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Research Article

ANTIOXIDANT ACTIVITY AND TOTAL POLYPHENOLS CONTENT OF CERTAIN HIGH VALUED MEDICINAL PLANTS OF TIRUMALA HILLS, ANDHRA PRADESH

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ABSTRACT
In present Investigation, the chloroform and ethanolic extracts of the leaves of *Syzygium alternifolium* (Wt.), *Pimpinella tirupatiensis*, *Leucas indica var nagalapuramiana* high valued plants of Tirumala hill ranges of Eastern Ghats, Andhra Pradesh were used for the study of measurement of total polyphenol content and assay of free radical scavenging activity. The aim of the present study was to determine the polyphenol and antioxidant activity of contents of crude extracts from *Syzygium alternifolium* (Wt.), *Pimpinella tirupatiensis*, *Leucas indica var nagalapuramiana*. Among three plants were studied, *Pimpinella tirupatiensis* ethanolic extract showed high amount (1.44±0.04) of polyphenol content and exhibited better antioxidant activity (84.76±1.64%) compared to *Syzygium alternifolium* and *Leucas indica var nagalapuramiana* respectively, against free radicals produced from DPPH. The use of *Syzygium alternifolium*, *Pimpinella tirupatiensis*, *and Leucas indica var. nagalapuramiana* leaves as antioxidants is a promising alternative to the use of synthetic antioxidants and seems to be the most promising species for further investigation in order to identify the compounds responsible for their activity.

Key Words: *Total Phenol Content, Antioxidant Activity, DPPH*

INTRODUCTION
Molecular oxygen in its ground state is essential to life on earth and it is relatively stable molecule that does not directly cause damage to living cells. In contrast whenever ground state O_2 molecule receive extra energy or electrons, it produce a variety of ROS(Reactive oxygen species) such as singlet oxygen ($1O_2$), hydroxyl radicals (OH^-) that causes oxidative damage to various cell components of living cells including lipids, proteins and nucleic acids (Halliwell and Gutteridge, 1990; Buxton *et al*., 1988; Asada, 1994; Apel and Hirt, 2004). ROS production and accumulation in living cells cause number of diseases, in such conditions dietary intake of antioxidants are needed to neutralization of the free radicals to remove the harmful effect of oxidative stress. The growing interest of consumers in substances of natural origin in addition to the increasing concern surrounding potentially harmful synthetic additives has resulted in the use of aromatic plants, their extracts and essential oils, as functional ingredients in the pharmaceutical, food and feed industries (Christaki *et al*., 2012).

The need of looking for new sources of substances, which, accessible from everyday food, could also influence unfavorable aging processes in the human body. Therefore, it seems to be a good aim to conduct investigations in isolation and characterization of biologically active components of plant origin, safe for people and showing high antioxidant activity if added to food**.** The most frequently encountered natural antioxidants in plants are Phenolic acids, Flavonoids such as Quercetin, catechin, rutin, Anthocyanins-Delphinidin, Tannins-Procyanidin, ellagic acid, tannic acid (Pokorny 2007). Medicinal plants, fruits, grains are rich in antioxidants such as polyphenol compounds with strong antioxidant activity (Kitts *et al*., 2000; Muselik *et al*., 2007 and Wang and Jiao 2000). Antioxidants have been used in food as additional compounds for centuries. They are mainly used to delay free radical accumulation, hence strengthening the oxidative stability of the food (Halliwell 1995 and Giese 1996). In this present investigation we assigned to analyze the physiochemical analysis of three endemic plants in Tirumala hills. Leaves of *Syzygium alternifolium* (Wt.) (Figure A), *Leucas indica var.nagalapuramiana* (Figure B),

*Pimpinella tirupatiensis (*Figure C) were analyzed and explored for the challenging phototherapeutics against ROS production.

Figure A: *Syzygium alternifolium* (Wt.) | **Figure B: *Leucas indica var.Nagalapuramiana*** | **Figure C: *Pimpinella tirupatiensis***

Table 1: List of Selected Taxa with its ethnic usage and description of the plant.

