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Enhancement Of Phytochemical Activity In Orange Juice Using Giloy (Tinospora Cordifolia)

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Abstract: *The present investigation was carried out to develop and standardize value added* orange juice *by incorporating giloy (*Tinospora cordifolia*) leaves extract at varying concentrations of 10, 15 and 20 per cent. Tinospora cordifolia, a medicinal plant of the Menispermaceae family, is well known for its potent bioactive compounds and significant therapeutic applications, including immunomodulatory, anti-inflammatory, and antioxidant activities. Phytochemical evaluation revealed a statistically significant ($p<0.05$) increase in alkaloids, flavonoids, tannins, and steroids with higher levels of giloy supplementation. DPPH radical scavenging assay demonstrated a progressive enhancement in antioxidant capacity across all supplemented formulations, with the highest value recorded in Type-III. However, sensory analysis using a 9-point hedonic scale indicated that increasing concentrations of giloy adversely affected organoleptic attributes such as taste, aroma, texture, and overall acceptability. Type-I (10% GLP) exhibited the most favorable sensory profile while also offering improved phytochemical and antioxidant benefits over the control. The study concludes that supplementation of orange juice with 10% giloy leaf extract represents an optimal formulation that balances enhanced nutritional and functional properties with acceptable sensory quality. This formulation has potential for commercialization as a health-oriented functional beverage that meets consumer preferences and supports dietary interventions for improved health and wellness.*

Key words: *Giloy, value added, bioactive, antioxidant, sensory etc.*

INTRODUCTION

Tinospora cordifolia is a semi-evergreen, deciduous climbing shrub commonly located on the trunks of large trees such as mango and neem. It is capable of thriving in various soil types, ranging from acidic to basic, under conditions of average moisture. The stems of the plant exhibit moisture and thickness, featuring prominent aerial roots that extend from the branches with differing diameters. The younger stems are characterized by a green hue and smooth surfaces, whereas the older stems present a light brown coloration. The shrub is a succulent, twining, rapidly growing vine characterized by green branches that turn brown with age. It features aerial roots and ovate, juicy, acute membranous young leaves with a round petiole, measuring 5 to 14 cm in diameter (Jain et al. 2021; Verma et al., 2021). Giloy is a readily accessible household herb known for its significant medicinal properties. It is recognized as a versatile rejuvenating herb commonly utilized as a tonic (Kapur et al., 2008). Giloy is a member of the Menispermaceae family and is known by various names in India, including giloy, guduchi, and amrita. It is highly regarded in Ayurveda and traditional medicine for its notable therapeutic properties (Singla et al., 2010; Wesley et al., 2008). All components of Tinospora, including leaves, stem, fruits, and roots, are utilized as a nutraceutical. The utilization of stems and leaves as dietary supplements enhances health and functions as both therapeutic and preventive measures. The entire plant of Tinospora serves as a substantial source of nutrients, including essential macro- and micro-nutrients such as Zn, Mn, Cl, K, Ca,

Ti, Cr, Fe, Co, Ni, Cu, and Br. Additionally, it contains various phytochemicals that significantly contribute to enhancing the necessary enzymatic activities. This substance serves as a significant source of nutrients and phytochemicals, utilized as a beneficial dietary supplement for both humans and animals (Upadhyay et al., 2010). The plant contains nutraceutical agents that contribute to its well-known properties, including immunomodulation, hepato-protection, anti-inflammatory effects, antipyretic action, antispasmodic benefits, and memory enhancement (Nagarkatti et al., 1994). The herb's rejuvenating qualities have led to its widespread designation as 'Amrita,' signifying elixir. Ayurvedic Giloy formulations are created following the established procedures by practitioners (Sangeetha et al., 2013).In compared to the stem and roots, the leaves are abundant in vitamin C, minerals, and phytochemicals (Singh et al., 2006). The 'Guduchi-Satva' formulation, derived from the stem core, is nutrient-dense, including fat (0.14 g/100 g), protein (0.64 g/100 g), dietary fibers (0.16 g/100 g), energy (288.8 cal/100 g), calcium (70 mg/100 g), and iron (9.7 mg/100 g) (Sangeetha et al., 2013). Tinospora serves as a beneficial dietary element that contributes to nutrition, holistic health, and the prevention of various diseases. Incorporating Tinospora stems and leaves into the diet is recommended for the maintenance and enhancement of health. Tinospora may serve as a promising source of natural antioxidants and an important dietary supplement. Tinospora may serve as a valuable nutritional resource for enhancing bodybuilding efforts and strengthening the human immune system.

