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# EVALUATION OF SECONDARY METABOLITES FROM SOME MEDICINAL PLANTS BY SPECTROPHOTOMETRIC METHOD

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***Abstract:*** This study mainly focused on evaluation or quantification of secondary metabolites by spectrophotometric method. Plant extract was prepared in alcoholic extract for quantification. Quantitative analysis was performed with the help of UV-vis spectrophotometer. The highest quantity of secondary metabolites was found in *Barleria prionitis* than *Ceasalpinia bonduc* and *Leonotis nepetifolia*. The highest amount of total phenol, ortho-dihydric phenol, tannin, and leuco-anthocyanin in *Barleria prionitis* was found 10 µg/gm, 9.5 µg /gm, 1.6 µg /gm and 0.055 µg /gm respectively. While *Caesalpinia bonduc* showed highest amount of anthocyanin (0.0325 µg /gm) than that of Barleria prionitis and Leonotis nepetifolia. Quantity of flavanol is high in *Leonotis nepetifolia* (0.86 µg /gm). It was concluded that *Barlelia prionitis* having more secondary metabolites which gives medicinal property to plant and may use as a traditional medicine.

***Index Terms :*** Secondary metabolites; Spectrophotometry; *Barleria prionitis; Caesalpinia bonduc; Leonotis nepetifolia.*

## 1. Introduction

Plant kingdom has always acted as sources of medication in all parts of the world. Hence the use of plants and their drugs is as old as origin of man himself, although the plants have been used by human from the ancient times. Various studies have pointed out that many drugs that are used and produced on commercial scale have come from folk-use and use of plants by various people of different religion. Medicinal plants play a vital role in health care system of human being and animal. Medicinal plants have their value due to substance present in various plant tissue. The substance used as therapeutic agent or an active ingredient for medicinal preparation. Medicinal plants are rich source of bioactive compound and thus serve as important row material for drug production. The medicinal plant contributes substantially to health, cultures, integrity and local economics, particularly among the tribes. All plants containing active compounds are important. The beneficial effects of plant materials typically result from the combination of secondary products present in the plants. In plants these compounds are mostly secondary metabolites such as alkaloids, cyanogenic glycoside, flavonoids, saponin, steroids and terpenoids, which are synthesized and deposited in

specific parts or in all parts of the plant. These specialized compound from secondary metabolites are essential to However, secondary plant metabolites are useful in the long term, often for defence purpose, and give plants characteristics such as colour.

Plants are playing an important role in medicinal world today as a source of medicine since ancient time. A number of medicinal plants and their purified constituent show beneficial therapeutic potential. Plant have been used as source of medicine since ancient time. The curative properties of medicinal plants are mainly due to the presence of various complex chemical compounds in different compositions which occur as secondary metabolites. These secondary metabolites produce specific physiological action on human body (Saxena et. al., 2018). The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led scientists to investigate the antimicrobial activity of medicinal plants. Likewise, the use of synthetic antioxidants is suspected to cause or promote negative health effects, hence stronger restrictions are being placed on their application and a trend to substitute them with naturally occurring antioxidants is developing (Nisa et. al., 2013).

Secondary plant metabolites are organic compound produce by the plant cell through metabolic pathway derived from the primary metabolic pathway. These compounds do not play direct role in growth and development. They also differ from primary metabolites (Rahab and Amira 2018). Secondary metabolites are often found in only particular plant species or related plant species, but primary metabolites are found throughout plant kingdom.

Several portions of *Barleria prionitis* individually or jointly administrated successfully by traditional practitioners specifically against fever, severe pain, asthma, ulcer etc. and also play significant role as anti-microbial, free radical scavenging, gastro liver protective agent (Talukdar et.al., 2015).

HPTLC studies confirmed the presence of flavonoids, diterpenes, phenolic compounds and neuroceutical studies revealed the presence of carbohydrates, proteins and amino acids (M. Kamalam et.al., 2013).

## 2. Material and methods:

*2.1. Collection of plants*

*Barleria prionitis* and *Caesalpinia bonduc* plants were collected from botanical garden of Govt. Vidarbha Institute of Science and Humanities, Amravati and *Leonotis nepetifolia* plant were collected from farm area of village Zada Tq- Dhamangaon rly. Dist.- Amravati Maharashtra.

