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QUANTITATIVE STUDY OF TOTAL PHENOLICS, FLAVONOIDS AND PHYTOCHEMICAL TEST'S IN *BOERHAVIA DIFFUSA* AND *EUPHORBIA HIRTA*

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ABSTRACT

Polyphenols comprise of large group of secondary metabolites like phenolics and flavonoids. These polyphenols are known antioxidants and are a subject of interest for long due to their benefit to human health and preventing many diseases. Identification and extraction of phenolics and flavonoids from plant tissue is hence gaining importance in health and medical research. Present study focuses on extraction and spectrophotometric quantitaion of phenolics and flavanoids from methanolic extract of *Boerhavia diffusa* and *Euphorbia hirta* (root, leaf and flower). Our result showed higher phenolic and flavonoid content in leaf and flower of *Boerhavia diffusa* and *Euphorbia hirta* respectively. Phytochemical tests with both the plant extracts showed presence of flavonoids, alkaloids and glycosides while terpenoids were only found to be present in *Boerhavia diffusa*.

KEYWORDS: *Flavonoids, Phenolics, Phytochemicals, Antioxidant, Medicinal plants.*

INTRODUCTION

Flavonoids and the other phenolic compounds are commonly known as plant secondary metabolites or phytochemical with aromatic ring bearing at least one hydroxyl groups. More than 8000 naturally occurring phenolic compounds from plants have been reported and half of these phenolic compounds are flavonoids representing as aglycone, glycosides and methylated derivatives.[1,2] These phytochemical substances are present in nutrients and herbal medicines.[3] Flavonoids and phenolics have been reported for their effective antioxidants, anticancer, antibacterial, cardioprotective, anti-inflammation, immune system promoting agents, for pharmaceutical and medical application.[4,5,6] For decades, the research studies focusing on flavonoids and the other phenolic compounds from medicinal plant species have increased Considerably because of their versatile benefits for human health.[7,8,9,10] *Boerhavia diffusa* and *Euphorbia hirta* are medicinally important herbaceous plants commonly found in Goa. *Boerhavia diffusa* is commonly known as punarnava in vernacular language and belongs to the family Nyctaginaceae.[11] The plant is widely dispersed, occurring throughout India, the Pacific, and southern United States and is known for its anti-inflammatory and expectorant properties. In ayurvedic medicines, Punarnava is said to be a good cure for rheumatoid arthritis (RA), analgesic, cataract, chronic conjunctivitis, blepharitis, intestinal colic, kidney disorders, asthma and jaundice.[11]

Euphorbia hirta is commonly called as asthma-plant belongs to family Euphorbiaceae. It is a pantropical weed, distributed throughout the hotter parts of India and Australia, often found in waste places along the roadsides.[12] This plant shows antibacterial, anti-inflammatory, antimalarial, galactogenic, antiasthmatic, antidiarrheal, anticancer, antioxidant, antiferlity, antiamoebic and antifungal activities. Further research is going on to find out more active constituents of *Euphorbia hirta*.[13,14] There are many other traditional uses of *Euphorbia hirta* in Ayurveda which serves as the basis for further studies.[12]

Considering the importance of phytochemicals as Antioxidants, health promoting and pharmaceutical effects, the present work was planned to undertake quantitative study of phytochemicals in medicinally important herbs such as *Boerhavia diffusa* and *Euphorbia hirta.*

MATERIALS AND METHOD

Sample collection: Fresh plant material of *Boerhavia diffusa* and *Euphorbia hirta* (root, leaf and flower) belonging to family Nyctaginaceae and Euphorbiaceae respectively were collected in the month of October from the college campus. The specimens were authenticated by the Department of Botany, Chowgule College and are maintained in the Department herbaria (Fig 1).

Figure 1: Euphorbia hirta and Boerhavia diffusa

Sample extraction[15]: Plant material (500g)was sun dried and homogenized overnight in 1L of methanol, filtered through Whatman filter paper No.1. The filtrate was subjected to rotary vacuum evaporator for solvent evaporation. The residues were re-dissolved in 500mL of methanol and stored at 4˚C until use. This extract was used for analysis of phenolic and flavonoid content.

Quantitation of phenolic and flavonoid content

Total phenolic content[16]: Total phenolic content was determined spectrophotometrically. Plant extract (0.5mL) was mixed with 0.5mL of Follin-Ciocalteu reagent (diluted with distill water 1:1). After 5min of incubation at room temperature, 2mL of 20% Sodium Carbonate (Na_2CO_3) was added. The tube contents were further incubated at room temperature followed by absorbance measurement at 650 nm. Gallic acid was used as standard.

