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# Evaluation of angiospermic epiphytes for the presence of bioactive phytochemicals with special reference to phenol and tannins

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**Abstract**
Plants are the major source of large amount of bioactive compounds viz., phenols, tannin, alkaloids, saponins and flavonoids. Secondary metabolites produced by angiospremic epiphytes have been known to relieve various diseases in both plants and human being. Phytochemical analysis of leaf extract of selected angiospermic epiphytes like *Cuscuta reflexa* Roxb. *Viscum orientale* Wild, *Cymbidium bicolor* (L.) Sw., *Bulbophyllum propinquum* Kraenzl. *Hoya ovalifolia* Wild & Arnott and *Dendrophthoe falcata* (L. f.) Ettingsh indicated the variation in all the plants chosen. Total phenols and tannins content in both methanolic and aqueous extracts were found varied species wise and proved to be the good source of natural bioactive compounds may be used for the management of crops diseases caused by fungi.

**Keywords:** Angiospermic epiphytes, phytochemical analysis, bioactive compounds, methanolic extract

## Introduction
Phenolics are the aromatic organic compounds possessing one or more aromatic rings with one or more hydroxyl groups, they consist of simple phenols, benzoic and cinnamic acid, coumarins, lignins, lignans and flavonoids. Substantial developments in research focused on the extraction, identification and quantification of phenolic compounds as medicinal and dietary molecules have occurred over the last 25 years. Phenolics are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, they are known to occur in all part of plants and are said to be resistance to diseases. The chemical composition and chemical structures of active extract components are important factors governing the efficacy of natural bioactive compound. For instance, it has already been reported that phenolic compounds act as antifungal, antioxidants and anticancer agents [1, 2].
The secondary metabolism of some plant species considered as a biosynthetic pathway for the Chemical compounds like alkaloids, terpenoids, flavonoids, glycosides, tannin, etc, which are responsible for the beneficial properties, also in traditional medicine system used in the treatment of many diseases worldwide. Tannins are a heterogeneous mixture of natural polymeric phenolic compounds with a high molecular weight, which are most abundant secondary metabolite in plants. Tannins are amorphous, astringent substances occurring widely in the bark, wood, leaves, and resinous exudations of plants. They are water-soluble phenolic compounds which occur widely in vascular plants typically binds to salivary proteins to generate a perception of astringency. Tannins form colloidal solutions in water, which have a variety of biological activities. Total tannin determination was carried out by many reporters through spectrophotometry after oxidation of the analyte with the Folin–Denis or Folin–Ciocalteu reagent in alkaline medium [3].
The isolation and identification of phenol and tannin compounds from extracts of different plants have been emphasized due to their action as good natural source of bioactive compounds and has become a major area of health and medical related research. Considering these aspects, in the present study, some angiospermic epiphytes extracts were used for the extraction and estimation of total phenols, tannins spectrophotometerically, along with that the qualitative analysis of alkolids, saponins and flavonoids.

## Materials and Methods
### Collection of plant materials
Leaves of angiospermic epiphytes viz., *Cuscuta reflexa* Roxb., *Viscum orientale* Wild, *Cymbidium bicolor* (L.) Sw., *Bulbophyllum propinquum* Kraenzl., *Hoya ovalifolia* Wild & Arnott and *Dendrophthoe falcata* (L. f.) Ettingsh were collected from different locations in and around Mysore district of Karnataka state in India and were washed with running tap water,

cut into pieces and dried under shade at room temperature (28 ± 2 ºC). The dried plants leaves were powdered using mechanical grinder and stored in brown bottles till further use.

**Extraction of plant sample**

50g of powdered plant materials extracted with 70% methanol and distilled water using soxhlet extractor, separately, maintained at 65ºC for 72h. The extracted solvent was filtered using Whatman No.1 filter paper and dried. The residue of each sample was stored in the clean bottles in the refrigerator until further use. The phytochemical analysis of each sample was done following the procedures as described in many reports [4 ,5, 6].

**Test for Phenols**

2ml of distilled water was added to 1ml of plant extract followed by few drops of 10% ferric chloride solution. Formation of blue or green color indicated the presence of phenols.

**Test for Tannins**

2ml of 5% ferric chloride solution was added to 1ml of plant extract, greenish black to dark blue color formed indicated the presence of tannins.

