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Research Article

COMPARATIVE AMINO ACID PROFILING OF *PTEROCARPUS SANTALINUS* AND *BOSWELIA OVALIFOLIOLATA* BARKS OF TIRUMALA HILLS, EASTERN GHATS, ANDHRA PRADESH

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ABSTRACT
In this study, investigations were done for the comparative occurrence of amino acid compositions in barks of endemic trees of tirumala hills of eastern ghats of Andhra Pradesh . The barks of *Pterocarpus santalinus* and *Boswelia ovalifoliolata* were selected and screened. The presence of the determined aminoacids tabulated explains the utilisation of *Pterocarpus santalinus* and *Boswelia ovalifoliolata* , bark extracts in pharmaceutical ,cosmetic and food additive products. Our study revealed that these plants are respectively rich in amino acids and have high protein levels and are potential supplements to overcome nutrient deficiencies. Comparison of colour factors obtained by the manual ninhydrin method depicted that all essential amino acids were observed in sufficient levels compared to FAO reference values.

Key Words*: Pterocarpus Santalinus, Boswelia Ovalifoliolata, Amino Acids, Tirumala Hills*

INTRODUCTION
An obvious advantage with the development of natural product chemistry is considered effective in discovering bioactive profile of plants of therapeutic importance. Serious attention of researchers working on natural products rely on phytochemical surveys being carried on for detecting diverse groups of naturally occurring phytochemicals of therapeutic usage. Plant foods have a complete amino acid Composition (John McDougall 2002). Chemically constituents may be therapeutically active or inactive. The application of chemical data to systematic has received serious attention of large number of biochemists and botanists. Amino acids are colorless ionic compounds that form the basic building blocks of protein. The application of investigated plant species in various drug preparations was based on their phytochemical content and their pharmacological activities (Masih and Singh, 2012). Efforts were made for the quantitative determination of the biochemical parameter .ie comparative profiling of amino acids for studying the biological efficacy of two medicinal plants *Pterocarpus santalinus* and *Boswelia ovalifoliolata* which were commonly used in various medicament preparations.

Biological Characteristics of Pterocarpus santalinus Bark
Pterocarpus santalinus L.f. , (*Leguminosae*.) endemic medicinal plant to tirumala hill ranges of eastern ghats ,Andhra Pradesh (Madhava chetty *et al*., 2011) (Fig 1.). It is a moderate sized deciduous tree with erect bole and dense rounded crown, conspicuous by its blackish brown bark resembling the skin of crocodile, divided into rectangular plates by deep vertical and horizontal cracks. A blaze on the bark exposes the white coloured sapwood which gradually turns red due to exudation of a red gummy juice. Heartwood is deep red in colour which on exposure turns to scarlet red. The uses of Bark and wood is used in different ailments. The wood of *P. santalinus* is highly valued for the manufacture of furniture, musical instruments, cosmetics, dye, and for medicinal purposes (Arunakumara *et al*, 2011).

Biological Characteristics of Boswelia ovalifoliolata Bark
Boswellia ovalifoliolata N.P.Balakr. & A.N.Henry (Burseraceae), vernacularly known as Konda sambrani or adavi guggilam, is a deciduous medium sized tree endemic to Seshachalam hills. The identifying characteristics of bark is presence of light green and dirt brown exfoliation into paper like pieces (Madhava chetty *et al*., 2011) (Fig .2). The stem bark is used as a mosquito repellent and its resin is used for external applications (Muniappan and Viraktamath, 2006).

MATERIALS AND METHODS

The plant specimens were identified and citation is given with the help of the floras of Gamble (1957), Thammanna *et al.*, (1994) and Madhava chetty *et al.*, (2011). Collected specimens were deposited in herbarium, department of botany, NBKR institutions, vidyanagar, Andhra Pradesh.

Fig.1 Bark & logs of *Pterocarpus santalinus.*

Fig .2 Habit of *Boswelia ovalifoliolata* and enlarged Bark

Plant extraction

Ten gm of the crushed barks of *Pterocarpus santalinus* and *Boswelia ovalifoliolata* powder was weighed and grounded in a pastel and mortar with a small quantity of powder was filtered and concentrated at room temperature for 2 hours. To these homogenate 5 to10 ml of ethanol (80%) was added, filtered and centrifuged. The extract was used for the quantitative estimation of total free amino acids. To 0.2, 0.4, 0.6, 0.8, 1.0 ml of extract 1.0 ml ninhydrin solution was added. The most sensitive and accurate method in our hands is a colorimetric ninhydrin method based on that of Moore and Stein (1948).