Total flavonoid content[17,18]: Total flavonoid content was determined spectrophotometrically. Plant extract (0.5mL) was mixed with 4mL distilled water and 0.3mL of 5% Sodium Nitrate ($NaNO_2$). The test tube contents were then incubated for 5 minutes. 0.3mL of 10% Aluminium Chloride ($AlCl_3$) was then added followed by 2mL of 1M Sodium Hydroxide (NaOH) solution making the final volume to 10mL by addition of distilled water. Absorbance was measured at 510nm with Quercetin as standard.

Phytochemical tests[19,20,21]

Phytochemical tests from plant extracts of *Boerhavia diffusa* and *Euphorbia hirta* (root, leaf and flower) were performed. Test for flavonoids, alkaloids, glycosides and terpenoids were carried out based on standard methods.

Test for flavonoids: Plant extract (1mL) was mixed with few drops of dilute Sodium Hydroxide (NaOH) solution to get intense yellow colour. Few drops of dilute acid (HCl) were then added. The solution turned colourless indicative of the presence of flavonoids.

Teat for alkaloids: Plant extract (0.2g) was mixed with 1mL 2% Sulphuric acid (H_2SO_4) and incubated for 2 minutes at room temperature. A few drops of dragendorff's reagent was added to the extract filtrate and mixed. Appearance of blue black colour indicated the presence of alkaloids.

Test for glycosides: Plant extract (0.5mL) was mixed with 2mL glacial acetic acid and 2 drops of Iron Chloride ($FeCl_3$) solution. This was followed by addition of few drops of tetraoxosulphate (VI). Formation of purple Colour layer indicated the presence of glycosides (digitoxose sugar).

Test for terpenoids: Plant extract (0.5g) was mixed with 2mL of chloroform and 5mL of sulphuric acid (H_2SO_4). Appearance of reddish brown colour at the interface indicated the presence of terpenoids.

STATISTICAL ANALYSIS

All experiments were repeated thrice independently with similar results. Data shown are expressed as mean ± standard deviation (S.D) with readings of three samples per tissue. Data analysis was based on one-way analysis of variance (ANOVA). All statistical analysis were performed using Microsoft Excel Version 2010.

RESULTS

Total phenolic content

The results of total phenolic content in *Boerhavia diffusa* and *Euphorbia hirta* are shown in *figure 2*.

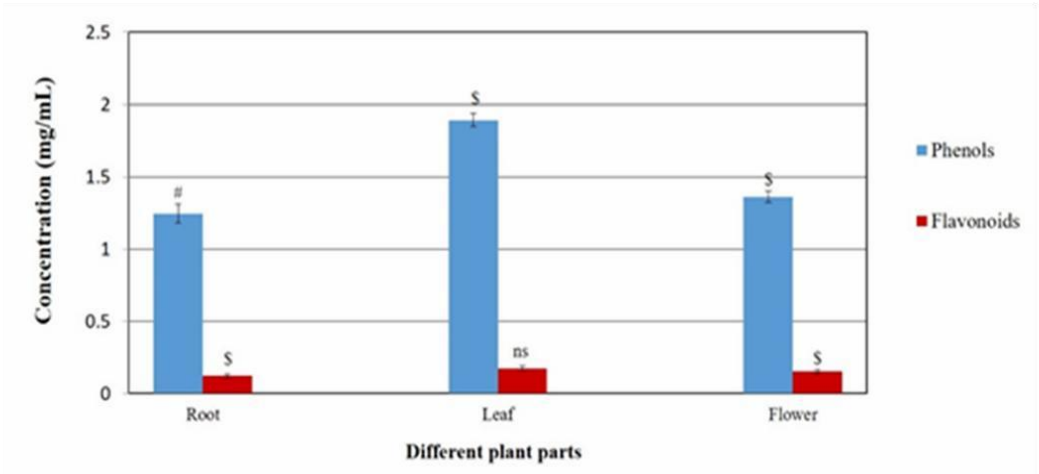

Figure 2: Total phenolic and flavonoid content in Boerhavia diffusa

The values are mean of 3 experiments ± S.D. Statistical data shows significant difference at $^{\#}P = 0.05\text{-}0.01$, $^{\$}P = 0.001\text{-}0.005$ & $^{ns}P > 0.1$

Our results with methanolic extract showed higher phenolic content in leaf (1.649 and 2.077mg/mL) and flower (1.593 and 1.996mg/mL) extracts of *Boerhavia diffusa* and *Euphorbia hirta* respectively than in its root extract.