*2.2. Preparation of Plant Extract*

1 gm fresh plant material was grinded with 10 times volume of 80% ethanol. Centrifuge at 1000 rpm for 5 min. and supernatant was taken for analysis. Supernatant solution was evaporated to dryness and residue was dissolved in 10 ml distilled water. Stored at $4^0$ C for further use. (Thambavani S, Kumar SR. 2011)

*2.3. Estimation of Total Phenol*

2 ml of plant extract was taken in test tube and volume was makeup to 5 ml with distilled water. 0.5 ml of folin-ciocalteu reagent was added. After 3 minutes 2 ml of 2% sodium carbonate solution was added. Test tube was placed in boiling water bath for 1 minutes. After cooling the solution absorbance was measured at 650 nm against the reagent blank. (Thambavani S, Kumar SR. 2011)

*2.4. Estimation of Ortho-dihydric Phenol*

1 ml of plant extract was taken in test tube then 1 ml of 0.5 N HCL, 1 ml of Arnow's reagent, 10 ml of distilled water and 2 ml of 1 N NaOH solution was added (pink colour appears). Then absorbance was measured at 515 nm. Against reagent blank. (Thambavani S, Kumar SR. 2011)

*2.5. Estimation of Flavanols*

1 ml of plant extract was taken in 25 ml of cap conical flask, 1 ml of distilled water was added. 4 ml of vanillin reagent from burette was added rapidly (within 10-15 sec.) to flask A, and 4 ml of 70% $H_2SO_4$ to flask B. reagent blank was prepared in flask C containing 4 ml of vanillin reagent and 2 ml of distilled water. Both the flasks A and B was shaken in water bath for below the temperature $30^0$C. flasks was kept at room temperature for 15 minutes and absorbance of flasks A, B and C was measured at 500 nm against 47% $H_2SO_4$. (Thambavani S, Kumar SR. 2011)

*2.6. Estimation of Tannin* (vanillin hydrochloride method)

1 gm of fresh plant material was grinded in 50 ml of ethanol. Centrifuged at 1000 rpm for 5 min. and supernatant was collected. 1ml of supernatant solution was taken in test tube and 5 ml of vanillin hydrochloride reagent was added quickly. After 20 min. absorbance was measured at 500 nm. (Thambavani S, Kumar SR. 2011)

*2.7. Estimation of Anthocyanin*

1 gm of fresh plant material was grinded with alcohol. Centrifuged at 1000 rpm for 5 min. and supernatant was collected. 1ml of supernatant solution was taken in test tube and 3 ml of HCL in aqueous methanol, 1 ml of anthocyanin reagent was added to sample solution and incubated in dark for 15 minutes. Absorbance was measured at 525 nm against blank. (Thambavani S, Kumar SR. 2011)

*2.8. Estimation of Leuco-anthocyanin*

1 gm of fresh plant material was grinded in ethanol and then centrifuged at 1000 rpm for 5 minutes and supernatant was collected. 1 ml of supernatant solution was pipette out in test tube. The volume was reduced to 0.5 ml by keeping the test tube in hot water bath so that the sample does not contain more than 0.5 ml of ethanol. After that 0.5 ml distilled water and 10 ml of leuco-anthocyanin reagent was added. Test tubes was heated in water bath at 97±10$^0$C for 3 minutes without covering the tubes. Then test tubes covered with glass stopper and heating was continued for 40 minutes. Test tube was cooled down under running tap water. Absorbance was measured at 550 nm. (Thambavani S, Kumar SR. 2011)

## 3. Result and discussion:

In present investigation *Barleria prionitis* contains 10000µg/gm of total phenol, 9500 µg/gm of ortho-dihydric phenol, 200 µg/gm of flavanol, 1600 µg/gm of tannin, 2 µg/gm of anthocyanin and 55 µg/gm of leuco-anthocyanin. Aiswarya and Ravikumar (2014) detected phenolic compounds and tannin by ferric chloride test and alkaline reagent test for flavonoids. Amit Kapoor et.al., (2014) also reported positive results for phenolic compounds in methanol and water extract, and flavonoids in chloroform, methanol and water extract.

*Caesalpinia bonduc* contains 8100 µg/gm of total phenol, 4500 µg/gm of ortho-dihydric phenol, 860 µg/gm of flavanols, 1120 µg/gm of tannin, 32.5 µg/gm of anthocyanin and 50 µg/gm of leuco-anthocyanin. V. Subramani et.al., (2014) reported presence of flavonoids in ethanol extract along with this they also reported antimicrobial activity of secondary metabolites from *Caesalpinia bonduc*.

We found 5100 µg/gm of total phenol, 3400 µg/gm of ortho-dihydric phenol, 100 µg/gm of flavanols, 120 µg/gm of tannin, 2 µg/gm of anthocyanin and 12.5 µg/gm of leuco-anthocyanin in *Leonotis nepetifolia*. Imran S. et al., (2012) reported presence of flavonoids (1.47±0.11mg/100gm of dry weight), phenols (1.20±0.21mg/100gm of dry weight) and tannin (0.11±0.81mg/100gm of dry weight). We found nearly same results with their observations.

Previous study showed only presence and absence of secondary metabolites for *Barleria prionitis* and *Caesalpinia boduc*, but present study shows specific amount of secondary metabolites.

