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NUTRACEUTICAL AND ANTIOXIDANT ACTIVITY OF *CLITORIA TERNATEA* EXTRACTS

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Keywords:

Butterfly pea flower, Blue tea, Anthocyanin

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ABSTRACT: *Clitoria ternatea* was selected because it possesses various medicinal compounds of great significance based on its usage as an ayurvedic drug to cure various diseases. This study helps to understand the significance of plant sources that possess pharmacological activities and is good for health on regular consumption. A comparative study was thus conducted to analyze the potential antioxidant activity by experimental quantifying the certain bioactive compounds present in the aqueous and methanolic extracts of the fresh and dry flowers of the blue flowered variety of *Clitoria ternatea*. This study showed that all the extracts possessed desirable antioxidant activity examined by DPPH assay and peroxide value assessment due to considerable amounts of secondary metabolites like flavonoids, phenols, steroids *etc.*, determined quantitatively during the phytochemical analysis. Although the dry methanolic extract showed positive results overall during the phytochemical analysis, the fresh aqueous extract of *C. ternatea* showed the highest antioxidant property with 63.63% scavenging activity.

INTRODUCTION: One of the most important key markers linked to a variety of progressive pathological disorders, such as neurological dysfunction, cancer, ageing, and endocrine diseases, is oxidative stress due to an increase in free radical levels [1]. Free radicals are produced in the body as products of normal metabolism due to exposure to radiation and other pollutants. Because they are so effective, they can damage parts of cells and become infected with various diseases [2]. Free radicals are usually reduced by active processes in the body, including antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) as well as small molecules found in nutrients (vitamin E, vitamin C, carotene, - flavonoids, glutathione, uric acid), and taurine) [3].

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In general, obese people risk high oxidative stress due to the disruption of antioxidant protection systems and strong production of free radicals. Obesity is also associated with a decrease in antioxidant activity indicated by low levels of antioxidant enzymes. A food regimen excessive in antioxidants may also lessen the chance of many sicknesses (which includes heart disease and different cancers) [4]. Antioxidants release loose radicals from the body's cells and prevent or reduce the damage as a result of oxidation. Reactive oxygen species are scavenged by multiple naturally occurring antioxidants found in plant sources. From ancient times, herbal extracts have been regarded as potent antioxidants.

Butterfly pea (*C. ternatea*) is a pod with deep roots, long climber with five leaflets, deep blue or white flowers. From ancient times "Shankhpushpi" has been known as the famous Ayurveda drug. It was also reported as a brain tonic to improve memory and intelligence. It is considered a Medhya-Rasayana in Ayurvedic texts. The roots are used as a diuretic and the seeds as cathartic. According to

Kancheepuram a district in Tamil Nadu, headaches and constipation are treated orally by mixing the root powder with water. The flower petals are used to treat eye diseases. The whole plant is used as a remedy for snake bites. The Tripura tribe of Bangladesh believed CT roots are used for UTI, to treat a burning sensation in the urinary tract, lack of urination and frequent urination. In the Uttara Kannada region in Karnataka, CT root juice is applied to the body to reduce fever." Root ash is used for facial care [5].

When the colored antioxidant flower (blue) is boiled in water it is used as a beverage, which is more commonly known as blue tea. Blue tea has a pleasant taste and texture. In addition to that, it is a non-caffeine beverage, which may have overcome many of the side effects of drinking coffee regularly, like anxiety, insomnia, digestive problems, etc. Blue Tea is rich in antioxidants, which makes it a great drink to include in detox diet. The earthy flavor of butterfly-pea flower tea is said to enhance mood. Tea is said to have anti-depressant effects that may also reduce anxiety symptoms. It is also known to stimulate the brain and help you stay strong and happy throughout the day. The flavonoids in blue tea can also stimulate collagen production, helping to improve and maintain skin firmness. The butterfly pea flower is good for hair, as it contains anthocyanin [6].

This study helps to understand the significance of the beneficial compounds present in blue tea of nutraceutical importance necessary for good health by incorporation as part of regular dietary intake.

MATERIALS AND METHODS:

Plant Source: The blue flowers of *Clitoria ternatea* was collected from Kalyan Nagar, Bengaluru Urban, India.

Preparation of Plant Extract:

Dry Aqueous Extract: The *Clitoria ternatea* flowers were rinsed 2-3 times with tap water. The washed flowers were kept in sunlight for drying to evaporate the water content. The sample was ground into a fine powder after drying using a mechanical blender. Powdered plant materials (1g) were extracted with distilled water (10 ml) to obtain aqueous extract of the dry sample. The extracts were filtered and used.

