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In Vitro Antioxidant Potential of Blue Pea Flower (*Clitoria ternatea)* and Tanner's Cassia Flower (*Senna auriculata)* Infused drink

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Abstract: Antioxidants play a pivotal role in mitigating oxidative stress and enhancing overall well-being. This study evaluates the in vitro antioxidant potential of an infused drink formulated using two medicinal flowers—Clitoria ternatea (Blue Pea) and Senna auriculata (Tanner's Cassia). Two variations were developed: Variation 1 (3:2 ratio of Blue Pea to Tanner's Cassia) and Variation 2 (2:3 ratio). The bioactive components were identified using Gas Chromatography–Mass Spectrometry (GC-MS), while nutritional and phytochemical properties were analysed through standard procedures. Antioxidant activity was assessed using DPPH radical scavenging and FRAP assays. Results indicated that Variation 2, with a higher concentration of Senna auriculata, demonstrated superior nutritional content and antioxidant activity. Conversely, Variation 1 showed higher levels of total phenolics, flavonoids, and anthocyanins, reflecting a richer phytochemical profile. In conclusion, both formulations offer substantial antioxidant benefits and present a promising natural beverage option.

Keywords: In vitro, Clitoria ternatea, Senna auriculata, infused drink, antioxidants, oxidative stress, phytochemicals.

I. INTRODUCTION

Oxidative stress refers to an imbalance between elevated levels of reactive oxygen species (ROS) and a low activity of antioxidant mechanisms [1]. ROS are highly reactive molecules that have unpaired electrons capable of causing cellular damage in the human body. [2]. This in turn, can increase the risk and onset of chronic physiological conditions such as atherosclerosis, hypertension, diabetes mellitus, Alzheimer's disease, idiopathic pulmonary fibrosis, cancers, rheumatism, urinary tract infections, and kidney diseases [3].

Antioxidants refers to any substance that delays, prevents, or removes oxidative damage to a target molecule. In other words, they are inhibitors of oxidative stress caused by free radicals. These antioxidants play an important role in the scavenging of the ROS (free radicals) thereby preventing oxidative stress [4],[5].

Fruits and vegetables are widely recognized for their rich antioxidant and phytochemical content. However, an often-overlooked yet valuable source of these beneficial compounds is edible flowers. Edible flowers are a rich source of phytochemicals such as flavonoids, phenolic acids, anthocyanins, essential vitamins, and minerals that exert health benefits, through their antioxidant and anti-inflammatory properties [6].

Clitoria ternatea flowers also known as Blue Pea flower are used worldwide as ornamental flowers, traditionally used in Ayurvedic medicine and in the preparation of tisane teas. This flower contains various phytochemicals such as tannins, phlobatannin, carbohydrates, saponins, triterpenoids, phenols, flavonoids, flavanol, glycosides, proteins, alkaloids, anthraquinone, anthocyanins, cardiac glycosides, Stigmast-4-ene-3,6-dione, volatile oils, steroids, kaempferol, and quercetin [7]. The flower also exhibits other pharmacological effects such as hypolipidemic, anticancer, anti-inflammatory, analgesic, antipyretic, and antidiabetic effects [8] , [9].

Senna auriculata flowers also known as Tanner's Cassia flower is a traditional medicinal plant used majorly in the Ayurveda and Siddha medicinal system. This flower has been used in the treatment of diabetes, asthma, rheumatism, dysentery, skin disease, and metabolic disorders. The phytochemicals present in this flower include alkaloids, anthraquinone, flavone glycosides, sugar, saponins, phenols, terpenoids, flavonoids, tannins, steroids, palmitic acid, linoleic acid, and ferulic acid. The present study was focussed on the formulation of an infused drink using *Clitoria ternatea* and *Senna auriculata* flower and assessing the synergistic in vitro antioxidant potential of the infused drink, to explore a food-based approach to address oxidative damage [10] , [11].

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II. REVIEW OF LITERATURE

A. Oxidative stress and Diseases

The word "Oxidative stress" refers to an imbalance between oxidants and antioxidants in favour of oxidants, potentially leading to cellular and tissue damage. In other words, oxidative stress is a state where oxidative forces exceed the antioxidant systems due to an imbalance between them [12]. Oxidative stress is considered harmful as the reactive oxygen species (ROS) can attack biological molecules such as carbohydrates, proteins, lipids, and the DNA of cells. Risk factors responsible for the development of oxidative stress include lifestyle choices, environmental factors, ageing, and physical inactivity. It is also involved in the pathogenesis of life style related conditions such as atherosclerosis, diabetes mellitus, ischemic diseases, cancers, hypertension, and neurodegenerative diseases [13].