Total flavonoid content

The results of total phenolic content in *Boerhavia diffusa* and *Euphorbia hirta* are shown in *figure3*. Our results with methanolic extract showed higher flavonoid content in leaf (0.175 and 1.996 mg/mL) and flower (0.152 and 0.423mg/mL) extracts of *Boerhavia diffusa* and *Euphorbia hirta* respectively than in its root extract.

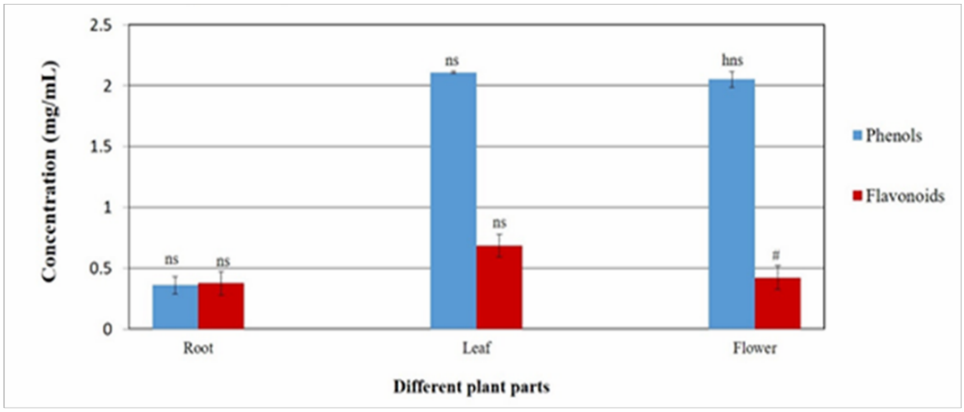

Figure 3: Total phenolic and flavonoid content in Euphorbia hirta

The values are mean of 3 experiments ± S.D. Statistical data shows significant difference at $^{\#}P = 0.05\text{-}0.01$, $^{ns}P > 0.1$ & $^{hns}P > 5$

Quantitation of total phenolics and flavanoids are shown in Table 1.

Table 1: Total phenolic and flavonoid content in *Boerhavia diffusa* and *Euphorbia hirta*

Plant extract	Total phenols (mg/mL)			Total flavonoids (mg/mL)		
	Root	Leaf	Flower	Root	Leaf	Flower
Boerhavia diffusa	1.033±0.2184	1.649±0.2456	1.593±0.2527	0.122±0.0088	0.175±0.1062	0.152±0.0305
Euphorbia hirta	0.152±0.0305	2.077±0.0453	1.996±0.118	1.996±0.118	1.996±0.118	0.423±0.135

Phytochemical tests

Our results on phytochemical tests with the plants under study are shown in Table 2, Fig 4. The result showed that both the plants *Boerhavia diffusa* and *Euphorbia hirta* showed presence of flavonoids, alkaloids and glycosides while terpenoids were only found to be present in *Boerhavia diffusa*.

Table 2: Phytochemical tests

Sr. no.	Chemical constituents	Observations	Inference					
			Boerhavia diffusa			Euphorbia hirta		
			Root	Leaf	Flower	Root	Leaf	Flower
1.	Alkaloids (Dragendorff's test)	Orange red ppt	+	+	+	+	+	+
2.	Glycosides	Brown ring formation	+	+	+	+	+	+
3.	Flavonoids	Yellow colour	+	+	+	+	+	+
4.	Terpenoids	Reddish brown colour	+	+	+	–	–	–