2 ml of distilled water Contents test tube were heated in boiling water bath for 20 minute to which 5 ml of the 80% ethanol was added and the contents were mixed. After 15 min the intensity of the purple colour against a reagent blank on a spectrophotometer at 570 nm was recorded. The colour is stable for 1hour. The reagent blank as above by taking 0.1 ml of 80% ethanol instead of the extract was prepared. The absorbance of the solutions was read at 570 nm. The unknown sample was then extrapolated from the calibration curve. Different amino acids will move different distances up the paper depending upon their relative solubilities in the two solvents, allowing for separation of amino acid mixtures. The Rf value of an amino acid is the ratio of the distance traveled by the amino acid from the origin to the distance traveled by the solvent from the origin.

Determination of amino acids by Paper chromatography

The techniques of paper chromatography were applied to the separation of mixtures of amino acids, It was extended by the use of two-dimensional chromatography, in which a different solvent was used in each direction (John Sturman and Applegarth 1985). The extract was spotted on whatman No.1 chromatographic grade filter paper along with standardized mixture of known amino acids on the same chromatogram. The chromatogram were developed using n-butanol : acetic acid : water (80:20:10). The spot of amino acids were visulised by spraying with Ninhydrin and were identified by their characteristic colour.

RESULTS

In *pterocarpus santalinus* bark amino acids such as Histidine , Leucine, Lysine, ϒ-Methylene glutamine, Phenylalanine, Proline , Serine, Threonine, Valine , Aspartic acid, Arginine , Asparagine, α-Alanine, β-Alanine , Cysteine, Cystine, Glutamic acid, Glycine are Present. Glutamine is not detected (Table 1)

In *Boswelia ovalifoliolata* bark Histidine, Leucine, Lysine, Proline Valine, Arginine, Asparagine, α-Alanine, β-Alanine, Cysteine, Cystine Glutamic acid, Glutamine, Glycine are Present. ϒ-Methylene glutamine, Serine, Phenylalanine, Aspartic acid, Threonine are not detected (Table 1).

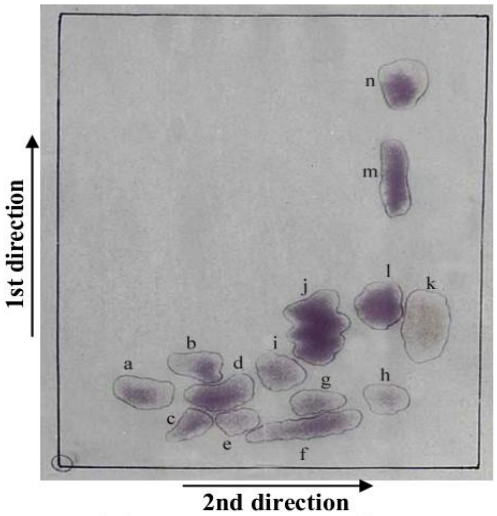

Fig 3. ***Pterocarpus santalinus***

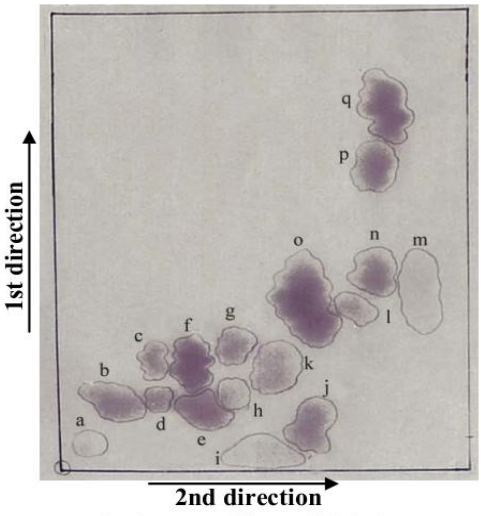

Fig 4. ***Boswellia ovalifoliolata***

O : Origin Solvents used: 1st direction : Sec. butanol : Formic acid : Water (75 : 13 : 12 v/v/v)
2nd direction : Buffer : Phenol (1: 2 v/v).

Pterocarpus santalinus
a. aspartic acid, **b**. glutamic acid, **c**. α–alanine, **d**. serine, **e**. glycine, **f**. threonine, **g.** lysine, **h**. asparagine, **i**. histidine, **j**. arginine, **k**. cystine, **l.** ϒ-methylene glutamine **m**. proline, **n**. β-alanine, **o**. cysteine, **p**. phenylalanine, **q**. leucine.

Boswellia ovalifoliolata
a. glutamic acid, **b**. α–alanine, **c**. glycine, **d.** asparagine, **e.** leucine, **f.** histidine, **g**. arginine, **h**. glutamine, **i.** cystine, **j**. cysteine, **k**. proline, **l.** β-alanine, **m**. valine, **n**. Lysine.