B. Antioxidants – An overview

Antioxidants are substances that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate [14] , [15]. The National Cancer Institute, United states of America, has defined antioxidants as "A substance that protects cells from the damage caused by free radicals. Free radicals are unstable molecules made by the process of oxidation during normal metabolism." The role of antioxidants in the body is very crucial as it can scavenge free radicals and protect the body against diseases such as cardiovascular diseases, neurodegenerative diseases, autoimmune diseases, liver diseases, renal diseases and more.

C. Flowers as a source of antioxidants

Flowers are increasingly recognized as rich sources of antioxidants, offering potential health benefits. Numerous studies conducted have investigated the antioxidant properties of various flowers. Flowers can serve as a valuable source of natural antioxidants, offering significant health benefits. Various flowers have demonstrated potent antioxidant activity through in-vitro assays such as DPPH, ABTS, and FRAP, showing their ability to scavenge free radicals effectively. The presence of bioactive compounds like flavonoids, terpenoids, and polyphenols further supports their potential as natural antioxidant sources. These findings highlight the promising role of flowers in preventing oxidative stress-related diseases such as cancer, diabetes, and cardiovascular disorders. In conclusion, flowers can be explored as a sustainable and natural alternative to synthetic antioxidants for pharmaceutical, nutraceutical, and functional food applications [16]. As flowers are said to possess antioxidant potential, the present study involves a blend of two edible flowers namely, Blue Pea flower and Tanner's Cassia flower.

D. Blue Pea Flower (Clitoria ternatea)

Clitoria ternatea (Sangu Poo in Tamil), commonly known as Butterfly Pea, Blue Pea, is an herbaceous medicinal plant belonging to the Fabaceae family. This is a native flower of tropical Asia, and cultivated in tropical and subtropical regions. This flower is primarily known for its vibrant blue flowers and unique shape. The acute toxicity of Clitoria ternatea flower extracts using Albino Wistar rats were studied. In this study, aqueous ethanol extracts of the flowers were administered orally at a dose of 2000 mg/kg body weight. The results showed no evidence of mortality or changes in haematological parameters [17]. This shows that the flower extract was well-tolerated at this dosage level. These findings suggest that Clitoria ternatea flower extract may have a broad safety margin when consumed at moderate levels [14].

E. Tanner's Cassia Flower (Senna auriculata)

Senna auriculata (Avaram Poo in Tamil), commonly known as Tanner's Cassia flower, is a perennial shrub or a small tree that belongs to the legume family, Fabaceae. It is native to India and parts of Sri Lanka and is widely recognized for its therapeutic properties, especially in traditional medicine. In a study the acute toxicity of *Senna auriculata* was studied by administering varying doses of 500, 1,000, 2,000, and 5,000 mg/kg body weight of *Senna auriculata* extracts on Albini Wistar rats [18]. Throughout the observation period, no mortality, or toxicity signs such as restlessness, respiratory depression, convulsions, or coma were observed, even at the highest dose of 5,000 mg/kg. This finding suggests that the aqueous extract of *Senna auriculata* exhibits a high safety margin in acute settings.

III. RESEARCH METHODOLOGY

An *in vitro* experimental research design was used to evaluate the antioxidant potential of the infused drink. Purposive sampling also known as judgmental or selective sampling was used in this study to ensure selecting the most relevant samples for the study.

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The *Clitoria ternatea* and *Senna auriculata* flowers used to formulate the infused drink were selected based on the bioactive compounds and therapeutic potential of the flowers. Additionally, only home grown, pesticide free flowers were selected to improve the quality of the infused drink. The analysis for the present study was conducted in Affyclone Laboratories Pvt. Ltd., an ISO certified laboratory located in Chromepet, Chennai. Sensory evaluation was conducted in the Food Science Lab of the Department of Home Science, Women's Christian College, Chennai. The present study was approved by the Institutional Ethics Committee of the Department of Home Science, Women's Christian College, Chennai.