Plant	Vernacular Name	Habit, Habitat, Distribution, Leaf Characteristics
Syzygium alternifolium (Wight) Walp. **(Figure A)** Myrtaceae	Konda neredu, Mogi, Adavi neredu.	Deciduous tree, Enemic to seshachalam hills, common on Tirumala hills of open dry deciduous forests. Leaves are thick.
Leucas indica var. nagalapuramiana (Chandrab. and Sriniv.) **(Figure B)** Labiatae	Chinna poola tummi	Erect herb. Endemic to chittoor Dt and occasional on hill slopes among grasses in Tirumala hills and at srikalahasti, nagalapuramiana areas, Leaves linear, lanceolate, entire, slightly undulate or serrulate, puberulous a both sides. Endemic to Tirumala hills only.
Pimpinella tirupatiensis Bal. and Sub. **(Figure C)** Apiaceae	Konda kothimeera, Konda dhaniyalu	Herbaceous plant growing gregariously on slopes of higher hillocks and in patches of dense mixed forests with an altitude about 1080 to 1240 mts. Lower leaves simple cordate, serrate in architecture.

MATERIALS AND METHODS

Plant Material and Preparation of Extracts

Leaves of *Syzygium alternifolium* (Wt.), *Leucas indica var.Nagalapuramiana*, *Pimpinella tirupatiensis*, was collected and washed with water to remove dust from leaves. The material was air dried under light exposure (30^0C) for 10 days then pulverized to course powder and stored in an airtight container for further use. The chloroform and ethanol extracts were prepared individually by soaking 100mg of leaf power in to 1000ml of Chloroform and ethanol for 48-72 hrs. The extracts were filtered through what man filter paper (125mm) and concentrated by using a rotary evaporator. The concentrated chloroform and ethanol extracts were used for further experiments by storing at 4^0C.

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Determination of Antioxidant Activity (DPPH-scavenging activity)

The free radical scavenging activity of plant extracts against DPPH (2, 2-Diphenyl-1-picryl hydroxyl radical) was performed according the method of (Brand-Williams *et al.*, 1995 and Ayoola *et al.*, 2006).

The capacity of each plant ethanolic extract to scavenge free radicals was determined by spectrophotometric measurement for the ability of the testing samples to scavenge the model free radical DPPH. The extracts were diluted in to different concentrations 100, 200,400 and 800 µg/ml in respective solvents. Ascorbic acid used as standard and it was diluted in 50,100,150, 200 and 250µg/ml. To 1ml of extract 3ml of methanol was added and followed by 0.5ml of 1mM DPPH was added and then allowed to incubation for 30min in dark. The radical-scavenging activity was expressed in terms of the extract concentration (μgmL^{-1}). Reading was measured by using UV spectrophotometer at 517nm. Ethanol was used as blank as control.

$$\% \text{ DPPH radical scavenging activity} = \left[\frac{\text{Absorbance of Control - Absorbance of test sample}}{\text{(Absorbance of Control)}} \times 100\right]$$

