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BIOCHEMICAL SCREENING OF LIPIDS OF SELECTED HIGH VALUED MEDICINAL PLANTS OF TIRUMALA HILLS

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ABSTRACT
This study aimed to screen the presence of lipids in *Syzygium alternifolium* and *Terminalia pallida* the high valued medicinal plants of Tirumala hills. TLC studies detected and confirmed the presence of lipids, Phospholipids, glycolipids. Screening of these high valued medicinal plants have an important role in enhancing the human health by synergetic functions with naturally available lipids.

KEYWORDS: Lipids, *Terminalia pallida* , *Syzygium alternifolium.*

INTRODUCTION

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cos *et al*., 2006). Plants synthesize a wide range of hydrophobic compounds, generally known as lipids. Lipid profiling is an emerging strategy that needs to be extended so that routine lipid profiling in plants will cover usual essential additional lipids. Glycolipids in higher plants mainly consist of steryl glucosides (SG), sphingoglycolipids, and glyceroglycolipids.
These glycolipids are widely distributed in edible plants such as cereals, legumes, vegetables, and fruits (1,2). In previous studies on lipids were done by Galliard (1968 a and b) Walter *et al*,(1971) ; Kinsella (1971) Morrison (1975) ;Wade and Bishop (1978); Choudhury and Juliano (1980). Plant glycolipids have been quantified gravimetrically or colorimetrically after separating and isolating them with column chromatography or with thin-layer chromatography (TLC) (Kuksis, 1987). In this investigation the bioavailability of bioactive lipids and its constituents from selected botanicals have been made to achieve nutrient adequacy and to prevent and treat different diseases as medicaments and food supplements. Since these bioactive lipids are naturally available, we suggest for the development of cultivation of wild varieties for the essentiality of natural lipids for the preparation of medicines and human health care products. For this purpose we explored high valued medicinal plants for the screening of natural lipds in the Phytotaxa as suggested by Sreelathadevi (2011).

***Terminalia pallida* Brandis (Combretaceae)**
This is vernacularly known as Tella karaka, its English name as White gallnut tree. The habit is tree (Fig.1). Leaves alternate, sub-opposite, ovate, petiole short with glands beneath. Flowers small, green, in spikes. Calyx campanulate. Stamens 10 in 2 series. Ovary inferior, 1-called; ovules 2-3, pendulous. Fruit obovoid, very faintly 5-ridged when dry, glabrous, indehiscent. (Fig.2). Endemic tree of Tirumala hills. Distributed almost all places of Tirumala hills. Flwering anf fruiting stage is in the month from March-July. Fruit is used against cold and cough. Bark is anti-inflammatory; bark powder is applied externally and given internally to cure swelling Fruit powder is given with honey to cure pectic ulcers, fissures and to clear harshness of voice. (Madhava chetty *et al.*, 2011b)

***Syzygium alternifolium* (Wight) Walp. (Myrtaceae)**
This selected taxa is a synonym of *Eugenia alternifolia.* Vernacular name is Mogi, Konda neredu. A moderate sized deciduous tree, up to 12 m tall,bark greyish, slightly fissured (Fig. 1). Leaves sub-opposite rarely alternate, coriaceous, ovate-elliptic. Flowers cream or yellowish-white, sweet scented. Fruit is a berry, seeds globose. (Fig.3). Endemic tree to Tirumala hills. Located in almost all places of Tirumala. Flowering and fruiting stage is at March-June. Stem decoction (10 ml) is mixed with fruit powder (2 g) and is given 2 times a day for 15 days to regulate blood sugar levels. Fruits often given in controlling diabetes, duodenal and pectic ulcers and in bacillary dysentery. (Madhava chetty *et al.*, 2011a)