A. Procurement and preparation of the infused drink

Blue Pea flower (*Clitoria ternatea*) and Tanner's Cassia flower (*Senna auriculata*) were procured from a home garden located in Chennai, Tamil Nadu. Organic honey was purchased online. The flowers were washed thoroughly in RO water and then used for the study. The infused drink was prepared by steeping both fresh flowers in 100 ml of water for 8 hours at room temperature. Two Variations of the infused drink were prepared by varying the concentrations of the flowers. Variation 1 was prepared by steeping 3 grams of *Clitoria ternatea* flowers and 2 grams of *Senna auriculata* flowers in 100 ml of RO water at room temperature for 8 hours while Variation 2 involved steeping 2 grams of *Clitoria ternatea* flowers and 3 grams of *Senna auriculata* flowers in 100 ml of RO water at room temperature for 8 hours. Finally, 5 ml of organic honey was added to the infused drinks. The two variations of the infused drink are represented in fig. 1.

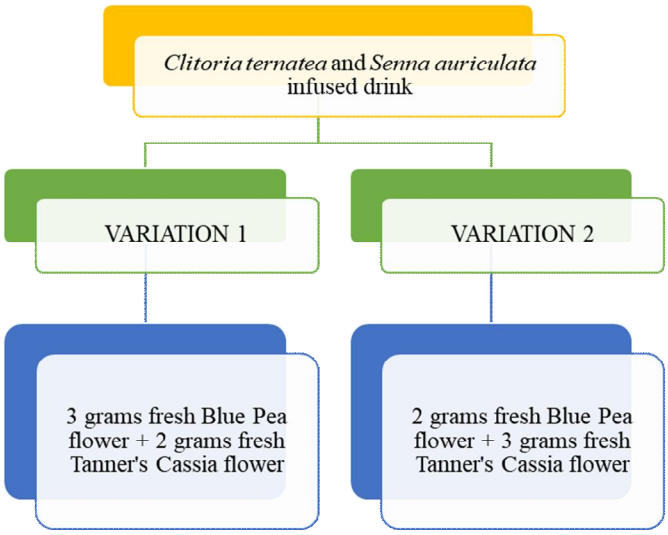


Fig. 1 Variations of the Blue Pea flower and Tanner's Cassia flower infused drink

B. Preparation of Extracts

The aqueous and ethanolic extracts of the infused drinks were prepared for both Variations. For aqueous extracts, Variation 1 combined 3g Blue Pea flower, 2g Tanner's Cassia flower, and 5 ml organic honey in 50 ml distilled water, while Variation 2 used 2g Blue Pea flower, 3g Tanner's Cassia flower, and 5 ml organic honey in 50 ml distilled water. The mixtures were boiled for 5 minutes, cooled to room temperature, filtered to remove residues, and condensed at 50ºC to obtain a gummy extract, which was used to quantify nutrients, phytochemicals, and assess antioxidant potential [19]. For ethanolic extracts, the same ingredient combinations were mixed in 50 ml ethanol and left to sit for 72 hours before filtering and condensing at 50ºC to yield a gummy extract [7], which was used for analysing bioactive components using GCMS.

C. Phytochemical and Nutritional Analysis of the Infused Drink

Bioactive compounds present in the infused drink was identified using Gas Chromatography – Mass Spectrometry method. The mass spectrum of unknown component present in the Blue Pea flower and Tanner's Cassia flower infused drink was compared with the spectrum of the known components stored in the NIST database. Standard AOAC protocols were used for the quantification of specific nutrients (Carbohydrates, fat, vitamin A, vitamin C, iron, and selenium) and phytochemicals (Total phenolic content, total flavonoids, total anthocyanin, and total antioxidant content).