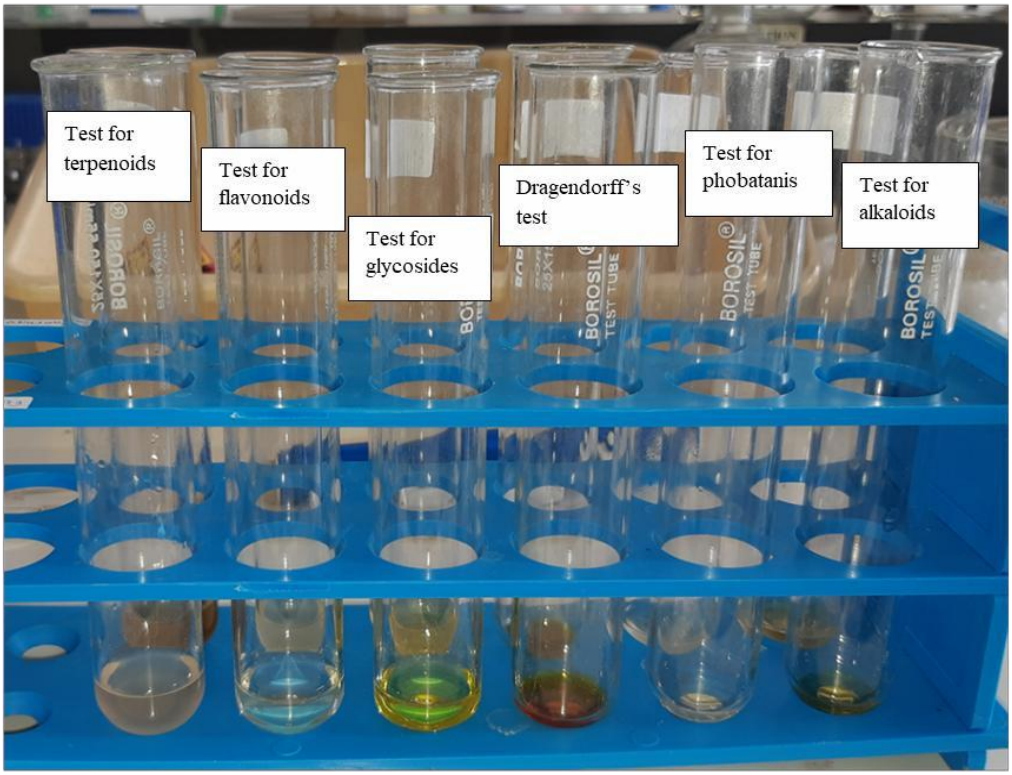

Figure 4: Various phytochemical tests

DISCUSSION

Number of medicinal plants contain, different types of bioactive compounds having antioxidant activity.[22,23,24] These antioxidants are known to play significant role in reducing the generation of free radicals. Using NADPH as the ultimate electron donor, low molecular weight antioxidants such as glutathione and ascorbate are synthesized in plants within the chloroplast stroma and cytosol.[25] These low molecular weight antioxidants, function as redox buffers interacting with numerous cellular components and influence plant growth and development. In addition, these antioxidants may influence gene expression associated with biotic and abiotic stress responses to maximize defense.[26]

Commercial antioxidants such as butylated hydroxytoluene (BHT), Propylene, tocopherol etc. are known for certain undesirable side effects. High-dose supplements of such antioxidants may be linked to health risks in some cases.[27] High-dose vitamin E supplements is linked to increased risks of hemorrhagic stroke (a type of stroke caused by bleeding in the brain) and prostate cancer. Hence there has been increasing interest amongst researchers towards antioxidants from plants and their use in medicine. In Unani and Ayurvedic medicines, plant derived medicines have been used for treating diseases and thus, phytomedicines are become natural blueprint for the development of a drug.[28]

In the present work, an attempt is made to quantitate and compare phenolics and flavonoid content in leaves, flower and root of medicinally important plants *Boerhavia diffusa* and *Euphorbia hirta*. Our study showed more phenolic and flavonoid content in leaves of *Boerhavia diffusa* and *Euphorbia hirta*. However, flavonoid content was found to be more in flower extracts.

Various reports also exist that indicate *Euphorbia hirta* and Boerhavia diffusa show increase in phenolic and flavonoid compounds and this increase has also been correlated to higher antioxidant activity in different parts *E. hirta* and *B.diffusa*.[29,30] Phenolic compounds form a complex redox reaction with phosphotungstic and phosphor-molybdic acid present in F.C reagent.[31] Antioxidant activity of plants appears to be due to the phenolic compounds or could be due to other antioxidant secondary metabolites such as vitamins, oils and carotenes. A similar correlation of phytochemicals with antioxidant activity is reported by us in our earlier reports in Moringa and Tulsi plants.[10,15] Role of polyphenolic compounds like flavonoids and flavanols in stabilizing lipid oxidation and anti-oxidant activity has been shown.[32] Anti-inflammatory, anti- carcinogenic properties of Flavonoids phenolic acids, tannins are shown to be associated with anti-oxidant activities.[33] Sub chronic and acute toxicity Study of *B. diffusa was tested* in albino mice by oral administration of the aqueous leaf extract. The aqueous extract showed progressive increase in body weight that was correlated with increased food and water intake by mice. Their study also reported that the aqueous leaf extract caused no toxicity to kidney and other organs of the treated mice.[34]

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CONCLUSION

From our results we conclude that, Phenolics and flavonoids were found to be present in both the plants. The study suggested that the leaves and flowers of *Boerhavia diffusa* and *Euphorbia hirta* contain maximum phenolic and flavonoid content than the roots. Phytochemical tests for flavonoids, alkaloids and glycosides were tested positive however, terpenoids were present only in *Boerhavia diffusa*. Further study towards characterization of biologically active drug in these plants is required. This will help to identify therapeutic agent in them with the potential to prevent or cure diseases due to oxidative stress.

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