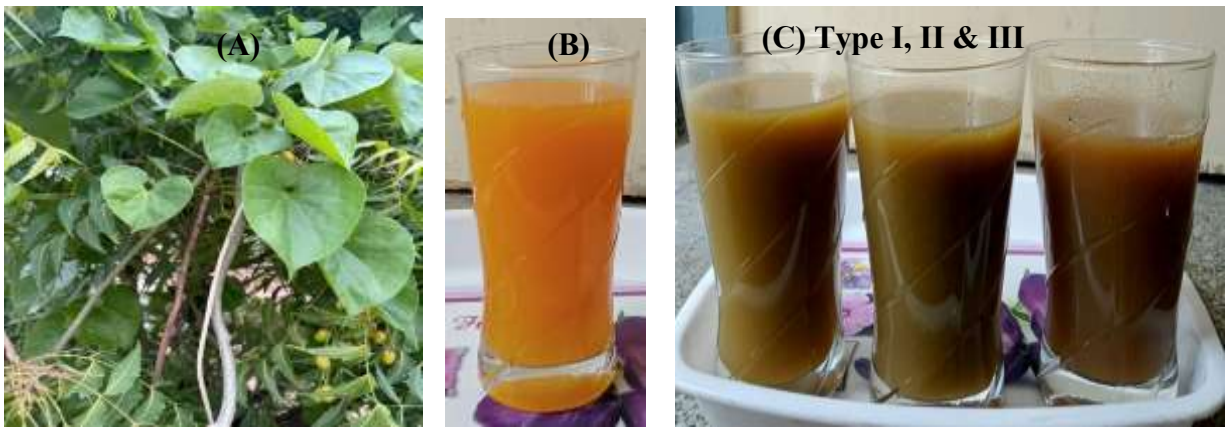

Figure A, B & C Giloy leaves, Orange juice (C) & Type I, II &III

Review of literature:

Sinha et al., 2004 revealed that Tinospora cordifolia demonstrates significant promise for the advancement of biopharmaceutical products aimed at treating a range of diseases. It functions as an immunomodulator. It helps in hepatosuppression, anti-allergic, anti-pyretic, anti-HIV, anti-diabetic, anti-cancer, anti-toxic, anti-asthmatic & anti-malaria, etc. The giloy plant possesses significant potential for the development of beneficial pharmaceuticals. The leaf extract of Giloy has demonstrated anti-HIV properties. While Mittal et al., 2014 found that biological extract derived from this plant is likely to be beneficial in the protection and treatment of various viral diseases in humans. Giloy is utilized for alleviating inflammation and injury in the mucous membranes of the digestive tract. The mechanism involves enhancing mucin production, which serves to safeguard the stomach and duodenum (Mittal et al., 2014; Badar, et al., 2005). Consuming fresh giloy juice contributes to enhanced immune function. This process improves the functionality of macrophages, the cells tasked with combating foreign entities and microorganisms, thereby facilitating a quicker recovery (Tamboli et al., 2021). Giloy enhances the body's resistance and stimulates the immune system Kapil & Sharma (1997) ; Kumari, 2012. The biological extract from the giloy plant is beneficial in protecting and treating various viral diseases in humans. It is also utilized in the treatment of chronic fever, diarrhea, cancer, jaundice, dysentery, bone fractures, pain, asthma, skin diseases, snake bites, poison from insects, and eye disorders (Sharma et al.,

2011) ; Kapil & Sharma (1997)). A diverse range of compounds that contribute to immunomodulatory effects and exhibit cytotoxic properties include 11-hydroxy muskatone, N-methyl-2-pyrrolidone, N-formylannanain cordifolioside, A. magnoflorine tinocordioside, and syringing (Sharma & Dabur, 2016). Giloy uses in in diabetes, stomachache, jaundice, urinary problems, skin ailments, and prolonged diarrhea and dysentery and attributes these health benefits to phytochemicals contained in giloy plant that comprise of several steroids, alkaloids, diterpenoid, lactones and glycosides (Verma et al. (2021). Some studies exhibited strong and effective anti-bacterial activity of giloy against gram-negative bacteria i.e. pseudomonas aeruginosa ATCC No.9027 and grampositive bacteria i.e. staphylococcus aureus ATCC No. 6538 Solanki & Goyal (2022) ; Estari et al. (2012).