D. Assessment of in Vitro Antioxidant Potential of the Infused Drink

The *in vitro* antioxidant potential of both Variations of the infused drink were assessed using 2,2-Diphenyl – 1 – picrylhydrazyl (DPPH) Assay and Ferric reducing antioxidant power (FRAP) Assay. Standard DPPH and FRAP assay protocols were used. The DPPH and FRAP activity were assessed for concentrations ranging between 20 µl and 100 µl. The IC50 values for both assays were calculated. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a commonly used method to assess the antioxidant activity of various substances such as food samples, plant extracts and pharmaceuticals. It is a very simple, rapid, and cost-effective method to use. This assay provides a quantitative measure of how well a sample can neutralize free radicals. DPPH is a stable free radical characterized by a deep violet colour. As an antioxidant donates an electron, DPPH radical is reduced and turns yellow or colourless. This change is usually measured using a UV-Vis spectrophotometer at 517 nm. The degree of colour change from purple to yellow or colourless is directly proportional to the antioxidant capacity of the sample [20]. The antioxidant activity for a DPPH assay is usually expressed as percentage of inhibition or IC50. IC50 value represents the concentration of sample required to scavenge or inhibit 50 per cent of the DPPH radicals [21]. The Ferric Reducing Antioxidant Power (FRAP) assay is a widely used method for evaluating the antioxidant capacity of various substances, including food samples, plant extracts, pharmaceuticals, and biological fluids. This assay is based on the single-electron transfer (SET) mechanism, where antioxidants in the sample donate electrons to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions. The reaction occurs in an acidic medium (pH 3.6), where ferric-tripyridyl triazine (Fe^{3+}-TPTZ) complex is reduced to Fe^{2+}-TPTZ, resulting in the development of an intense blue colour. This colour change is proportional to the reducing power of the sample, which is quantified by measuring absorbance at 593 nm using a UV-Vis spectrophotometer. The higher the absorbance, the greater the antioxidant potential of the sample. The FRAP assay is particularly advantageous because it is simple, reproducible, rapid, and does not require expensive reagents or complex reaction conditions. However, it only measures the reducing ability of antioxidants and does not account for radical scavenging or hydrogen-donating capacities, which are covered by other antioxidant assays like DPPH or ABTS. Despite this limitation, FRAP remains a valuable tool for assessing antioxidant potential in functional foods, medicinal plants, and nutraceuticals, providing crucial insights into their health benefits [22].

E. Data Analysis

Data gathered from the study were subjected to analysis of mean, standard deviation and t-test using Microsoft Word. Mean was used to calculate the average of specific nutrients and phytochemicals in the infused drink. Standard deviation was used to measure the variability in the mean values of nutrients and phytochemicals.

IV. RESULTS AND DISCUSSION

A. Nutrient analysis

The results of nutrient analysis of Variation 1 and Variation 2 of the infused drink are represented in table I. Variation 1 of the infused drink had higher quantities of carbohydrate and selenium content whereas, Variation 2 of the infused drink had higher quantities of fat, vitamin A, vitamin C, and iron. This indicates that, as the concentration of Tanner's Cassia flower increased, the nutrient content of the infused drink increased. According to a study done by [23], it was found that, *Clitoria ternatea* flower contains 2.2 per cent carbohydrates, 0.32 per cent protein, 2.5 percent fat, 2.1 per cent fiber and 92.4 per cent moisture. It contains approximately 3.09 milligrams of calcium, 2.23 milligrams of magnesium, 1.25 milligrams of potassium, 0.59 milligrams of zinc, 0.14 milligrams of sodium and 0.14 milligrams of iron for every gram of flower. Similarly, *Senna auriculata* flower contains approximately 10.54 per cent protein, 2.98 per cent fat, 2.08 per cent fibre, 12.37 per cent moisture content, 18.35 milligrams of zinc, 190.50 milligrams of iron for every gram of flower. As this flower has very high zinc and iron content, it could potentially prevent the occurrence of micronutrient deficiencies [11].

TABLE I
MICRONUTRIENT AND MACRONUTRIENT CONTENT OF THE INFUSED DRINK

Nutrients	Unit	Variation 1	Variation 2
Carbohydrates	g/100 ml	0.032	0.021
Fat	Per cent/100 ml	28 %	31 %
Vitamin A	mg/100 ml	49.43	75.81
Vitamin C	mg/100 ml	73.59	92.53
Iron	mg/100 ml	0.096	12.56
Selenium	mg/100 ml	0.088	0.056

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B. Identification of Bioactive Compounds using GC-MS

The results of Gas Chromatography – Mass Spectrometry for both variations of the infused drink are represented in table III and IV. The compounds identified belonged to the phytochemical classes such as phenols, flavonoids, lactones, fatty acids, glycosides, triterpenoids, steroids, and alkaloids. Bioactive compounds that were identified in both Variations of the infused drink were 2,3-Butanediol [R- (R, R)], Glyceraldehyde, Dihydroxyacetone, Isosorbide Dinitrate, 2(3H)-Furanone, dihydro-4-hydroxy-, 1,2,3-Propanetriol, 1-acetate, Acetoxyacetic acid, nonyl ester, α-D-Galactopyranoside, methyl, Melezitose, and 4-O-Methylmannose.