**Test for alkaloids**

2ml of HCL was added to 2ml of plant extract, further added with a few drops of Mayer's reagent. Appearance of green color confirmed the presence of alkaloids.

**Test for Flavonoids**

1ml of 2N NaOH was added to 2ml of plant extract, yellow color indicated the presence of flavonoids.

**Test for Saponins**

To 2ml of plant extract, 2ml of distilled water was added and shaken well for 15minutes, formation of foam confirmed the presence of saponins.

**Determination of total phenolic content in the plant extracts**

The concentration of phenolics in plant extracts was determined by spectrophotometric method as described by Singleton *et al.* [7]. The reaction mixture were prepared by mixing 0.5 ml of methanolic extract with 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 20% $NaHCO_3$ solution. Blank was prepared**,** containing 0.5ml methanol, 2.5ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5ml of 20% of $NaHCO_3$ solution. The samples were thereafter incubated at room temperature for 45minuts. Similarly the reaction mixtures were prepared using aqueous extracts and for all the samples, the absorbance was recorded using spectrophotometer at 650nm. The samples were prepared in triplicates for each analysis and the mean value were calculated based on the standard curve obtained using gallic acid solution at serially increased concentration at 20, 40, 60, 80 and 100mg/ml and the content of phenolics in extracts were expressed in terms of gallic acid equivalent (mg of GA/g of extract).

**Determination of tannin content in the plant extracts**

The tannins in the plant extracts were determined using Folin - Ciocalteu method. For this purpose 0.1ml of the sample was added to the solution containing 7.5 ml of distilled water, 0.5ml of Folin-Ciocalteu phenol reagent, 1ml of 35% $Na_2CO_3$ and was diluted to 10ml with distilled water. The mixture was shaken well and kept at room temperature for 30min. A set of reference standard solutions of Gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier and the standard curve was plotted by recording their absorbance at 725nm using a visible spectrophotometer. Similarly, absorbance for test solutions were also measured against the blank at 725nm. The data were compiled and the tannin content was expressed in term of mg of GAE/g of extract as described in other reported by Tambe and Bhambar [8] and the results were tabulated based on the average of triplicates.

**Results and Discussion**

Angiospermic epiphytes used in this study showed the occurrence of various bioactive compounds such as phenols, tannins, flavonoids, saponins and alkaloids. Table 1 revealed the qualitative analysis of bioactive secondary metabolites in the epiphytes extracts (Table 1). Both aqueous and methanolic extracts of all the plant species showed the occurrence of phenols, tannins, alkaloids, saponins and flavonoids. However, flavonoids in *C. reflexa*, saponins in *V. orientale*, phenols and flavonoids in *C. bicolor*, flavonoids in *H. ovalifolia* were not observed. The comparative analysis of the active compounds in the plant extracts is variation in the color intensity indicated the varied concentrations of the compounds which was marked as additional + marks parenthesis as shown in the table (Table 1).

**Table 1:** Phytochemical analysis of methanol extract of some angiospermic epiphytes

| Angiospermic epiphytes | Presence of bioactive compounds in Methanol extract of selected angiospermic epiphytes | | | | |
|---|---|---|---|---|---|
| | **Tannin** | **Phenols** | **Alkaloids** | **Flavonoids** | **Saponins** |
| *Cuscuta reflexa* | + (++) | + (+) | + (+) | _ | + |
| *Viscum orientale* | + (+++) | + (+++) | + (++) | + (+) | _ |
| *Cymbidium bicolor* | + | _ | + | _ | + (+++) |
| *Bulbophyllum propinquum* | + (+) | + | + | + | + (+) |
| *Hoya ovalifolia* | + (+) | + (+) | + | _ | + (+++) |
| *Dendrophthoe falcata* | + (++) | + (+++) | + (+) | + (+) | + (+) |

(-) The absence of the compound; (+) the presence of the compound; (++), (+++) Increased intensity of color