materials and methods:

procurement and processing of giloy

Fresh giloy leaves were procured from locally available sources. All other ingredients and packaging materials required for product development were purchased from the local market in a single batch. The collected giloy leaves were thoroughly washed with tap water followed by distilled water to remove surface impurities. The leaves, dried in a hot air oven at 50 ± 5°C. Once dried, the materials were ground into fine powder, sieved through a 60-mesh sieve, and stored in airtight containers. Giloy juice was prepared by mechanically crushing and pressing the cleaned leaf pieces.

Methods for preparation of orange juice with giloy

The fruit (orange) was washed, manually peeled, cut into halves with sterile knife using hand gloves and seeds of orange were removed. The cut oranges were pressed with a hand juicer squeezer to extract the juice. The juice was clarified manually using a sterile muslin cloth to obtain a clear juice. After completing the preliminary work to standardize the recipe for beverages various blends of juices with GLP was developed. The different ratio was taken for various treatments. These treatments was 100 orange juice [T0], 90 (O):10 (G) [T1], 85 (O) :15 G [T2] and 80 (O) :20 G [T3].

Antioxidant Activity

Sample Extraction

Antioxidant compounds were extracted by following the method of Serrano et al. (2007). Finely ground, moisture-free sample powder (300 mg) was treated with 5 mL of methanol: water (80:20, v/v) and shaken at 150 rpm for 30 minutes. The mixture was centrifuged at 5000 × g for 10 minutes at 4°C. The supernatant was collected, and the extraction was repeated using another 5 mL of the same solvent. Both supernatants were pooled, filtered through Whatman No. 1 filter paper, and used for antioxidant assays.

DPPH Free Radical Scavenging Activity

The antioxidant activity of the extracts was evaluated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay, as described by Brand-Williams et al. (1995) and modified by Tadhani et al. (2009).

Reagents used included:

- Trolox standard solution (10 mg/100 mL distilled water)
- DPPH solution (15.77 mg in 200 mL methanol, O.D. adjusted to 1.0 at 517 nm)
- Methanol

For the assay, different aliquots of sample extract were diluted to 1 mL with methanol. Each was mixed with 3 mL of DPPH reagent and incubated at 37°C for 20 minutes. Absorbance was recorded at 517 nm against methanol as blank. Control consisted of methanol treated the same way as the samples. A standard curve was prepared using Trolox (10–40 µg/mL).

Per cent inhibition was calculated using the equation:

% Inhibition= (Ac−Ae/Ac)×100

Where, Ac = absorbance of control and A_e = absorbance of extract.

The antioxidant capacity (mg Trolox equivalents per 100 g) was calculated as:

DPPH (mg TE/100 g) = (X/Y×Standard Conc.×Volume made/Aliquot taken×100/Sample taken× dilution factor

Where X = sample inhibition (%), Y = standard inhibition (%).

Phytochemical Evaluation

Tannin Content

Tannins were estimated using the Folin- Ciocalteu method (Ejikeme et al., 2014). To the sample extract, 7.5 mL of distilled water, 0.5 mL Folin-Ciocalteu reagent, and 1 mL of 35 per cent sodium carbonate solution were added. The volume was adjusted to 10 mL with distilled water. After 30 minutes of incubation at room temperature, absorbance was measured at 700 nm. A calibration curve was generated using tannic acid (20–100 µg/mL), and results were expressed as mg tannic acid equivalents per gram of dry weight (mg TAE/g).

Flavonoid Content

Flavonoids were quantified using the aluminium chloride colorimetric method (Sofora, 1993; Harborne, 1973). Sample aliquots were adjusted to 5 mL with distilled water, and sequentially treated with 0.5 mL of 5 per cent sodium nitrite, 0.6 mL of 10% aluminium chloride, 2 mL of 1N sodium hydroxide, and 2.1 mL of distilled water. The pink color formed was measured at 510 nm. A standard curve was prepared using rutin (50–200 µg/mL). Total flavonoid content was expressed in mg rutin equivalents per 100 g (mg RE/100 g) using the formula:

TFC (mg RE/100 g) = (Standard OD/Sample OD×Standard Conc.×Volume made/Aliquot × 100/Sample taken×Dilution factor ÷1000)

Alkaloid content

Alkaloids were estimated using the method described by Hikino et al. (2014). Five grams of sample were subjected to Soxhlet extraction with methanol. The methanolic extract was evaporated and the residue was dissolved in 5 mL of 2N HCl. One mL of this solution was mixed with 5 mL phosphate buffer (pH 4.7) and 5 mL bromocresol green (BCG) solution, and the complex formed was extracted with chloroform. The chloroform layer was collected and diluted to 10 mL. Absorbance was read at 415 nm. Total alkaloids were expressed as mg berberine chloride equivalents per gram (mg BCE/g).

Steroid content

Steroids were quantified based on the method of Ejikeme et al. (2014). To 5 mL of extract, 2 mL of 4N sulfuric acid, 2 mL of 0.5 per cent iron (III) chloride and 0.5 mL of 0.5 per cent potassium hexacyanoferrate (III) were added. The mixture was heated in a water bath at 70 ± 2°C for 30 minutes with intermittent shaking, then diluted to 10 mL with distilled water. Absorbance was measured at 780 nm against a reagent blank. Steroid content was expressed in absorbance units or quantified using a suitable standard.

Sensory evaluation of value-added products developed by adding giloy leaves powder:

Organoleptic evaluation of developed products was done. The value - added products were sensory evaluated by a semi-trained panel of 10 judges using 9-point hedonic scale (Ranganna, 1986).

Statistically analysis: All the data was analysed using one way- ANNOVA in OP- STAT and SPSS, 2016

Results & Discussion

DPPH content of orange juice developed using giloy leaves powder

The antioxidant activity of orange juice supplemented with varying concentrations of giloy (Tinospora cordifolia) was measured using the DPPH radical scavenging assay. The results showed a consistent increase in antioxidant potential with the addition of giloy extract (Table 1). The control sample, which contained 100 per cent orange juice, recorded a DPPH activity of 35.27 per cent. While Type-I, which had the lowest level of giloy addition, exhibited a slightly higher antioxidant activity of 36.05 per cent. This significantly ($p<0.05$) increasing trend continued in Type-II and Type-III samples, which showed values of 36.63 per cent and 37.13per cent, respectively. These findings indicate that the inclusion of giloy extract contributes positively to the antioxidant capacity of orange juice. The enhanced activity in the supplemented samples is likely due to the bioactive compounds present in Tinospora cordifolia, which are known for their free radical scavenging properties. Among the formulations, Type-III demonstrated the highest antioxidant activity, making it the most effective in enhancing the functional quality of the beverage (Figure 2).

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Table 1: DPPH content of orange juice supplemented with giloy leaves powder (%, on dry weight basis)

Treatment	DPPH Activity (mean ± SEm)
Control (OJ:100)	35.27 ± 0.13^{d}
Type-I	36.05 ± 0.07^{c}
Type-II	36.63 ± 0.07^{b}
Type-III	37.13 ± 0.05^{a}
C.D. (P<0.05)	0.29

Values are Mean ±SE of three independent determinations. NS- Non-significant
Mean with the same letter within a column is not significantly different.
OJ: Orange Juice
Type-I: GLP@10%; Type-II: GLP @15%; Type-III: GLP @20%

