

Original Research Article

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## Screening of Flavonoids in Selected High Valued Medicinal Plants (HVM) of Tirumala, India

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### ABSTRACT

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Flavonoids are the potent therapeutical biochemicals which occur in almost all plant taxa at variable amounts. In this present article based on the ethnobotanical claims and literature survey we have investigated the presence and quantified the important flavonoids in selected taxa inhabiting Tirumala hills. Paper chromatography technique were used for the detection and the flavonoids like rutin, myricetin, quercetin, kaempferol, luteolin, apigenin, orientin and vitexin were detected with the help of  $R_f$  values and colour reactions with chromogenic spray reagents in different propositions. A total of 20 high valued medicinal plants (HVM) reported in the present study has wide number of flavonoids and are greatly used by the various ethnic and folklore communities for curing numerous diseases or ailments.

### Introduction

Flavonoids are a large group of polyphenolic compounds showing have antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities (Kumar and Pandey, 2013).

The growing body of scientific evidence indicates that flavonoids play a beneficial role in disease prevention, however further research in new therapeutic flavonoids should be discovered in different species of plant kingdom and pharmacological assays clinical and epidemiological trials are greatly needed for the dietary benefits (Manipal *et al.*, 2017). There has been increasing interest in the research of flavonoids from dietary sources,

due to growing evidence of the versatile health benefits of flavonoids through epidemiological studies. As occurrence of flavonoids is directly associated with human daily dietary intake of antioxidants, it is important to evaluate flavonoid sources in locally available plant taxa and we have put scientific efforts for thoroughly screening for phytochemical constituents.

High valued medicinal plants (HVM) are the most potent medicinal plant taxa used since ancient times to treat different disorders and diseases and are still in the process of mining the therapeutical compounds till to date (SreeLatha Devi, 2017)

The present studies is done on evaluation of Flavonoids in the twenty important

indigenous high valued selected medicinal plants of Tirumala hills in Chittoor district of Andhra Pradesh based on ethnobotanical information collected from different tribal medicobotanical informants of Nakkala, Irula, Yanadi communities.

## Materials and Methods

Twenty important indigenous high valued selected medicinal plants of Tirumala hills in Chittoor district of Andhra Pradesh was selected for the studies on the quantitation of flavonoids. Table 1 represents the plant taxa, parts used selected for the flavonoid screening were documented according to the Tribal informants' prescriptions and formulation.

Plant species were collected from different areas of Tirumala and was authenticated by SKM Basha. The plant specimens were identified and plant nomenclature is given with the help of the floras of Gamble (1957), Rangacharyulu (1991), Pullaiah and Chennaiah, (1997) and Madhava Chetty et al., (2008). The final identification was confirmed by comparing the specimens with the authentic specimens available at various botanical institutions of Madras Herbarium (MH), Coimbatore; Central National Herbarium, Howarah (CAL). All the mounted herbarium specimens were classified according to genera wise and were kept in species folders. The identified specimens were housed in the herbarium, department of botany, NBKR Medicinal Plants Research Centre, NBKR Science and Arts College, Vidyanagar, Nellore district.

Preparation of extracts were followed according to the methodology followed by Mitta *et al.*, (2014). The crude water extract was used for the present study. Quantitative analysis of flavonoids was followed according to Maniplal *et al.*, (2017). The flavonoid compounds were extracted following the method of Markham (1982).

About 2 g plant powder was dried at 40°C and taken in a boiling test tube. This was added in 18 ml of methanol and 2ml of water (9:1) shaken well and was kept for about 24 hours at room temperature. The upper clear solution of the extract was transferred to another test tube. To the remaining residue in the test tube, again 10 ml of methanol and 10 ml of water (1:1) was added, stirred well and the contents were kept for 24 hours. The clear extract thus obtained was pooled up with the earlier sample. The combined extract was mixed well and filtered through the cotton. Later the filtrate was evaporated to 1/3 of the original volume and the resultant aqueous extract was taken into a separatory funnel and then extracted with 10 ml of CHCl<sub>3</sub>. This process was repeated 3 to 4 times. All the chloroform extracts were combined and evaporated to dryness under vacuum in a rotary evaporator. Later the dried residue was dissolved in 1 ml of 95% alcohol which was stored at low temperature in a dark until used.

Finally, few ml of water extract is added to conc. HCl and Mg powder. The presence of flavonoids can be identified by the development of pink or magenta or red coloured foam.

