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EFFECT OF CLITORIA TERNATEA (BLUE BUTTERFLY PEA) FLOWER EXTRACT ON ANTIOXIDANT ACTIVITY USING FENTON REACTION

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ABSTRACT

A tropical flower with the common names Butterfly pea flower and Bunga telang, Clitoria ternatea is renowned as one of the primary suppliers of polyphenols with potent anti-oxidant characteristics. Natural blue colourants benefit from the abundance of anthocyanin chemicals produced by Clitoria ternatea. As the least stable and most quickly damaged anthocyanins after extraction are those that give this colour, bluish colouring agents are now the most challenging to produce. Their stability frequently depends on a amount of operational factors, including sun exposure, high temperatures, and pH shifts. In addition to its colour properties, anthocyanins. They were recognized as a source of beneficial atoms as a result of its antioxidant abilities and advantageous health impacts, which include antibacterial, anti-cancer, and anti-obesity properties.. Various qualitative phytochemical tests were done for the presence of alkaloids, glycosides, carbohydrates, flavonoids etc. It was observed that 50% ethanol extract of Clitoria ternatea contains carbohydrates, proteins, glycosides, steroids and sterols, anthraquinones and triterpenoids. In the present study the methanol extract Clitoria ternatea was evaluated for antioxidant activity by the OH free radical scavenging activity using Fenton reaction. This study was conducted to investigate the effect of Clitoria ternatea extract using Fenton reaction. The dried fruit of Clitoria ternatea was extracted with methanol using a Soxhlet extractor. The total phenolics content of bark as determined by Fenton reaction and was found to be good antioxidant activity as dose depended manner. The antioxidant activity of plant extract was carried put with ascorbic acid as a standard reducing agent. All the analysis was made with the use of UV-Visible spectrophotometer. The Clitoria ternatea extract, there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. The result suggest that the Clitoria ternatea extract can be used as food antioxidant together with the improvement of food palatability. Further studies are in processing of analyzing the synergic association of extract with synthetic antioxidant and to identify compounds with antioxidant activity in cinnamon extracts. In conclusions the present study indicated that Clitoria ternatea extract may be a potential source of a natural antioxidants.

KEYWORDS: Clitoria ternatea; fruit; extraction; methanol; phenolic content; flavonoid content; antioxidant activity.

1. INTRODUCTION

Clitoria ternatea, also referred to as a permanent twiner is the butterfly pea blossom that relates to the Fabaceae family. This flower can develop quickly; therefore gardens and the wild frequently have it blooming (Mukherjee P.K. et al., 2008) With several common names, Clitoria ternatea is widely cultivated in numerous nations for examples, Aparajita (India), Pukingan (Philippines), Ang Chan (Thailand), 'bunga telang' (Malaysia), Aparajita (India), as well Die Dou (China) (Kosai et al., 2015, Subramanian et al., 2011, Mukherjee, 2008) Since Clitoria ternatea has numerous cultivars with different bloom hues, it is renowned for being a beautiful decorative climber and regarded as an must-have ornamental plant for garden enthusiasts (Luengwilai, 2019, Oguis et al., 2019). Its petals came in two main hue varieties: blue and white (Landim Neves et al., 2021, Taranalli, et al., 2000, Kazuma, et al., 2003) Different anthocyanins' presence in the flower's chemical makeup is primarily what causes the variety of petal colours. The "Double blue" line flower specifically was noted for assembling a large number of polyacylated anthocyanins with ternatins. (Lakshan, et al., 2019, Kazuma K et al., 2003) while the line of white petals appeared to lack anthocyanins (Al-Snafi, 2016). Considering the anthocyanins that have gathered in the petals of the Clitoria ternatea flower, which can provide a brilliant blue colour (Figure. 1), It's commonly used