Data pertaining to the quantification of phenol and tannin in selected epiphytes were presented in the table (Table 2). Data revealed the varied level of both phenol and tannins, varies with respect to different epiphytes. None of the sample indicated the zero level of phenol and tannin. Both aqueous and methanol extracts showed less content of phenol and tannin compared to methanol extract except in case of *H. ovalifolia* extract. In all the cases methanol was found to be more effective in the extraction of phenol and tannin, which was 2 to 3 fold more compared to aqueous extract. But it was found to be reverse in case of *H. ovalifolia*, where the aqueous solution was proved its efficacy over the usage of

methanol for extraction of phenol and tannin. Among the epiphytes *D. falcata* extract showed high content of phenol followed by *V. orientale* and *C. reflexa*. However, tannin content was found to be more in case of *V. orientale*, followed by *D. falcata* and *C. reflexa*. Comparatively, *C. bicolor* extract indicted the less yield of both phenol and tannin in both the extracts. The varied content of the bioactive secondary metabolites is probably due to their varied component wise accumulation. It might also be due to adaptation of plant during their life time to the surroundings which varies from species to species. It's perhaps due to change in their structure which make differences in the release of such compounds in to the used solvents, ultimately depending upon the occurrence of number of –OH groups in them. The differences in the yield of phenol and tannin may also due to the nature of cross linking of bioactive compounds with other macro molecular in the plants, which may require more time of refluration which might have also depending upon the type of solvent used for extraction.

**Table 2:** Determination of Phenols and Tannin content of angiospremic epiphytes extract expressed in term of gallic acid equivalent (mg of GA/g of extract)

| Angiospermic epiphytes used for extract | Occurrence of total phenol and tannin in epiphytes extract (mg of GA/g of extract) | | | |
|---|---|---|---|---|
| | Total phenols* | | Total tannin* | |
| | Aqueous Extract | Methanol extract | Aqueous Extract | Methanol Extract |
| *Cuscuta reflexa* | 9.02 ±0.03 | 31.1 ±0.12 | 10 ±0.08 | 42.3 ±0.09 |
| *Viscum oreintale* | 22.03 ±0.20 | 41.3 ±0.05 | 32.06 ±0.12 | 58.36 ±0.12 |
| *Hoya ovalifolia* | 24 ±0.09 | 15.1 ±0.12 | 34.2 ±0.12 | 17.3 ±0.12 |
| *Bulbophyllum propinquum* | 14.06 ±0.07 | 28.13 ±0.08 | 17.16 ±0.07 | 33.2 ±0.12 |
| *Cymbidium bicolor* | 13.13 ±0.07 | 18.03 ±0.20 | 18.23 ±0.10 | 21.3 ±0.12 |
| *Dendrophthoe falcata* | 43.16 ±0.07 | 54 ±0.07 | 54.26 ±0.07 | 55.13 ±0.12 |

*Each value represents the average of triplicates ± S. E.S. E. = Standard Error

Plants secondary metabolites are important for the plant to intact with its environment and defense against herbivores and pathogens, often they confer protection against environmental stresses [9]. In the present study varied level of secondary metabolites was recorded species wise. This differences in their quantity may be due to dependency of epiphytes variedly with respect to their physiohgcial and developmental stage as described [10]. Differences in the phenol and tannins among the epiphytes screened may be also be due to the local geo-climate seasonal changes, external conditions such as light, temperature, humidity which might have affected their synthesis and accumulation. Similar findings were reported by Morison and Lawlor [11] in *Panax ginseng*. Occurrence of saponins in a few epiphytes represents the adoptative strategy to environmental stress leading to tolerance to adversed conditions. This is in parallel to the observations of Szakiel *et al.* [12] with respect to the accumulation of saponins in different parts of *Panax ginseng*. Qualitative and quantitative difference in the vegetative parts of the epiphytes in perhaps also due to their growth in $CO_2$ rich environment. Similar findings were reported by Idso and Idso [13] in plants pertaining to increased accumulation of phenols and tannins under elevated $CO_2$ environment. Increase in polyphenols under increased salinity has been reported in several plants [14, 15]. The observations made in the present studies are thus in support of these reports.