The chromatograms after unidimensional development were removed from the chromatographic chambers and dried at room temperature. The dried sheets were observed under U.V. light and the fluorescent spots were identified. The papers while being exposed to ammonia were observed under U.V light and the new fluorescent spots were also marked and sprayed with chromogenic spray reagents for the detection of flavonoid compounds. The flavonoid compounds were identified with their R<sub>f</sub> values, colours and with those of authentic samples by co-chromatographic techniques.

Solvents used for detection of flavonoids are  
1) Iso-propyl alcohol: Ammonia (25%):

Water (8:1:1) and 2) Conc. Hydrochloric acid: Acetic acid: Water (3:30:10). Chromogenic spray reagents are a) Diazotized sulphanilic acid b) 1% Alcoholic aluminium chloride

### Results and Discussion

A total of 20 taxa reported in the present study are selected for phytochemical investigation which are widely used by the various ethnic and folklore communities for curing numerous diseases or ailments, have yielded several therapeutic agents of known chemical structures are reported in detail in the present research work. Pictorial identity of HVM is given in figure 1.

Based on the  $R_f$  values and Colour reactions on paper chromatogram detection of flavonoids has been screened and identified. Table 2 represents the flavonoid screening according to the Retention factor value and colour obtained.

Solvents used for detection of flavonoids are 1) Iso-propyl alcohol: Ammonia (25%): Water (8:1:1) and 2) Conc. Hydrochloric acid: Acetic acid: Water (3:30:10). Paper chromatogram studies revealed the occurrence of flavonoid compounds rutin, myricetin, quercetin, kaempferol, luteolin, apigenin, orientain and vitexin (Table 2).

**Table.1** Selected HVM taxa of ethnobotanically used in and around Tirumala Hills

Sl. No.	Botanical name	Family	Part used
1.	<i>Polycarpaea corymbosa</i> (L.) Lam.	Caryophyllaceae	Leaves
2.	<i>Shorea tumbuggaia</i> G.Don	Dipterocarpaceae	Bark
3.	<i>Boswellia ovalifoliolata</i> Bal. and Henry	Burseraceae	Paperry Bark
4.	<i>Soymida febrifuga</i> (Roxb.) A.Juss.	Meliaceae	Fruit pulp
5.	<i>Indigofera barberi</i> Gamble	Fabaceae	Complete plant
6.	<i>Rhynchosia beddomei</i> Baker	Fabaceae	Complete plant
7.	<i>Sophora interrupta</i> Bedd.	Fabaceae	Inflorescence
8.	<i>Terminalia pallida</i> Brindis	Combretaceae	Leaf + Fruits pulp (1:1)
9.	<i>Syzygium altenifolium</i> (Wight) Walp.	Myrtaceae	Inflorescence
10.	<i>Opuntia dillenii</i> (Ker.-Gawl.) Haw.	Cactaceae	Fruit + Flower (1:2)
11.	<i>Trianthema decandra</i> L.	Aizoaceae	Complete plant
12.	<i>Gisekia pharnaceoides</i> L.	Gisekiaceae	Leaf
13.	<i>Enicostema axillare</i> (Lam.) Raynal	Gentianaceae	Root
14.	<i>Pisonia aculeata</i> L.	Nyctaginaceae	Leaf
15.	<i>Celosia polygonoides</i> Retz.	Amaranthaceae	Immature Leaf
16.	<i>Trichuriella monsoniae</i> (Wight) Walp.	Amaranthaceae	Stem +Leaf (1:3)
17.	<i>Polygonum glabrum</i> Willd.	Polygonaceae	Complete plant
18.	<i>Givotia moluccana</i> (L.) Sreem.	Euphorbiaceae	Stem Bark
19.	<i>Stemona tuberosa</i> Lour.	Stemonaceae	Complete plant
20.	<i>Smilax perfoliata</i> Lour.	Smilacaceae	Leaf

**Table.2** Flavonoids: R<sub>f</sub> values and Colour reactions on paper chromatograms

Compound	R <sub>f</sub> values in solvent		U.V. fluorescence		Sulphanilic reagent	1% Alcoholic aluminium chloride
	1	2	Without NH <sub>3</sub>	With NH <sub>3</sub>		
Luteolin	0.44	0.66	Dull yellow	Yellow	Light red	Pale yellow
Apigenin	0.61	0.83	Red brown	Red brown	Pink	None
Orientin	0.78	0.02	Yellow	Yellow green	Grey	None
Vitexin	0.91	0.06	Dull yellow	Yellow	Bright red	None
Rutin	0.03	0.35	Orange brown	Yellow	Green	Yellow
Muyricetin	0.07	0.28	Yellow	Bright yellow	Light green	Grey yellow
Quercetin	0.26	0.41	Yellow	Light yellow	Bright yellow	Yellow
Kaempferol	0.37	0.54	Green yellow	Bright yellow	Orange	Yellow