Dry Methanolic Extract: The *Clitoria ternatea* flowers were rinsed 2-3 times with tap water. The washed flowers were kept in sunlight for drying to evaporate the water content. Using a mechanical blender, the sample was ground into a fine powder after drying. Powdered plant materials (1g) was extracted with 80% methanol (10 ml) to obtain methanolic extract of the dry sample. The extracts were filtered and used.

Fresh Aqueous Extract: 5 g of the freshly plucked *Clitoriaternatea* flowers was weighed. Using a mortar and pestle, the sample was ground finely and extracted with distilled water (10 ml) to obtain an aqueous extract of the fresh sample. The extracts were filtered and used.

Fresh Methanolic Extract: 5g of the freshly plucked *Clitoria ternatea* flowers was weighed. Using a mortar and pestle, the sample was ground finely and extracted with 80% methanol (10 ml) to obtain a methanolic extract of the fresh sample. The extracts were filtered and used.

Quantitative Phytochemical Analysis:

Quantitative Determination of Flavonoids: 10 g of the plant sample was filtered with 100 ml of 80% aqueous methanol at room temperature. The filtrate was transferred to a water bath, and the solution was evaporated to dryness. The sample was measured until constant weight was obtained.

Quantitative Determination of Tannin Content: 1g of sample was weighed and taken in a mortar and pestle, 10ml methanol was added and homogenized. The homogenized extract was centrifuged at 6000rpm for 15 minutes at room temperature. The supernatant was collected in a measuring cylinder, and its volume was noted and used for estimation. 0.2 to 1.0ml aliquots of standard tannic acid were pipetted in test tubes. The volume was 1 ml with distilled water in each test tube. 5ml of Folins–Denis reagent and 1ml of sodium carbonate solution were added to all the tubes. The absorption was read at 700nm against a blank [7].

Quantitative Determination of Phenol Content: 1g of sample was weighed and transferred into a mortar and pestle, 10 ml of 80% ethanol was added and homogenized.

The homogenized extract was then centrifuged at 10,000 rpm for 10 minutes. The supernatant was carefully transferred into a measuring cylinder and the volume was noted. 0.2 to 1.0ml aliquots of standard catechol were taken in test tubes. The volume in each test tube was made up to 3ml with distilled water. 0.5ml of FC reagent was added and incubated at room temperature for 3 minutes. 2ml of 20% sodium carbonate was added and incubated at room temperature for 20 minutes. The absorbance was measured at 650nm against a suitable blank [8].

Quantitative Determination of Total Steroid Content: 1 ml of the plant extract was added to 10 ml of volumetric flask, and 2 ml of H_2SO_4 (4 N) and 2 ml of ferric chloride (0.5% W / V) was added to the extract. 0.5 ml solution of potassium hexacyanoferrate (III) (0.5%) was added. The mixture was heated in a water bath at 70 ± 20°C for 30 minutes. The volume was made up to the mark with distilled water, and the absorption was measured at 780 nm against a suitable blank. Cholesterol was used as a standard with varying concentrations (10, 20, 40, 80, 160 µg/ ml) for absorption and standard curve. From the standard graph, the amount of steroid samples in absorption values were calculated and expressed as cholesterol equivalents (mg /g) [9].

Quantitative Determination of Anthocyanin Content: 1ml of the plant extract was transferred into a volumetric flask to prepare two dilutions of the sample, one adjusted volume with potassium chloride buffer (pH 1.0) and the other with sodium acetate buffer (pH 4.5). These dilutions were equilibrated for 15 minutes. The absorbance of each dilution was measured at 510 nm and 700nm, respectively, against a blank containing distilled water. All the absorbance was taken between 15 minutes to 1 hour of the sample preparation. [10]Absorbance of the diluted sample was calculated by the formula:

$$A = (A510 - A700)\ pH1.0 - (A510 - A700)\ pH4.5$$

Antioxidant Activity:

DPPH Assay: This experiment was carried out with ascorbic acid (10 mg/ml) as the standard. The standard was pipetted out in 200-1000 microliters aliquots in various test tubes, and the volume was brought up to 1000 microliters with distilled water. 3ml of DPPH (2 mg/ml) was added to the mixture. This combination was incubated for 10 minutes at room temperature. The tube contents were spectrometrically read at 517nm after incubation [8]. The formula for calculating DPPH radical scavenging activity (percent) was:

$$\text{DPPH radical scavenging activity (percent)} = [(\text{OD of control} - \text{OD of the sample}) / \text{OD of control}] \times 1009$$

Determination of Peroxide Value: In a conical flask, approximately 5.0g of fresh oil (or 0.20.5g of old oil) was weighed out. Incubated at room temperature for 5 minutes. 15ml of the solvent solution [Glacial acetic acid: Chloroform (6:4)] swirled to dissolve the oil. 0.25ml saturated KI solution was pipetted out and whirled for 1 minute (light brown color is observed). Titrated with 0.01M sodium thiosulphate ($Na_2S_2O_3$) until the solution turned pale yellow, then 15ml pure water was added. Titration was continued with 1ml Starch (1%) indicator solution until the blue hue faded (endpoint). A blank determination against 0.01M Sodium thiosulphate (15ml Glacial acetic acid: Chloroform solution + 0.25ml KI + 15ml distilled water) was performed [8].

$$\text{Peroxide value} = S \times M / \text{Sample weight (g)} \times 100$$

Where S = volume (ml) of $Na_2S_2O_3$ solution used in the titration after correction for the blank titration and M= molar concentration of the sodium thiosulphate solution.

RESULTS AND DISCUSSION:

Phytochemical Analysis:

Quantitative Determination of Flavonoid Content: Flavonoids are plant secondary metabolite that acts as powerful antioxidant to exert functions like anti-diabetic, anti-inflammatory, anti-allergic, anti-viral, vasodilatory effects, eye disorders, Alzheimer's. Flavonoids can protect against oxidative stress, reducing the oxidation of low-density lipoproteins. Flavonoids present in blue tea improve skin health due to the presence of collagen. The flavonoid content in both fresh and dry flowers of butterfly pea flower was estimated, and the results obtained showed the amount of flavonoid in the dry sample was significantly higher than that of the fresh sample of *Clitroria ternatea* flowers.

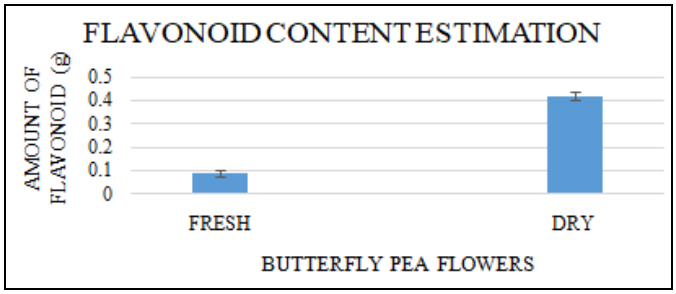

FIG. 1: ESTIMATION OF FLAVONOIDS IN BUTTERFLY PEA FLOWER. The experiment was performed in triplicates and the results are presented as mean ± SD.

Quantitative Determination of Tannin Content: Tannins (commonly called tannic acid) are polyphenols found in many plants. They are water-soluble and have been reported to be the main contributors to decrease food intake growth rates. Tannic acid in both methanolic and aqueous extract of fresh and dry sample of blue tea was estimated by plotting a standard graph for tannins. The dry aqueous extract showed the highest amount of tannic acid i.e., 59.693 mg followed by dry methanolic extract with 48.037 mg followed by fresh methanolic extract with 38.957 mg and fresh aqueous extract had least amount of tannic acid i.e., 10.377 mg.

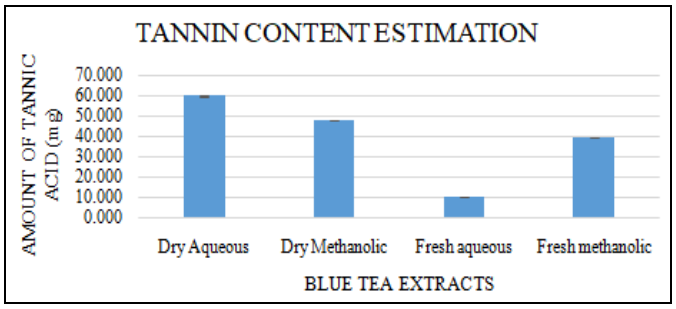

FIG. 2: AMOUNT OF TANNIN (MG) IN EXTRACTS. The experiment was performed in triplicates and results are presented as mean ± SD.

