlling growth and development. Natural variations of metabolites between different plants species in the same genus or family have been studied extensively (Wink, 2016). At present we performed the experiments choosing total metabolites as the target to see the difference between related species. The majority of plants used for medicines were collected from wild areas including forest regions. In the present investigation, we examined the chemical constituents and metabolites of two important medicinal plants i.e. *Boswellia serrata* and *Boswellia ovalifoliolata*.

Boswellia serrata Roxb. ex Colebr. is an important medicinal plant which is located in most of

the parts of India. In South India, it is available in several places including lower hill slopes of Tirumala and Talakona of Andhra Pradesh. *B. serrata* consists predominantly of various group of boswellic acids (Cameron and Chrubasik, 2014). It is widely used for joint arthritis as well as to cure diabetes (Ammon, 2019). Another medicinal plant namely *Boswellia ovalifoliolata* Balakr. & Henry is endemic to Seshachalam hill range of Eastern ghats in Andhra Pradesh, India (Latheef et al., 2008). Extracts of the endemic plant generally used to treat as antioxidant, anticancer and also used as larvicide (Devi et al., 2012). The extracts of these plants can be used to kill mosquitoes and used as a repellent. Both the plants belong to family Burseraceae and showed resemblances as well as differences with respect to morphology and medicinal properties. Both are gum yielding plants and consists of unique chemical constituents. Hence extract of these plants used to isolate gum and also used to cure diseases which relieve physical suffering by the tribal people (Thammanna et al., 1994). The selected species were widely used in industrial practices recently for medicinal purpose to cure numerous diseases and analysis of chemical constituents work has been under progress.

By seeing the different properties of these plants, we extended the natural variation approach by choosing these two important medicinal plants i.e. *Boswellia serrata* and *Boswellia ovalifoliolata* to check the level of various metabolites, chemical constituents and comparative analysis were carried out. To screen comprehensive metabolite profiling of the plants, bark and leaf extracts were used in liquid chromatography. Liquid chromatography is one of the potential analytical tools for the identification of metabolites in plant samples (Sampaio et al., 2016). Further, these two plants belong to the same family and genus, and resulting data may relate to available phenotypic information. So the present study aimed to do the quantitative as well qualitative analysis of total metabolites and chemical constituents in both *B. serrata* and *B. ovalifoliolata.*

MATERIALS AND METHODS

For the present work, two important medicinal plants namely *Boswellia ovalifoliolata* and *Boswellia serrata* were collected from forest areas in different places of Tirumala hills, Chittoor District of Andhra Pradesh. The botanical identification of the plants and herbarium work as well seasonal physiology studies were carried out by referring regional and local floras (Gamble, 1957; Narayana Rao et al., 1988; Pullaiah et al., 2001; Madhava Chetty et al., 2015). As mentioned above and also in our field trips we found that most of the tribal people used these two plants for medicinal purpose. After seeing the ethnomedicinal importance, work has been carried out to examine the metabolite variation in these plants.

Technically we used liquid chromatography (LC-MS/MS) to check the quality and quantity of total metabolites and comparative studies were performed. To perform this work, the known identity of metabolites is prerequisite for a suitable metabolic assessment. Liquid chromatography has become one of the popular analytical tools for screening and identification of metabolites in any biological sample particularly advanced ultra-performance liquid chromatography (UPLC). UPLC posses the same principle as HPLC and the basic difference is in the designer of the column material particle size which less than 2-µm, for efficient separation. The plant collection was carried out with same age group plants in the same season at a time. An equal amount of leaf and bark materials were prepared using different solvents for this work. 1.0 gm of plant tissue was grinded with liquid nitrogen and mixed with metabolite extraction buffer which contains methanol, chloroform and water and further homogenized for 5 min. Centrifugation was carried out at 10,000 rpm for 10 min at 4°C and the supernatant was collected and 1 ml each sample was dried using speed vacuum. Later all the samples were reconstituted in a mixture of methanol and 0.1% formic acid. 10.0 µL injection volume was used on BEH C18 UPLC column for separation of metabolites. We used 0.1% formic acid in MS Water and 0.1% FA in MS grade CAN as buffers. All the samples then go through ESI-QTOF and the raw data details were acquired. Table 1 explains the details and conditions of chromatography which was used for the present study. All the statistical work has been carried out using a personal computer.