**Table.3** Flavonoids detected in the selected HVM of Tirumala Hills

Compound	<i>Polycarpaea corymbosa</i>	<i>Shorea tumbergaia</i>	<i>Boswellia ovalifoliolata</i>	<i>Soymida febrifuga</i>	<i>Indogofera barberi</i>	<i>Rhynchosia beddomei</i>	<i>Sophora interrupta</i>	<i>Terminalia pallida</i>	<i>Syzygium alternifolium</i>	<i>Opuntia dillenii</i>	<i>Trianthema decandra</i>	<i>Gisekia pharnaceoides</i>	<i>Enicostema axillare</i>	<i>Pisonia aculeata</i>	<i>Celosia polygonoides</i>	<i>Trichuriella monsoniae</i>	<i>Polygonum glabrum</i>	<i>Givotia moluccana</i>	<i>Stemona tuberosa</i>	<i>Smilax perfoliata</i>
Rutin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Myricetin	+	+	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Quercetin	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Kaempferol	+	+	+	+	-	-	-	+	-	+	+	-	+	+	+	+	+	+	+	+
Luteolin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Apigenin	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-
Orientin	+	-	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Vitexin	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-

+ = Present; - = absent

**Fig.1** High valued Medicinal Plants: **A:** *Polycarpaea corymbosa*; **B:** *Shorea tumbuggaia*; **C:** *Boswellia ovalifoliolata*; **D:** *Soymida febrifuga*; **E:** *Indigofera barberi*; **F:** *Rhynchosia beddomei*; **G:** *Sophora interrupta*; **H:** *Terminalia pallida*; **I:** *Syzygium altenifolium*; **J:** *Trianthema decandra*; **K:** *Stemona tuberosa*; **L:** *Polygonum glabrum*