Quantitative Determination of Phenol Content: Phenolic compounds are a group of secondary metabolites that is derived from Pentose phosphate, zthe Shikimic acid pathway, Phenyl propanoid pathway in various plants. These compounds are involved in the interaction of biotic and abiotic factors. They also play an important role in the development of the plant, pigment biosynthesis and various other plant activities. Phenolic compounds play an important role as dietary supplements in preventing oxidative stress damage and recent studies have proved to have desirable antioxidant effect [11].

The total phenolic content of both methanolic and aqueous extract of blue tea sample was estimated using Folin-Ciocalteu reagent, and it was calculated using a standard curve of catechol and is expressed in terms of catechol equivalents per gram of plant extract. The phenolic content estimated was higher in the dry extracts than in blue tea.

The total phenolic content was found to be higher in both aqueous and methanolic extract of a dry sample of *Clitoria ternatea* (0.573 and 0.645 mg of catechol equivalence, respectively), while the fresh aqueous extract showed low levels of phenols, i.e., 0.273 ± 0.006 of mg of catechol equivalence.

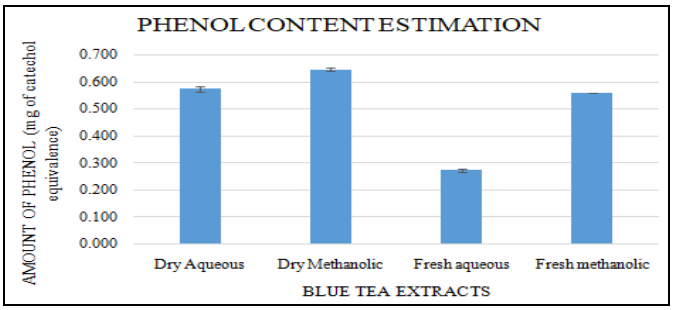

FIG. 3: AMOUNT OF PHENOL IN BLUE TEA EXTRACTS. The experiment was performed in triplicates and results are presented as mean ± SD.

Quantitative Determination of Total Steroid Content: The total steroid content in the sample was estimated using cholesterol as a standard steroid. The amount of the steroid in the sample as per absorbance values was calculated and expressed as cholesterol equivalents(µg/g).

A desirable amount of steroid content was found in both aqueous and methanolic extracts of fresh and dry sample of *Clitoria ternatea*. Dry methanolic extract of blue tea was found to contain the highest amount of steroid.

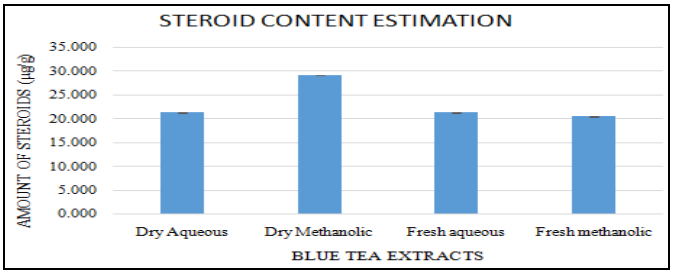

FIG. 4: AMOUNT OF STEROIDS IN BLUE TEA EXTRACTS. The experiment was performed in triplicates and results are presented as mean ± SD.

Quantitative Determination of Anthocyanin Content: Anthocyanins are grouped under flavonoids, they are water soluble phytochemicals which are responsible for pigments like red, purple, blue in fruits and vegetables.

The total anthocyanin content in the plant sample was determined by pH differential method. The anthocyanin pigments in the sample undergo reversible structural changes with pH change which is marked by difference in absorbance. The colored oxonium form levels are higher at pH 1.0 whereas the colorless hemiketal form predominates at pH 4.5. Hence, the pH differential method is an accurate and rapid method for estimating the total anthocyanin content even in the presence of any interfering compounds [12]. The total anthocyanin content obtained was expressed in mg/100g of sample.

All 4 extracts showed the desirable amount of anthocyanin content in them. But the highest amount anthocyanin was found in the dry methanolic extract of *Clitoria ternatea*. In contrast, the aqueous and methanolic extracts of fresh flower samples were found to have lower levels of anthocyanin.