as a food colouring (Nur, et al ., 2018, Luengwilai, (2019). secondary metabolites include kaempferol, glycosides, myricetin, flavonols, and the phenolic acids are present. and anthocyanidins (Luengwilai, (2019) was considerably demonstrated by phytochemical analysis to the flower sections. In contrast to other therapeutic plants, the anthocyanin pigment found in Butterfly flowers gives them more antioxidant qualities. These effects include antidiabetic, antibacterial, anticancer, and anti-inflammatory properties (Taranalli, et al., 2000). Clitoria ternatea is rich in these bioactive compounds. Extraction was the first step in removing antioxidants from the plant. These extraction efficiency is affected by a number of factors, including sample size of particles, solvent types, operating circumstances, extraction techniques, and phytochemical chemical makeup. (Do, et al., 2014). Perhaps because Ethanol is commonly used as an extraction solvent because it has been determined to be safe (GRAS) for use in food and medicinal applications. Additionally, a number of research show that the optimal organic solvent mixture for isolating or recovering the plant-based phenolic compounds is an aqueous mixture. Quantitating the phytochemical properties from the flower petals, such as their overall amount of flavonoids and phenols, total anthocyanin content, and antioxidant activities, was necessary to complete this task in order to gain a deeper understanding of the potential uses of Clitoria ternatea. (Zhang, et. al., 2019) Flavonoids and phenolic compounds are recognised for their strong antioxidant properties. Recent study investigated the phenolic composition of Clitoria ternatea extracts and found a wide range of phenolic substances, likeas quercetin, kaempferol, and catechin. These substances have the ability to scavenge free radicals, shielding cells from oxidative harm. (Jimenez- Moreno et al., 2019) In addition to its direct antioxidant activity, it has been demonstrated that Clitoria ternatea increases the activity of natural antioxidant enzymes. Recent study by Tang et al. (2020) demonstrated that administration of Mice's levels of catalase (CAT) and superoxide dismutase (SOD) were elevated by Clitoria ternatea extract. These enzymes break down dangerous Reactive Oxygen Species (oxygen species that are reactive) (ROS), which are essential to the body's defence against oxidative stress. Antioxidant activity is among the principal characteristics that has drawn scientific attention. Antioxidants are substances that assist in counteracting harmful the body's free radicals, shielding cells from cellular oxidative damage as well as possible harm. In this article, we shall look into the Clitoria ternatea flowers' capacity as antioxidants. To do this, we will compare studies on the Clitoria ternatea flowers' antioxidant activity in ethanol and ethyl acetate extracts using conventional techniques. In this research paper, Using approved methods; we will look into the antioxidant capacity of Clitoria ternatea flowers in different extracts.

2. MATERIALS AND METHODS

Plant material – Clitoria ternatea Blue Butterfly pea Flower extract was collected from Local Market, Raipur (Chhattisgarh), India.

Chemicals and Reagent samples – The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany) and other. Sample absorbances were read using a Lambda 532 nm, UV Spectrometer made by Varian.

Preparation of extract - Dried powdered of *Clitoria ternatea Blue Butterfly pea Flower extract* (10 g) were extracted by continuous mixing in 100 ml 50% methanol, 24 h at room temperature. After the filtration process, methanol was evaporated until only water remained through evaporation on water bath at 60-70 0c temperature. The final extract was kept in air tied box.

Deoxyribose assay to assess OH -radical scavenging activity

The OH- radical scavenging activity of *Clitoria ternatea Blue Butterfly pea Flower extract* (10–100 ug/ml) was determined according to the deoxyribose method reported of Halliwell, *et al*., (1987). In the protocol the presence of 100 lM EDTA. $FeCl_3$, H_2O and ascorbic acid were prepared in degassed H_2O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 lM EDTA, 1 mM H2O2, 100 lM L-ascorbic acid, 100 lM $FeCl_3$, H_2O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Samples was kept in incubation at 38º C, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture after that samples was heated in a boiling water bath for 15 min. After the samples were cooled, the absorbances were read at 532 nm. The IC_{50} value of the plant extract was compared with that of ascorbic acid, which was used as the standard. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage of inhibition of hydroxyl radical was calculated as follows.

$$\% \text{ Inhibition} = \text{Abs: } \frac{532 \text{ nm Control Abs. - 532 nm sample Abs.} \times 100}{532 \text{ nm Control Abs}}$$

Antioxidant capacity of test compounds was expressed as IC_{50}, the concentration necessary for 50% inhibition concentration of TBARS.

3. RESULT

The results of the effects of the examined *Clitoria ternatea Blue Butterfly pea Flower extract* as well as control solutions on OH- radical production. They show that all extract of *Clitoria ternatea Blue Butterfly pea Flower extract* and control solutions as a DMSO inhibited the production of OH- radicals. The % of free racial scavenging activity of hydro-methanolic extract of *Clitoria ternatea Blue Butterfly pea Flower extract* presented in Table 1 have reducing power, the free radial OH- scavenging activity of the extract increases with increasing the concentration.

Table 1: Antioxidant activities of *Clitoria ternatea Blue Butterfly pea Flower extract* using Fenton reaction.

Constrictions (in µl)	% of inhibition	
	Ascorbic Acid	*Clitoria ternatea Blue Butterfly pea Flower extract*
10	22.71	17.21
20	38.13	23.99
30	46.12	30.45
40	52.30	41.40
50	61.35	52.15
60	73.10	63.60
70	79.52	67.12
80	82.28	72.55
90	86.17	78.55
100	90.42	83.25
Blank: 0.4320		