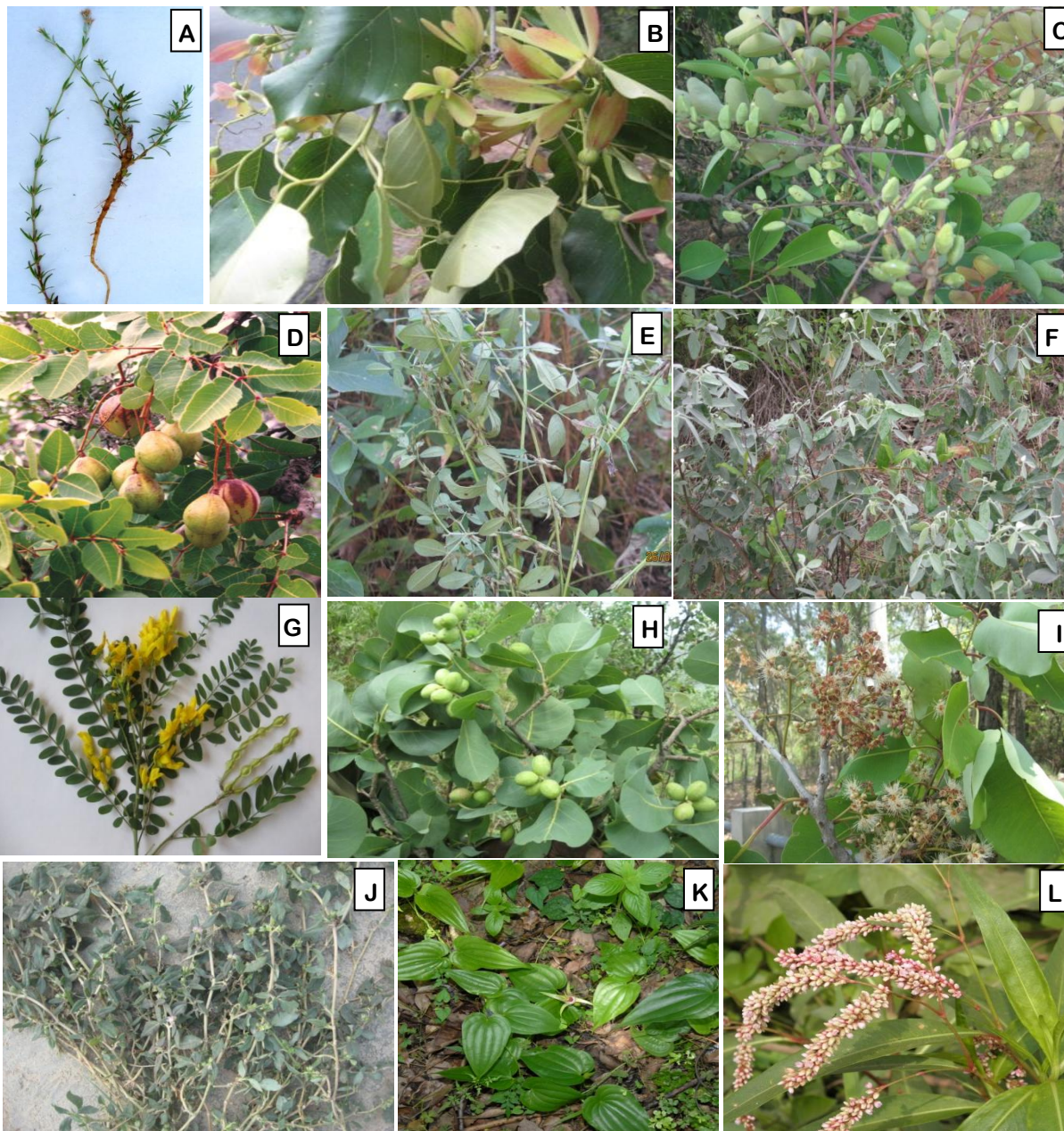


Table 3 represents the presence and absence of detected selected flavonoids in the extract of selected HVM viz., *Polycarpha corymbosa*, *Shorea tumbergaia*, *Boswellia ovalifoliolata*, *Soymida febrifuga*, *Indogofera barberi*, *Rhynchosia beddomei*, *Sophora interrupta*, *Terminalia pallida*, *Syzygium alternifolium*, *Opuntia dillenii*, *Trianthema decandra*, *Gisekia pharnaceoides*, *Enicostema axillare*, *Pisonia aculeata*, *Celosia polygonoides*, *Trichuriella monsoniae*, *Polygonum glabrum*, *Givotia moluccana*, *Stemona tuberosa* and *Smilax perfoliata*.

It was reported that the concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation. *Soymida febrifuga* contained all types of flavonoid compounds viz., rutin, myricetin, quercetin, kaempferol, luteolin, apigenin, orientin and vitexin.

Our previous investigations have revealed that the selected taxa are ethnomedicobotanicals high valued plants which can be utilized for household medications and pharmaceutical formulations (Sree Latha Devi and Basha (2013a and b). Research works by Mahendranath *et al.*, (2013) and Mitta *et al.*, (2014) justifies that Total flavonoid contents and flavonoids show antioxidant activity and biochemical potency.

The plant medicines for internal use prepared in their traditional manner involve simple methods such as hot or cold water extraction, extraction of juice after crushing and powdering of dried material, formulation of powder into pastes or pills via such a vehicle as water, butter milk, oil, honey and even fermentation after adding a sugar sours. These traditional herbal medicines were being produced using age old methods by the practitioners themselves who were able to identify the correct plant species.

Tirumala hills are a store house of very high valued medicinal plants of ethnobotanical information and it is true to extend the scientific efforts for thoroughly screening for phytochemical constituents. *Shorea tumbergaia*, *Boswellia ovalifoliolata*, *Soymida febrifuga*, *Indogofera barberi*, *Rhynchosia beddomei*, *Sophora interrupta*, *Terminalia pallida*, *Syzygium alternifolium* are some of the rare and endangered plants of Tirumala Hills. This research on screening of flavonoids in 20 selected taxa indicate that the flavonoids act as identification markers of genuinity in evaluation of ethnobotanicals and herbal drugs in the markets against adulterations also.

In conclusion this investigation revealed and proved to be highly beneficial which are used by the different ethnic and rural communities for a variety of diseases which proved that the tribal informants prescribed botanicals proves to be flavonoid rich potent phytomedicine. The present survey makes an important addition to the growing knowledge on ethnomedicobotany. When such high valued medicinal plants are frequently used, they may become rare, endangered, and even extinct in no time. So there must be a strong measure of conservation strategy to protect High valued medicinal plants for future generations for use.

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