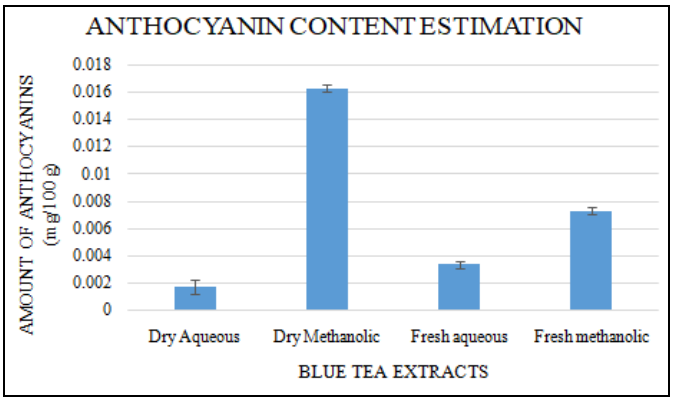

FIG. 5: AMOUNT OF ANTHOCYANIN IN BLUE TEA EXTRACTS. The experiment was performed in triplicates and results are presented as mean ± SD.

Antioxidant Assays: In the current study, two procedures were followed to measure the anti-antioxidant capacity of the test sample. DPPH and the determination of peroxide value showed that fresh aqueous extract of blue tea possess good antioxidant property with 63.63% scavenging activity and a decrease in the peroxide value, as it is proved that compounds with high free radical scavenging have lower peroxide value.

DPPH Assay: The antioxidant capacity of blue tea extracts was determined by measuring their ability to degrade DPPH radicals. The total antioxidant quantity of the extract was determined using % inhibition against the concentration of ascorbic acid. DPPH free radical donates electrons and provides maximum absorption at 517 nm (purple color). When antioxidants react with DPPH, it pair with a hydrogen donor (e.g., free radical scavenging antioxidant) and then reduce to DPPH, reducing absorption from DPPH. DPPH-H form results in decolorization (yellow color) with respect to the number of electrons accepted [13].

The amount of ascorbic acid equivalent was found to be 14.185 mg/g of plant sample. Free radical scavenging activity of 50 µg /ml ascorbic acid was found to be 47.72%. Free radical scavenging activity of 14.185 micrograms of ascorbic acid equivalent of plant extracts i.e. dry aqueous extract, dry methanolic extract, fresh aqueous extract and fresh methanolic extract was found to be 25.71%, 15.78%, 63.63%, and 33.33% respectively.

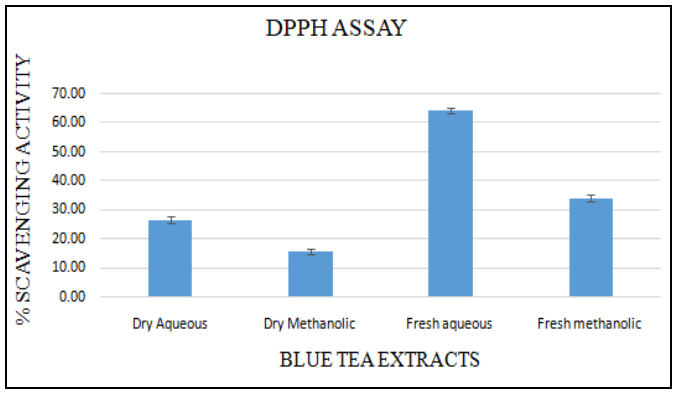

FIG. 6: % SCAVENGING ACTIVITY OF BLUE TEA EXTRACTS. The experiment was performed in triplicates and results are presented as mean ± SD.

Determination of Peroxide Value: The amount of peroxide indicates the first stage of fat deterioration or oil spoilage. It is also an indicator of determining the amount of hydroperoxides, which is the main product of oxidation of oils and fats. Oils with a peroxide value of more than 10 units are considered rancid. Lesser peroxide value indicates a good-quality of oil.

In this assay, aqueous and methanolic extract of both fresh and dry samples of *Clitoria ternatea* was taken to see their effect on the peroxides present in the sample. All four extracts were observed to have

a desirable effect on the oil. Fresh aqueous extract had a better effect in reducing the peroxide value (to 0.59%) than the other extracts, while dry methanolic extract had a comparatively higher peroxide value (28.67%).