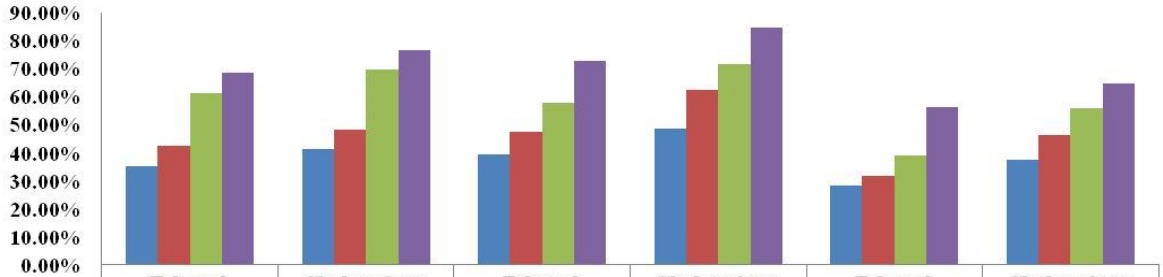
Determination of Total Phenol Content

Total phenol content in the chloroform and ethanolic extracts of *Syzygium alternifolium* (Wt.), *Pimpinella tirupatiensis*, *Leucas indica var.Nagalapuramiana* was determined with Folin-Ciocalteu reagent by using method of (Aiyegoro *et al.*, and Spanos and Worsted, 1990). 2.5ml of 10% Folin-Ciocalteu reagent and 2ml of Na_2Co_3 (2%w/v) was added to 0.5ml of each sample of plant extract solution (100, 200, 400 and 800µg/ml). The resulting mixture was incubated at 45^0C with shaking for 15min. The absorbance of the sample read at 765 nm using UV/visible light. The content of Phenolic compounds in each plant ethanolic extract was expressed as mg gallic acid equivalent (GAE) g^{-1} plant extract. Solvents used for extraction were glass distilled prior to use. Ethanol and chloroform used for activity assays were of spectrophotometric grade.

RESULTS

The present study examined the antioxidative activity of *Syzygium alternifolium* (Wt.), *Pimpinella tirupatiensis*, *Leucas indica var.Nagalapuramiana*

Fig-1: Free radicals scavenging activity



	Ethanol	Choloroform	Ethanol	Choloroform	Ethanol	Choloroform
	Syzygium alternifolium		Pimpinella tirupatiensis		Lucas indica	
100µg	35.24%	41.54%	39.54%	48.58%	28.47%	37.48%
200µg	42.65%	48.25%	47.57%	62.48%	31.75%	46.15%
400µg	61.45%	69.61%	57.64%	71.51%	39.14%	55.78%
800µg	68.54%	76.58%	72.94%	84.76%	56.48%	64.84%

Figure 1: Antioxidant properties of chloroform and ethanolic extracts of *Syzygium alternifolium* (Wt.), *Pimpinella tirupatiensis, Leucas indica var.Nagalapuramiana*.

The results are mean ± S.D. of six parallel measurements.

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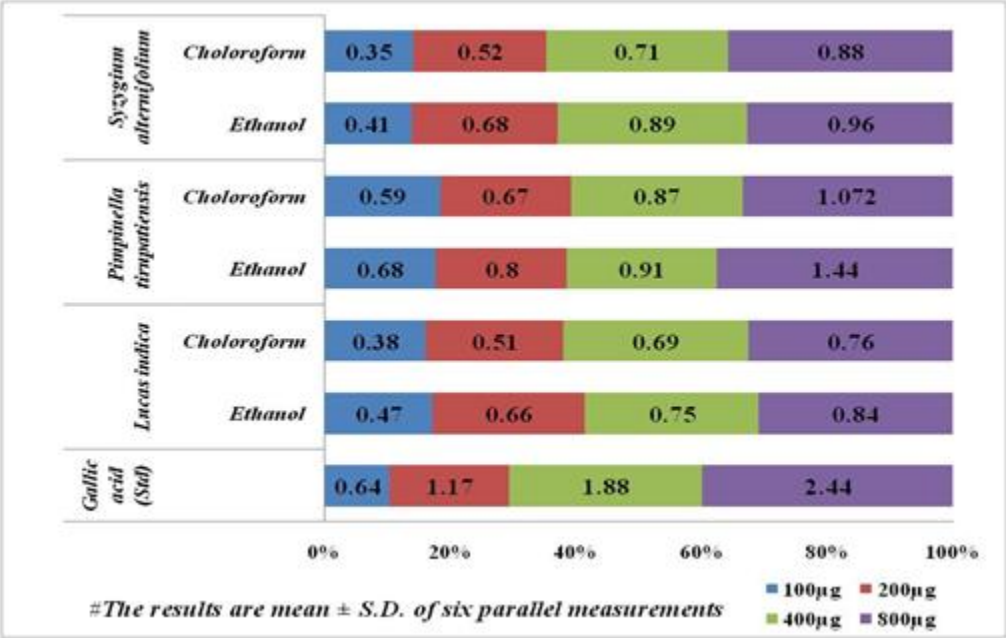
The percentage inhibition of scavenging activities of the chloroform and ethanolic extract tested plants (*Syzygium alternifolium* (Wt.), *Pimpinella tirupatiensis, Leucas indica var.Nagalapuramiana*) for DPPH was shown in Figure 1: The highest concentration of chloroform and ethanolic extracts of *Syzygium alternifolium* (68.54±1.64 and 76.58±0.27), *Pimpinella tirupatiensis* (72.58±0.65 and 84.76±1.64), *Leucas indica var.Nagalapuramiana* (56.48±0.28 and 64.84±0.82) showed high percentage of free radical scavenging activity when compared to other concentrations respectively. The ethanolic extracts of *Pimpinella tirupatiensis* showed highest (84.76±1.64) free radical scavenging activity when compared to the plants extracts (Figure 1)