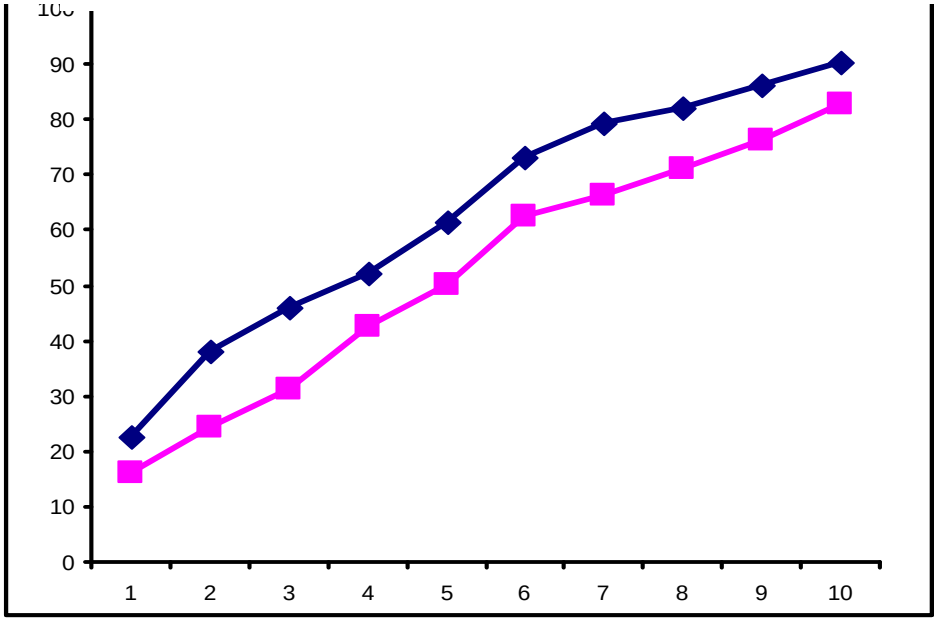

Fig. 1: Antioxidant Activity of *Clitoria ternatea Blue Butterfly pea Flower extract.*

4. DISCUSSION

The body's innate mechanism has many enzymes and nonprotein compounds that protect it from the free radicals and reactive oxygen species produced inside the body during normal metabolism and also due to external stimuli. Major compounds include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione, which also play a major role in detoxification and coordinate the body's antioxidant defense processes. The superoxide dismutase is a metalloprotein that scavenges superoxide anions. Catalase is a heme protein, localized in the peroxisome or the microperoxisome, which catalyzes the decomposition of H2O2 to water and oxygen and thus protects the cell from oxidative damage produced by H_2O_2. The glutathione peroxidase catalyzes the reaction of hydroperoxides, which reduces glutathione to form glutathione disulfide (GSSG) and the reduction product of the hydroperoxide. Glutathione reductase is involved in the regeneration of glutathione that has been converted to GSSG by oxidation and thiol transfer reactions. Glutathione, a major nonprotein thiol, is mainly involved in detoxification (Halliwell & Gutterridge, 1985).

The capacity to give electrons or hydrogen atoms to free radicals in order to displace them and stop harm from free radicals is the antioxidant capability. Anthocyanins show antioxidant activity both in vivo and in vitro. The antioxidant properties of phenol and extract of ethyl acetate from Clitoria ternatea flowers are thought to be accountable for the blue pea flower's ability to prevent cardiovascular and neurological conditions, cancer, and diabetes.

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Bioactive components present in Clitoria ternatea was determined using the GCMS method. The spectral analysis of the sample by the GCMS showed several peaks however compounds of interest possessing antioxidant, antibacterial and anti carcinogenic properties in Clitoria ternatea extract. The Clitoria ternatea extract was found antioxidant potential and having the ability to trigger cellular antioxidants, can be exploited for its use against a number of disorders including cardiovascular diseases, inflammation, and cancer.

5. CONCLUSION

Traditional medicine has been practiced in India for decades and is still widely practiced even today. The knowledge of medicinal plants is passed on based on indigenous knowledge system and orally by the traditional herbal practitioners form one generation to the next. The medicinal plants are extracted from trees and shrubs. The common practice is the use of the bark, roots and sometimes both. Medicinal plants have a wide range of pharmaceutical use in disease diagnosis etc. Experimental data revealed that there might be correlation between total phenolic and antioxidant capacity of different extracts of lemon grass.

In the present study, the phenolic content of Clitoria ternatea extract was found to be high which might have responsible for its antioxidant and free radical scavenging activity in the in vitro study models. Thus our results were congruent with the findings of others. Further studies can be designed to prove the antioxidant activity of Clitoria ternatea extract in experimental animal models and also an attempt can be made to analyze the phenolic antioxidants present in it. Phenolic compounds consist of one or more aromatic ring structures with an abundance of hydroxyl groups attached to them. Which have the ability to squelch free radicals, and that their structure is directly in connection with their antioxidant effect a single or many aromatic rings ts, we can confidently state that the phenolic, anthocynin and flavonoid components present in the extracts have antioxidative potential. We observed that medicinal potential of the Clitoria ternatea L. plant extracts chosen for Antioxidant activity has not been carefully examined. The present study aimed to determine the antioxidant activity of plant Clitoria ternatea L. This research would be very helpful in treating various kinds of oxidative stress and disease.

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