Epiphytes which have been screened might have been exposed to severe cold and light conditions during their growth and hence there is increased accumulation of phenolics and tannins. This is in similarities with the observations in other reports in apple trees. These reports Griffith and Yaish; Perez-Ilzarbe *et al.* [16, 17] also claimed their increased accumulation and incorporation in to cell wall in the form of lignin and suberin which are protective in function. Since the selected epiphytes are of different genera might have experienced the same and thus showed the different level of secondary metabolites. The positive correlation between accumulation of secondary metabolites with increased intensity of light have been reported Chalker-Scott [18] in several crops. In contract Larsson *et al.* [19] reported the less synthesis of tannins and phenolic glycosides in the willow foliages which were grown under shade. Similar condition played with the present selected epiphytes, which might have affected the varied accumulation of secondary metabolites in the foliages.

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**References**

1. Heinonen IM, Meyer AS, Frankel EN. Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. Journal of Agricultural and Food Chemistry. 1998; 46(10):4107-4112.
2. Roleira FM, Tavares-da-Silva EJ, Varela CL, Costa SC, Silva T, Garrido J *et al*. Plant derived and dietary phenolic antioxidants: anticancer properties. Food Chemistry. 2015; 183:235-258.
3. Tambe VD, Bhambar RS. Estimation of total phenol, tannin, alkaloid and flavonoid in Hibiscus tiliaceus Linn. Wood extracts. Research and Review Journal of Pharmacognosy and Phytochemistry. 2014; 2(4): 41-47.
4. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Internationale pharmaceutica sciencia, 2011; 1(1):98-106.
5. Kumar RS, Venkateshwar C, Samuel G, Rao SG. Phytochemical screening of some compounds from plant leaf extracts of *Holoptelea integrifolia* (planch.) and *Celestrus emarginata* (Grah.) used by Gondu tribes at Adilabad District, Andhra Pradesh, India. International Journal of Engineering Science Invention. 2013; 2(8):65-70.
6. Kumar Bargah R. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma* Gaertn. Journal of Pharmacognosy and Phytochemistry. 2015; 4(1):
7. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates

and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology, Academic press. 1999; 14(299):152-178.
8. Tambe VD, Bhambar RS. Estimation of total phenol, tannin, alkaloid and flavonoid in *Hibiscus tiliaceus* Linn. Wood extracts. Research and Review Journal of Pharmacognosy and Phytochemistry. 2014; 2(4): 41-47.
9. Bennett RN, Wallsgrove RM. Secondary metabolites in plant defence mechanisms. *New phytologist*, 1994; 127(4):617-633.
10. Ravishankar GA, Rao SR. Biotechnological production of phytopharmaceuticals. J Biochem. Mol. Biol. Biophys. 2000; 4:73-102.
11. Morison JIL, Lawlor DW. Interactions between increasing CO2 concentration and temperature on plant growth. Plant, Cell and Environment. 1999; 22(6):659-682.
12. Szakiel A, Pączkowski C, Henry M. Influence of environmental abiotic factors on the content of saponins in plants. Phytochemistry Reviews. 2011; 10(4):471-491.
13. Idso CD, Idso KE. Forecasting world food supplies: The impact of the rising atmospheric CO~ 2 concentration. Technology-elemsfors-journal of the fralkin institute then journal of science serving legislative regulatory and judicial systems. 2000; 7:33-56.
14. Parida AK, Das AB. Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Saf. 2005; 60:324-49. PMID: 15590011; DOI:10.1016/j.ecoenv.2004.06.010.
15. Navarro JM, Flores P, Garrido C, Martinez V. Changes in the contents of antioxidant compounds in pepper fruits at ripening stages, as affected by salinity. Food Chemistry. 2006; 96:66-73.
16. Griffith M, Yaish MW. Antifreeze proteins in overwintering plants: a tale of two activities. Trends in plant science. 2004; 9(8):399-405.
17. Pérez-Ilzarbe J, Hernández T, Estrella I, Vendrell M. Cold storage of apples (cv. Granny Smith) and changes in phenolic compounds. Zeitschrift für Lebensmitteluntersuchung und-Forschung A. 1997; 204(1):52-55.
18. Chalker-Scott L. Environmental significance of anthocyanins in plant stress responses. Photochemistry Photobiology. 1999; 70:1-9.
19. Larsson S, Wiren A, Ericsson T, Lundgren L. Effects of light and nutrient stress on defensive chemistry and susceptibility to *Galerucella lineola* (Coleoptera, Chrysomelidae) in two *Salix* species. Oikos. 1986: 47:205-10.