MATERIALS AND METHODS

Lipids were extracted according suggested by the method of Hoppe and Haitefuss (1974). About 5 g of immature fruits of *Syzygium alternifolium* and *Terminalia pallida* was homogenized in 110 ml of boiling solvent mixture containing 30 ml $CHCl_3$, 60 ml MeOH and 20 ml H_2O (1:2:0.8v/v/v) and the contents were filtered. The residual plant material was again re-extracted with 70 ml methanol and this was added to the first extract. The residue was again washed with 100 ml $CHCl_3$. After taking all the fractions into separator funnel and 90 ml water was added. The ratio of choloroform: methanol: water was now 2:2:1.8 and the extract separated into two distinct layers. The lower lipid containing chloroform layer was taken into a separate beaker. The upper water layer was treated 3 times with 50 ml of

chloroform successively. All the combined chloroform layers were evaporated in a vacuum by rotary evaporator to dryness at 40°C. Two ml of benzene was added to the residue to remove any traces of chlorophyll pigments. The final residue was dissolved in two ml of $CHCl_3$ and stored in a dark container until it was used. Quantitative determinations were made and Concentrations of phosphatides and glycolipids were separated by two dimensional chromatography

Fig 1.Tree Habit of *Terminalia pallida* and *Syzygium alternifolium*

Fig .2 Fruiting stage of *Terminalia pallida* **Fig .3 Fruiting stage of *Syzygium alternofolium***

Thin layer chromatography (TLC)

Thin layer plates were prepared by spreading slurry of silica gel-G (50 g in 100 ml distilled water) to 0.5 mm thickness on thin glass plates with the help of spreader (Desaga Co., Heidelberg, Germany; Model No. 611). The plates were air dried and stored at room temperature. The plates were activated by heating them at 110°C for 30 minutes in a hot air oven, just before use. About 1 g equivalent of lipid extract was spotted on TLC plates with the help of micropipette. The spot areas were dried immediately with the help of a hair drier. Then the plates were run in unidimensional ascending chromatography by using flat rectangular TLC glass chambers. The chambers were saturated with the developing solvents one day before the plates developed. Solvent systems are Chloroform: Methanol: Acetic acid: Water (170:25:25:3 v/v/v/v) and Acetone: Benzene: Water (91:30:8 v/v/v) . The plates were placed in the chambers and made airtight. The developed plates were removed and then dried at room temperature and exposed to iodine vapours to visualize all the lipid compounds. For the detection of various lipids separated on the TLC and were sprayed with the following chromogenic spray reagents with the help of an automizer. The lipids were identified by comparison of R_f values, colours and with those of the authentic samples by co-chromatography.

Chromogenic spray reagents.

The glycolipids and phospholipids were identified by using specific colour developing reagents as given by Skipski *et al.*, (1965). Glycolipids with Orcinol reagent *(*200 mg orcinol in 100 ml 75% H_2SO_4 was used for detection of lipid sugars). The plates were sprayed until the whole surface uniformly and obviously become moist and then heated in an oven at 100°C for 15 minutes. Ninhydrin was used to detect phospholipids contain free amino group like ethanolamine and serine. This spray consists of 0.25% ninhydrin in acetone lutidine (9:1 v/v). The other reagents are as follows

Phosphate reagent

Ammonium molybdate (16.0 g) was dissolved in 120 ml of distilled water (solution-I). A mixture of 40 ml of conc. hydrochloric acid, 1.0 ml of mercury and 80 ml (solution-I) was shaken for 30 minutes and filtered (solution-II). To the remaining solution-I, 200 ml of conc. H_2SO_4 followed by solution-II were added. The mixture was cooled and diluted with distilled water to make upto one litre.

Anthrone reagent

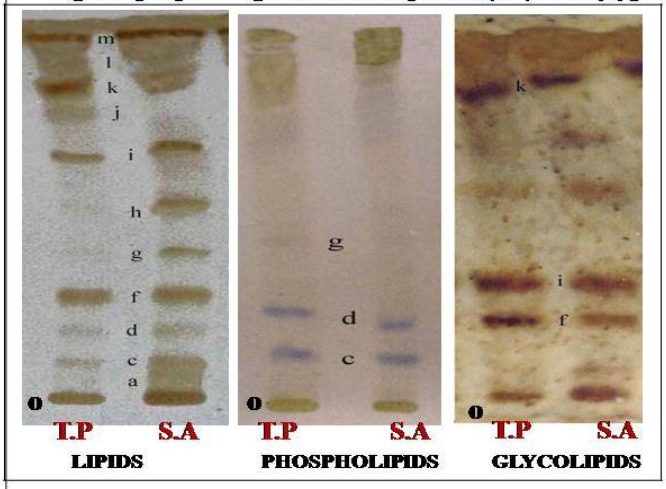
0.2% anthrone in conc, sulphuric acid was sprayed to the TLC plates and heating at 70°C for 20 minutes,