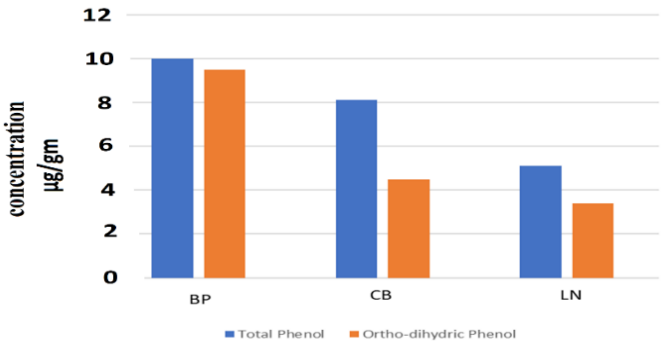


**Fig.1:** Quantitative analysis of Total phenol and Ortho-dihydric phenol

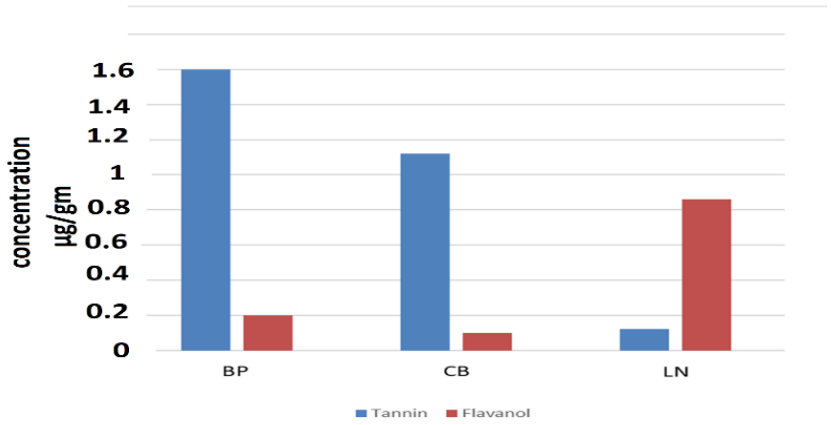


**Fig. 2:** Quantitative analysis of Tannin and Flavanol

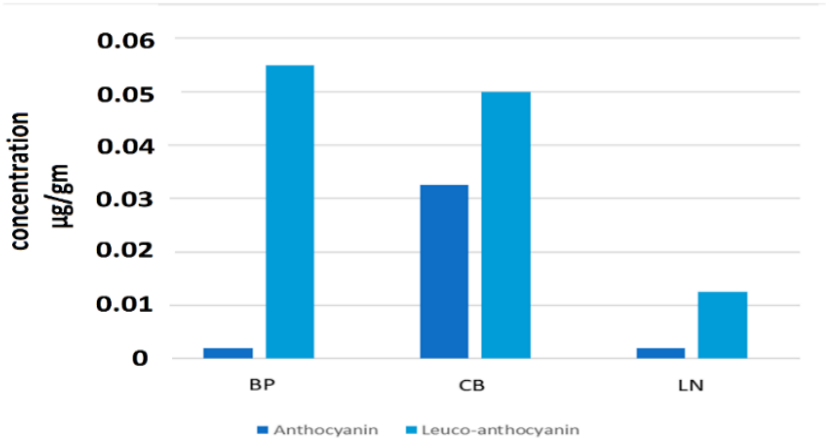


**Fig.3:** Quantitative analysis of Anthocyanin and Leuco-anthocyanin

| Sr. no. | Plant material | Secondary metabolites ( µg/gm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Total phenol | Ortho-dihydric phenol | Flavanol | Tannin | Anthocyanin | Leuco-anthocyanin |
| 1. | *Barleria prionitis* | 10 µg/gm | 9.5 µg/gm | 0.2 µg/gm | 1.6 µg/gm | 0.002 µg/gm | 0.055 µg/gm |
| 2. | *Caesalpinia bonduc* | 8.1 µg/gm | 4.5 µg/gm | 0.86 µg/gm | 1.12 µg/gm | 0.0325 µg/gm | 0.05 µg/gm |
| 3. | *Leonotis nepetifolia* | 5.1 µg/gm | 3.4 µg/gm | 0.1 µg/gm | 0.12 µg/gm | 0.002 µg/gm | 0.0125 µg/gm |

**Table:** Quantitative analysis of secondary metabolites

## 4. Conclusion:

Among all three plants *Barleria prionitis* shows highest amount of total phenol, ortho-dihydric phenol, tannin and leuco-anthocyanin. While *Cesalpinia bonduc* shows highest amount of anthocyanin and *Leonotis nepetifolia* shows more amount of flavanol. These plants were used traditionally in medicine from ancient period and it is authenticated that the medicinal property of these plants is due to presence of secondary metabolites.

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