Total Phenolic content of chloroform and ethanolic extracts of *Syzygium alternifolium* (Wt.), *Pimpinella tirupatiensis*, *Leucas indica* plants were recorded (Figure 2). The results are mean ± S.D. of six parallel measurements.

The presence of high amount of polyphenol content was observed at highest concentration (800µg/ml) of plant extracts. The amount of polyphenol content was noted in chloroform and ethanolic extracts respectively *Syzygium alternifolium* (0.88±0.04 and 0.96±0.07), *Pimpinella tirupatiensis* (1.07±0.07 and 1.44±0.04), *Leucas indica var.Nagalapuramiana* (0.76±0.07 and 0.84±0.04). The highest amount of Polyphenols content was observed in ethanolic extract of *Pimpinella tirupatiensis* (1.44±0.04) at 800µg of extract concentration.

Figure 2: Estimation of Total Phenolic Content.



DISCUSSION

Much attention is paid to both natural antioxidants and the search for new sources of these naturally occurring compounds. Thus a huge amount of work has been developed to find interesting sources of potentially safe natural antioxidants in many aromatic and medicinal plants. (Surveswaran 2007) Natural antioxidants are mainly less active, but it is advisable to determine the actual antioxidant activity under conditions identical with or very close to those of real applications (Evans 1997) .Generally free radicals are generated and degradation of various cellular macromolecule such as proteins, nucleic acids and lipids. It resulted severe damage of tissues and causes various disease conditions such as degenerative diseases and extensive lysis (Halliwell and Gutteridge, 2007). Today many synthetic drugs having good antioxidant activity were available in market, but they have adverse side effects. So there is need to consume natural antioxidants from nature (Yazdanparast and Ardestani, 2007 and Yazdanparast *et al.*,

2008). Many researchers were isolated many antioxidants from natural plants (Packer and Ong, 1997 and Jovanovic and Simic, 2000). leaves can be the source of polyphenolic compounds, which have strong antioxidative properties on the basis of above results obtain in present study, the ethanolic extract of *Pimpinella tirupatiensis* showed highest (84.76±1.64%) free radical scavenging activity at the same time large (1.44±0.04) amount of total polyphenol content. The presence of large quantity of total phenol may be enhances the free radical scavenging activity to the ethanolic extract of *Pimpinella tirupatiensis*. The presented research is also only one step in a wider set of experiments on the antioxidant properties of different extracts in different solvents The use of *Syzygium alternifolium, Pimpinella tirupatiensis, Leucas indica var.Nagalapuramiana* leaves as antioxidants is a promising alternative to the use of synthetic antioxidants and seem to be the most promising species for further investigation in order to identify the compounds responsible for their activity. It may also be concluded that several tests have to be applied in this kind of study for appropriate selection of plants for future research.

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