Bioactive compounds such as Propane, 1-isothiocyanato-, Butanoic acid, 2-methyl-3-oxo-, ethyl ester, 6-Hydroxy-2,6-dihydropyran-3-one, Pentanoic acid, 4-oxo-, 2(3H)-Furanone, 5-ethyldihydro-, 2-Isopropyloxan-4-ol, 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-, Phenol, 3,5-bis(1,1-dimethylethyl)-, 3-Deoxy-d-mannoic lactone, Crystalline Antibiotic, 1,2,4-Benzenetricarboxylic acid, cyclic 1,2-anhydride, decyl ester, Agaricic acid, Heptasiloxane, hexadecamethyl-, Acetamide, 2-phenylthio-N-benzyl-N-dodecyl-, and Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandro-) were present in Variation 1 but absent in Variation 2.

Similarly, bioactive compounds such as 5-Oxotetrahydrofuran-2-carboxylic acid, 2-(Isobutoxymethyl)oxirane, Melibiose, 3-Deoxy-d-mannonic acid, 1-Deoxy-d-mannitol, 6-Tridecanol, 3,9-diethyl-, Erythro-dl-O-ethylthreonine, iso-Butyl aldehyde propylene glycol acetal 2, d-Mannose, 1-Propanamine, N-methyl-N-nitroso-, Tris(tert-butyldimethylsilyloxy)arsane, Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl- , 2-Fluoro-3-trifluoromethylbenzoic acid, pentadecyl ester, 5,14,23-Octadecatrien-14,15-diol, and 1H-Benzoimidazole, 2-benzyl-1-isobutyl- were present in Variation 2 but absent in Variation 1.

From the findings of GC-MS analysis, it is therefore evident that Variation 1 of the infused drink has a better profile of bioactive compounds such as phenols, flavonoids, carbohydrates, lactones, fatty acids, glycosides, triterpenoids, steroids, and alkaloids with potent antioxidant properties and also other therapeutic properties such as antibacterial, antimicrobial, anticancer, hypo cholesterolemic, neuroprotective, anticonvulsant, hypoglycaemic, and anti-inflammatory properties compared to Variation 2. Thus, can play an important role in neutralizing free radicals, and may alleviate oxidative stress and related diseases such as cardiovascular diseases, neurodegenerative disorders, cancers, and diabetes mellites.

C. Quantification of phytochemicals

The results for quantification of phytochemicals for Variation 1 and Variation 2 of the infused drink are represented in table II. Variation 1 of the infused drink had a higher concentration of total phenolic compounds, total flavonoids, and anthocyanins, while Variation 2 showed an increased total antioxidant content. Thus, it was concluded that Variation 1 had a better phytochemical content due to its higher phenolic, flavonoid, and anthocyanin levels. These phytochemicals are known for their strong antioxidant properties, which may contribute to long-term health benefits [24]. Therefore, the rich phytochemical profile of Variation 1 of the infused drink suggests that it may offer consistent health benefits, making it the better choice for enhancing overall health and well – being.

TABLE II
QUANTIFICATION OF PHYTOCHEMICALS IN THE INFUSED DRINK

Phytochemicals	Variation 1	Variation 2
Total phenolic content (mg GAE/ 100 ml)	3.032 ± 0.10	2.597 ± 0.10
Total flavonoids (mg QE/100 ml)	77.28 ± 0.59	50.08 ± 0.40
Anthocyanin content (mg CGE/100 ml)	91.90 ± 0.58	81.87 ± 0.56
Total antioxidant content (mg AAE/100 ml)	354.63 ± 5.5	407.65 1.1

D. In vitro Antioxidant potential of the infused drinks

The DPPH assay was conducted to evaluate the free radical scavenging ability of both Variations of the infused drink. It was observed that the antioxidant activity of both Variations of the infused drink increased with increasing concentrations. This suggests that the antioxidant activity was dose dependent. Though both Variations exhibited antioxidant activity, it was not significant at concentrations ranging between 20 µl and 100 µl.