Table-1: UPLC conditions applied for the present study

S.No.	Ultra-Performance Liquid Chromatography (UPLC) Conditions
1	Acquity Waters UPLC system
2	75umx150mmx1.7um BEHC18 column
3	60 min separation

RESULTS AND DISCUSSION

The present results include metabolite variation between *Boswellia serrata* and *Boswellia ovalifoliolata* samples with both leaf and bark samples. In fact we estimated hundreds of chemical constituents between both the plant species, but limited information is documented. Apart from quantitative estimation, qualitative analysis results also revealed the novel compounds in both the species. Specifically certain metabolites exhibited less variation and some other demonstrated huge variation of both leaf and bark samples in *B. serrata* and *B. ovalifoliolata* were documented as follows.

Variations in metabolites of leaf samples

Present study exhibits the similarity and dissimilarity between *B. serrata* and *B. ovalifoliolata* particularly with metabolite and chemical constituent composition (Figs 1. and 2). Less variation was noticed in leaf samples specifically with Pyrimidine, Cannabinol, Phosphotyrosine, D-Glucosamine, R-Pantolactone and Delta1-Piperideine-2-carboxylate metabolites (Fig. 1). This data indicates that biochemical similarities in certain aspects between both plants.

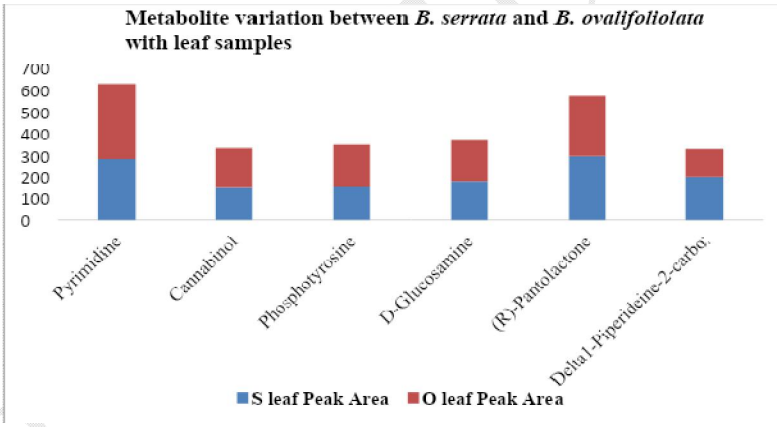

Figure 1-Low metabolite variation between leaf samples of *B. serrata* and *B. ovalifoliolata* (values in Y-axis indicates peak areas)

In contrast, high variations was noticed with certain metabolites which includes 1,2-Bis-O-sinapoyl-beta-D-glucose, UDP-3-O-(3-hydroxytetradecanoyl)-D-glucosamine, Hallactone B, Ginkgolide B, Z-Gly-Pro-Leu-Gly-Pro, Chebulagic acid, 12 ADT, Alpha, alpha'-trehalose 6-phosphate, 5-Amino-6-(5'-phospho-D ribitylamino) uracil and Kyotorphin (Fig. 2). This may be due the variation in genome content and growth levels including environmental conditions. Soltis and Klienbenstein (2015) discussed the reasons for metabolite variation in detail by choosing several plant examples. Moreover this work also enhances the knowledge on natural variation in metabolite accumulation and their profiles within the related species.

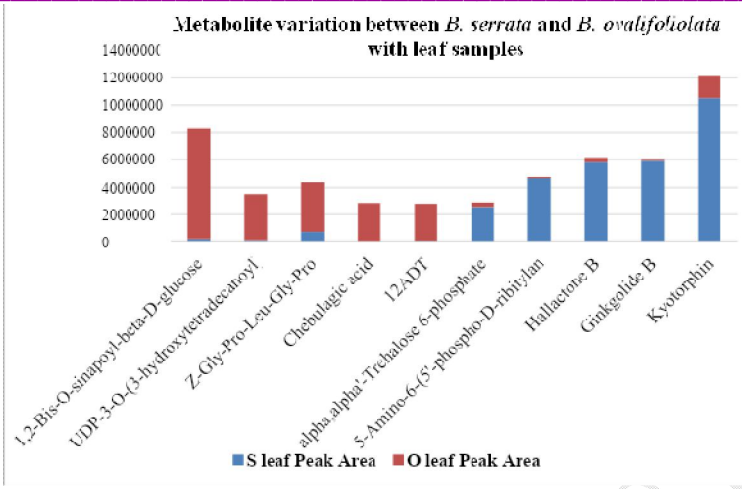

Figure 2-High metabolite variation between leaf samples of *B. serrata* and *B. ovalifoliolata* (values in Y-axis indicates peak areas)