RESULTS

The identified lipids in the two selected species are phosphatidic acid, phosphatidyl serine, phosphatidyl inositol, phosphatidyl choline, phosphatidyl ethanolamine, digalactosyl diglyceride, phosphatidyl glycerol, sulpho-quinovosyl diglyceride, diphosphatidyl glycerol, monogalactosyl diglyceride, steryl glycoside, unidentified galactolipid and unidentified lipid compound U_1.

The phosphatidic acid with simple chemical structure could be seen in *Syzygium alternifolium* only. Whereas digalactosyl diglyceride, monogalactosyl diglyceride, steryl glycoside and unidentified lipid U_1 are present in both the members of selected taxa. Thus, when the fruit extracts are analyzed by TLC, the results are illustrated by Fig 4. Most of the glycolipids gave blue-violet spots. After spraying, purple spots appeared on a white background implies presence of phospholipids. When anthrone reagent is sprayed the galactolipid gave green colour and sulpholipid (sulphoquinovosyl diglyceride) yielded violet spots. When Phosphate reagent sprayed, phospholipids yielded blue spots.

Fig.4. Lipid profiling of *Terminalia pallida* (T.P) and *Syzygium alternifolium* (S.A)



Lipids
a. Phosphatidic acid,
c. Phosphatidyl inositol,
d. Phosphatidyl choline,
f. Digalactosyl diglyceride,
g. Phosphatidly glycerol,
h. Unidentified galactolipid,
i. Sulpho-quinovosyl diglyceride,
j. Diphosphatidyl glycerol,
k. Monogalactosyl diglyceride,
l. Steryl glycoside,
m. unidentified lipid (U_1).

Phospholipids
c. Phosphatidyl inositol,
d. Phosphatidyl choline,
g. Phosphatidylglycerol,
j. Diphosphatedyl glycerol.

Glycolipids
f. Digalactosyl diglyceride,
i. Sulphoquinovosyl diglyceride,
k. Monogalactosyl diglyceride.

O: Origin and Solvents used: 1st direction: Chloroform : Methanol : Acetic acid : Water (170:25:25:3)

DISCUSSION

The discovery of new bioactive compounds depends on valid biological assays, while new chemistry can make the discovery of new biological targets possible **(**Kuo-Hsiung Lee ,2012). Plants synthesize a huge array of metabolites that cannot be revealed by any single methodology (Saito and Matsuda, 2010). Because there are significant differences in the known plant and animal lipids, however, a parallel initiative to discover all of the lipids in a plant system would probably yield valuable information about additional lipid targets. It is therefore timely and important to present plant researchers with this full set of tools needed to elucidate the particular roles of diverse high valued lipids. Changes observed in the fatty acids of galactolipids, the main component of the thylakoids exclusively found in chloroplasts were studied by Wintermans (1960). Monogalactosyldiacyl glycerol which is colorless to pale yellow anti-inflammatory activity on adult articular cartilage and in addition it has a cell anti-proliferative activity (Lenti , 2009).

Mono- and digalactosyl diglycerides (MGDG, DGDG) were the predominant acyl lipids Phospholipids are the primary bilayer forming lipids of cell membranes. In developing seeds they also were more abundant than the phospholipids. Food sources are Egg yolks, liver, soybeans and peanuts. Glycolipids are lipids with a carbohydrate attached. Their role is to provide energy and also serve as markers for cellular recognition. Glycolipids are edible plant sources. Glycolipids (glyco=sugar, lipid=fat), however, are different as well, because they contain no phosphates in comparison to phospholipids (Kuksis,1987). From our investigation we have quantified and documented the presence of phospholipids,glycolipids in our experimented taxa and in this work. We also recommend for further investigations for the lipid profiling of high valued medicinal plants of Tirumala as suggested by Sree latha devi (2011).

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