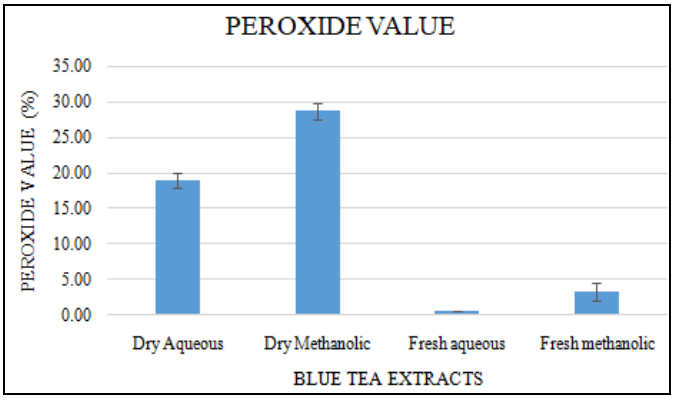

FIG. 7: PEROXIDE VALUE OF BLUE TEA EXTRACTS. The experiment was performed in triplicates and results are presented as mean ± SD.

CONCLUSION: From the present experimental study, it can be surmised that all the different extracts showed the presence of considerable amounts of various phytochemicals such as flavonoids, tannins, total phenols, total steroid, and anthocyanin. Among the different extracts, the dry methanolic performed better overall than the dry aqueous extracts and the fresh extracts. All the extracts had antioxidant activity however the dry methanolic extract showed maximum reduction in the peroxide value while the fresh aqueous extract showed highest scavenging activity. The variability in the results of different assays may be due to the presence of different types of phenols or other interfering elements. As oxidative stress is related to obesity and leads to other degenerative diseases such as diabetes, high blood pressure, and hyperlipidemia. Therefore, it may be helpful to incorporate pharmacologically active plants that have nutraceutical and antioxidant effects in our diet. This research may provide the basis for *in-vivo* research and a solid basis for future developments of generic herbal medicines or active ingredients that effectively prevent and treat diseases.

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CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Sindhi V, Gupta V, Sharma K, Bhatnagar S, Kumari R and Dhaka N: Potential applications of antioxidants – A review. Journal of Pharmacy Research 2013; 7(9): 828–35.
2. Lakshan SAT, Pathirana CK, Jayanath NY, Abeysekara WPKM and Abeysekara WKSM: Antioxidant and selected chemical properties of the flowers of three different varieties of Butterfly Pea (*Clitoria ternatea* L.). Ceylon Journal of Science 2020; 49(2): 195.
3. Halliwell B, Aeschbacht R, Loligert J and Aruoma OI: The Characterization of Antioxidants. Fd Chem Toxic 1995; 33(7): 601-17.
4. Gaman AM, Buga AM, Gaman MA and Popa-Wagner A: The role of oxidative stress and the effects of antioxidants on the incidence of infectious complications of chronic lymphocytic leukemia. Revista de chimie (Bucharest) 2019; 70(6): 2241-4.
5. Bhatia M and Kumar Gupta G: *Clitoria ternatea* (L.): Old and new aspects. Journal of Pharmacy Research [Internet]. 2010; 3(11): 2610–4.
6. Mehla J and Gupta P: *Clitoria ternatea* linn: A herb with potential pharmacological activities: Future prospects as therapeutic herbal medicine. Journal of Pharmacological Reports 2018; 3(1).
7. Keshab B, Deepak KD, Mayank T, Thirumeignanam and Rai SN: Tannin Assay 2008. DOI:10.13140/RG.2.2.29171.73763
8. Singh K, Kour A and Mohanty A: *In-vitro* and *Ex-vivo* studies on Antiobesity Leptin Activation Effects of *Saliva hispanica, Ocimum basilicum* and *Coriandrum sativum* Extracts. International Journal of Pharmaceutical Sciences Review and Research 2020; 65(2): 114–20.
9. Madhu M, Sailaja V, Satyadev T and Satyanarayana MV: Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. Journal of Pharmacognosy and Phytochemistry 2016; 5(2): 25–9.
10. Sutharut J and Sudarat J: Total anthocyanin content and antioxidant activity of germinated colored rice. International Food Research Journal 2012; 19(1): 215–21.
11. Kabera JN, Semana E, Mussa AR and He X: Plant Secondary Metabolites: Biosynthesis, Classification, Function and Pharmacological Properties. Journal of Pharmacy and Pharmacology 2014; 4(2): 377-392.
12. Lee J, Durst RW, Wrolstad RE, Barnes KW and Eisele: Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. J of A0AC International 2005; 88(5): 1269-78.
13. Molyneux P and Associates M: The use of the stable radical Diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin Journal of Science and Technology 2003; 26(2): 211-219

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