Variations in metabolites of bark samples

Similar to leaf samples, high and low levels of metabolite variation was found even with bark samples between both the plants (Figs. 3 and 4). Low levels of metabolite variations were noticed in a certain compound such as Sodium6-20-hydroxy-23-oxoergost-9(11)-en-3-yl sulfate, Meclofenamate sodium, Maleylpyruvate, L-Threonine O-3-phosphate, L-Leucine, 2-Bromomaleylacetate and 6-Acetamido-3-oxohexanoate (Fig. 3). Moreover some of the metabolites showed more variation in bark samples of both *B. serrata* and *B. ovalifoliolata* such as Stigmatellin A, beta-Carotene 5,6-epoxide, Isopimpinellin, Lipid A disaccharide, Xanthine-8-carboxylate, ADP, UDP-2-deoxyglucose, Vitamin D, 4-Methylcatechol and Kaempferol 3-(6''-sinapylglucosyl)-(1->2)-galactoside (Fig. 4).

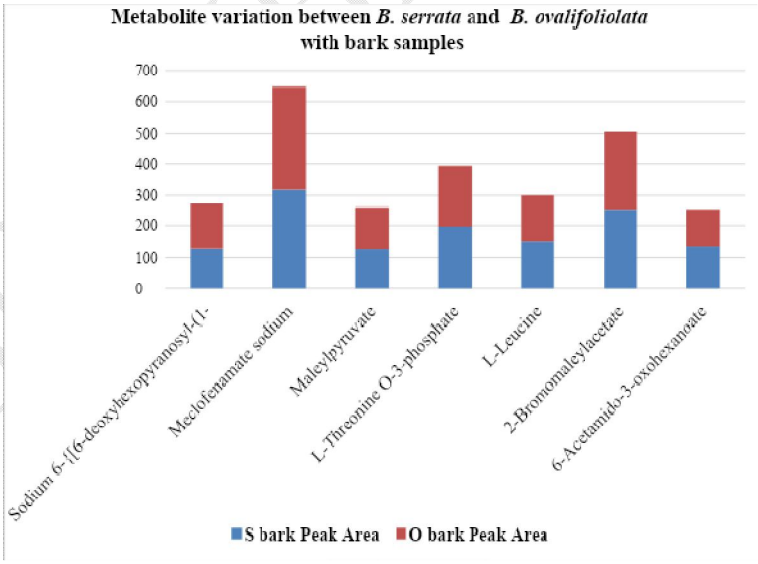

Figure 3-Low metabolite variation between bark samples of *B. serrata* and *B. ovalifoliolata* (values in Y-axis indicates peak areas)

The present data is helpful to justify the similarities and also variations in the growth pattern of both *B. serrata* and *B. ovalifoliolata.* Similar kind of metabolite variations was noticed by Van Treuren (2018) and Monchgesung et al. (2016) in lettuce and Arabidopsis species. They also reported that there

is every possibility of variation between cultivars of the same species. This kind of research may be useful to know the morphological as well as biochemical variations in cultivars of same species and also between the species.

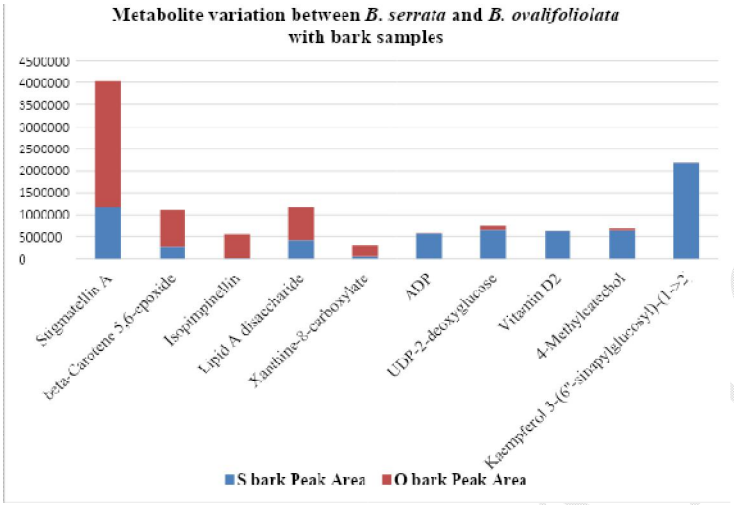

Figure 4-High metabolite variation between bark samples of *B. serrata* and *B. ovalifoliolata* (values in Y-axis indicates peak areas)

CONCLUSIONS

Present investigation exhibited the metabolite variation as well as similarities between *B. serrata* and *B. ovalifoliolata.* This kind of work may help to draw the conclusions for the genetic as well biochemical level significance of species and also useful for future research on evolutionary studies.

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