The IC50 value, which indicates the concentration required to scavenge 50 per cent of the DPPH radicals, was found to be 237.9 for Variation 1 and 216.88 for Variation 2. Since both IC50 values exceed 200 µl, a relatively higher concentration is needed to achieve significant antioxidant activity. A lower IC50 value reflects greater antioxidant potential, indicating that Variation 2 of the infused drink demonstrates stronger radical scavenging activity. This suggests that Variation 2 is more effective at neutralizing free radicals, thereby exhibiting greater overall antioxidant activity. The results for 2,2-Diphenyl – 1 – picrylhydrazyl (DPPH) assay is represented in fig. 2.

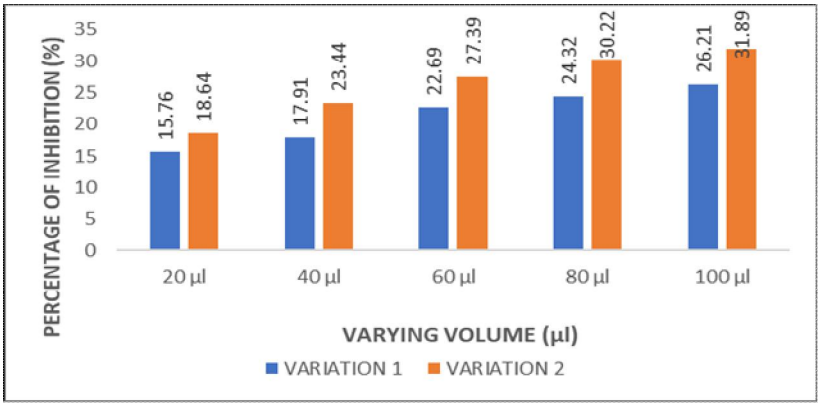


Fig. 2 Percentage of Inhibition of Variation 1 and Variation 2 of the infused drink

The FRAP assay was performed to assess the ferric reducing power of both Variations of the infused drink. The FRAP activity of both Variations 1 and 2 of the infused drinks, increases with increase in concentration. Therefore, both Variations of the infused drink exhibit a dose-dependent increase in antioxidant activity. Variation 2 had an IC_{50} of 39.44 µl, while Variation 1 had an IC_{50} of 46.64 µl. The lower IC50 value of Variation 2 suggests that it reaches 50 per cent inhibition at lower concentrations than Variation 1 indicating that Variation 2 is more potent in reducing ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions, thus exhibiting good antioxidant activity even at lower concentrations. The results for FRAP assay is represented in fig. 3.

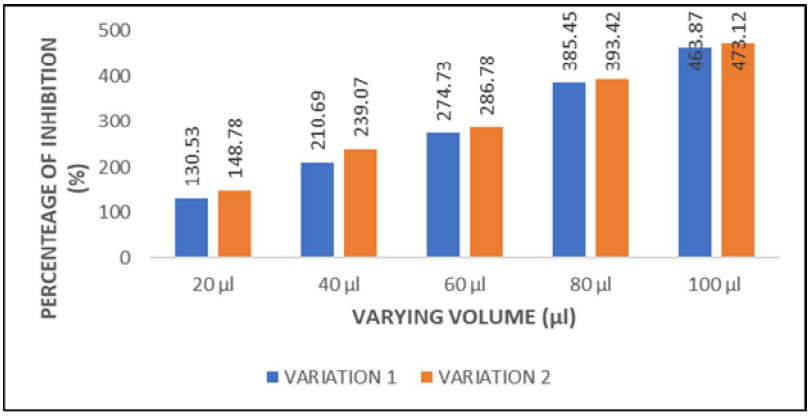


Fig. 3 FRAP activity of Variation 1 and Variation 2 of the infused drink

V. CONCLUSION

The findings highlight the distinct strengths of both Variations of the infused drink. Variation 1 demonstrated higher radical scavenging activity, indicating its strong potential to neutralize free radicals. On the other hand, Variation 2 showed significantly greater ferric reducing power, suggesting its enhanced ability to reduce oxidized compounds and maintain cellular balance. This implies that while Variation 1 may be more effective in preventing oxidative damage, Variation 2 could provide better support in restoring antioxidant balance within the body. Thus, both Variations present valuable antioxidant properties that can contribute to protection against oxidative damage. Both Variations offer notable antioxidant benefits, making them valuable for health. The choice between them ultimately depends on personal preference and taste. One can be benefitted from both Variations of the infused drink for its antioxidant potential.