Figure 3 & 4: Paper Chromatogram representing detected aminoacids

DISCUSSION

The potential of chemotaxonomy is new becoming increasingly obvious. In essence, this study is claimed to be the first of its kind in being able to demonstrate that in this work because of this nutritional profile, these materials make a significant dietary ,cosmetic,medicated contribution. The samples were subjected to qualitative amino acid by adopting standard methodology. The results showed the efficacy of ethonolic extracts are observed to be present in the investigated plants in variable proportions.

Applying the method directly to pieces of filter paper, there is a high and variable 'amino-acid' value, due mainly to the presence of absorbed ammonia. The colour response per mole of ammonia is about the same as that of an amino-acid (Fowden and Penney, 1950).

Ninhydrin, a powerful oxidizing agent, decarboxylates the alpha-amino acids and yields an intensely colored bluish purple product which is calorimetrically measured in 570nm. This reaction is unique among chromogenic reactions in that at pH 5.5 it results in the formation of the same soluble chromophore by all primary amines which react with amines, amino acids, peptides, proteins, and even ammonia (Mendelfriedman, 2004). Comparison of colour factors obtained by the manual ninhydrin method suggests that the progressive increase in these values may be due to the increase in distance

between the NH_2 and SH groups in the compounds, which minimizes the extent of the ring formation in the higher homologues.

Table 1: Profiling of the amino acids

AMINOACID	R_f values in solvent		Colour with ninhydrin	*Pterocarpus santalinus*	*Boswellia ovalifoliolata*
	1	2			
Histidine	0.08	0.52	Violet	+	+
Leucine	0.79	0.83	Deep violet	+	+
Lysine	0.12	0.43	Brown	+	+
ϒ-Methylene glutamine	0.30	0.71	Violet	+	--
Phenylalanine	0.60	0.90	Light violet	+	--
Proline	0.39	0.89	Yellow	+	+
Serine	0.20	0.23	Deep violet	+	--
Threonine	0.28	0.35	Deep violet	+	--
Valine	0.59	0.77	Deep violet	+	+
Aspartic acid	0.05	0.10	Light violet	+	--
Arginine	0.10	0.60	Violet	+	+
Asparagine	0.19	0.41	Violet	+	+
α-Alanine	0.26	0.28	Light violet	+	+
β-Alanine	0.42	0.77	Deep violet	+	+
Cysteine	0.38	0.61	Deep violet	+	+
Cystine	0.23	0.53	Violet	+	+
Glutamic acid	0.17	0.20	Violet	+	+
Glutamine	0.14	0.82	Violet	--	+
Glycine	0.09	0.38	Violet	+	+

The presence of the determined aminoacids tabulated explains the utilisation of *Pterocarpus santalinus* and *Boswelia ovalifoliolata* , bark extracts in pharmaceutical ,cosmetic and food additive products.

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REFERENCES

Arunakumara KKIU, Buddhi Charana Walpola,Siripala Subasinghe, and Min-Ho Yoon (2011) *Pterocarpus santalinus* Linn. f. (Rath handun): A Review of Its Botany, Uses, Phytochemistry and Pharmacology. *Journal of The Korean Society* for *Applied Biological Chemistry* **54**(4) 495-500
Fowden I and Penney JR (1950). Elimination of Losses in the Quantitative Estimation of Amino-Acid by Paper Chromatography, *Nature* **165** 846 - 847
Gamble JS (1957). Flora of the Presidency of Madras. Vol: 1-3 B.S.I. Calcutta.
John A Sturman and Derek A Applegarth (1985). Automated Amino Acid Analysis**,**Neuromethods ,springer protocols **3**: 1-27
John McDougall (**2002)**. Plant Foods Have a Complete Amino Acid Composition *Circulation.,**197***: 105.
Madhava Chetty, KK Sivaji and K Tulasi Rao. (2010). Flowering plants of Chittoor District, Andhra Pradesh, India. **2**nd edn. Student offset Printers, Tirupati.

Mendelfriedman (2004). Applications of the Ninhydrin Reaction for Analysis of AminoAcids, Peptides and Proteins to Agricultural and Biomedical Sciences. *Journal of Agricultural Food Chemistry*,**52**: 385−406

Moore S and Stein WH (1948*).* Photometric ninhydrin method for use in the chromatography of amino acids :*Journal of biological chemistry* 367-388.

Masih NG and Singh BS (2012) Phytochemical Screening of Some Plants Used in Herbal Based Cosmetic Preparations In: *Chemistry of Phytopotentials: Health, Energy and Environmental Perspectives* 111-112.

Peace RW, Gilani GS (**2005**.) Chromatographic determination of amino acids in foods. *The Journal of AOAC International* .**88(3)**:877-87.

Shaik Abdul Latheef, Beerkam Prasad, Middi Bavaji, Gangapatnam Subramanyam (2008). *Bioinformation* **2**(6) 260–262.

Thammanna P, KN Rao and K Madhava Chetty (1994). *Angiospermic Wealth of Tirumala*, T.T.D. Press, Tirupati.