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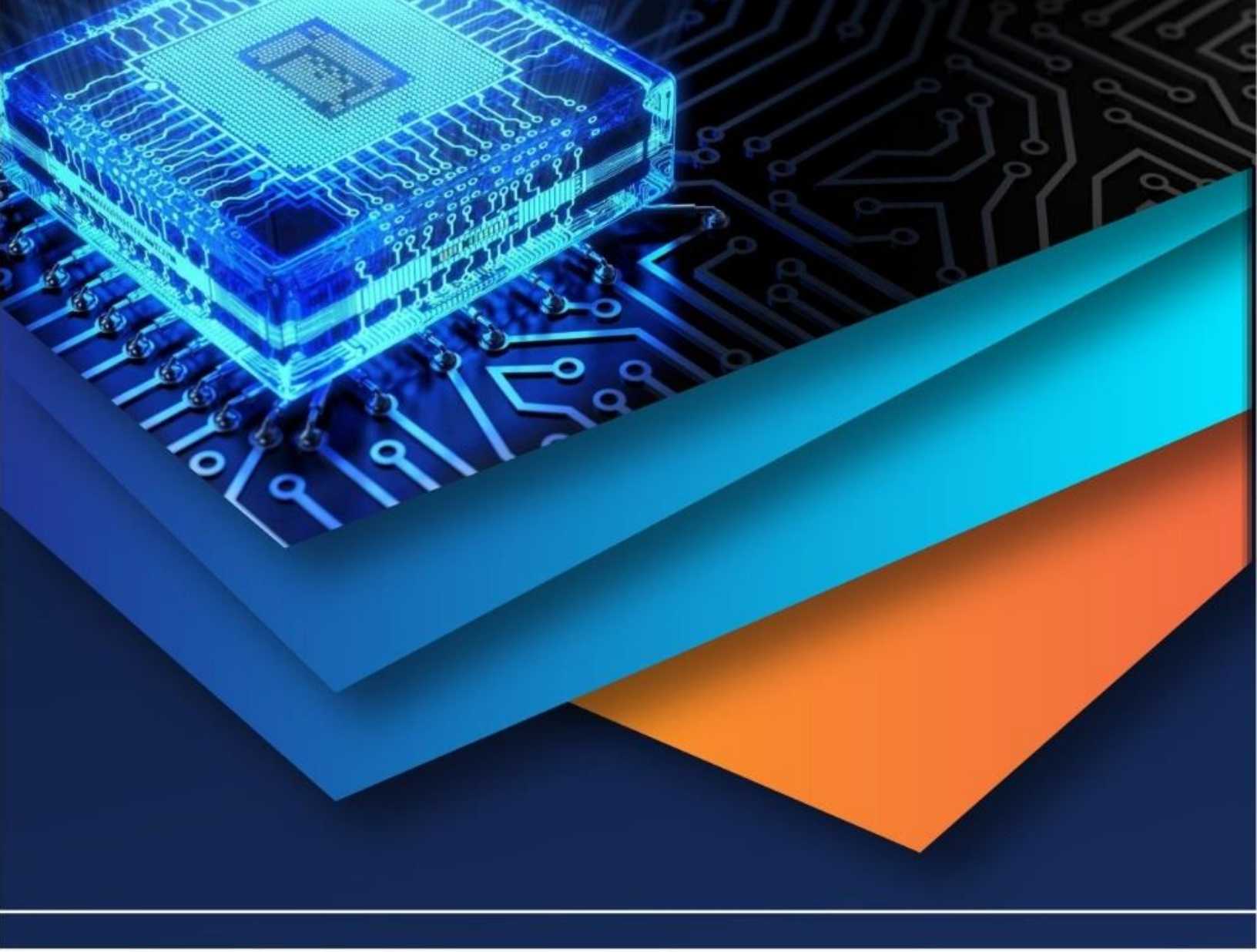
VI. ACKNOWLEDGMENT

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