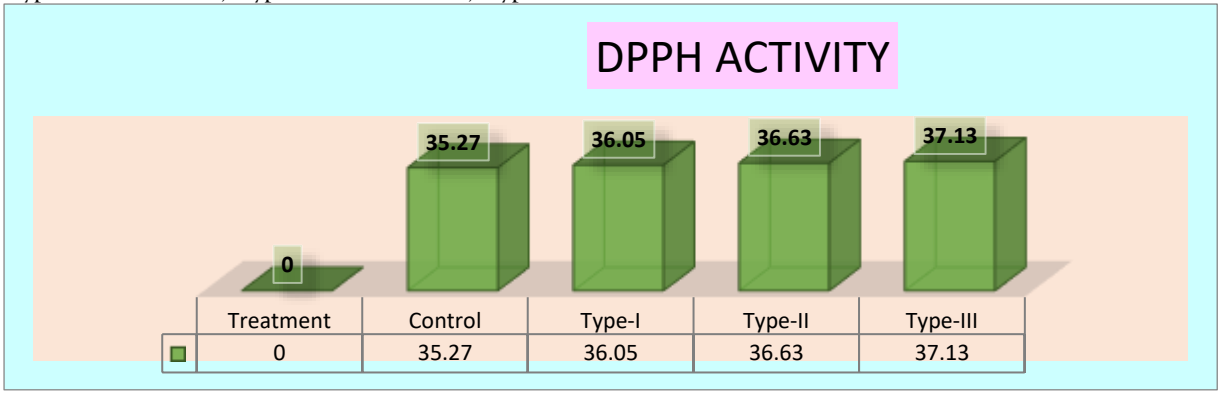

Figure 2: DPPH content of orange juice developed using giloy leaves powder

Table 2: Phytochemical evaluation of orange juice supplemented with giloy leaves powder (%, on dry weight basis)

Orange juice	Alkaloid	Tannins	Flavonoids	Steroids
Control (OJ:100)	1.13 ± 0.06^{d}	1.71 ± 0.04^{d}	1.74 ± 0.04^{d}	0.16 ± 0.03^{d}
Type-I	2.08 ± 0.05^{c}	1.96 ± 0.02^{c}	2.23 ± 0.09^{c}	0.33 ± 0.12^{c}
Type-II	3.15 ± 0.06^{b}	3.01 ± 0.10^{b}	3.03 ± 0.05^{b}	0.45 ± 0.13^{b}
Type-III	3.55 ± 0.08^{a}	3.84 ± 0.06^{a}	3.91 ± 0.06^{a}	1.18 ± 0.06^{a}
C.D. (P<0.05)	0.22	0.20	0.22	0.24

Values are Mean ±SE of three independent determinations. NS- Non-significant
Mean with the same letter within a column is not significantly different.
OJ: Orange Juice
Type-I: GLP@10%; Type-II: GLP @15%; Type-III: GLP @20%

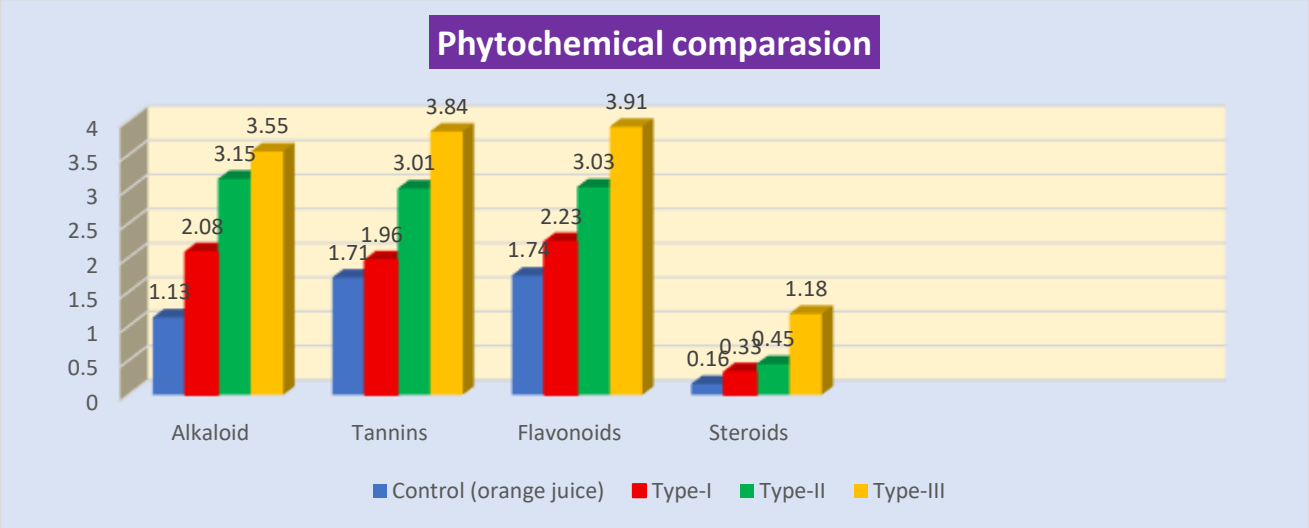

Figure 3: Phytochemical composition of giloy supplemented orange Juice

The phytochemical profile of orange juice fortified with giloy (Tinospora cordifolia) was evaluated to determine changes in the levels of key bioactive compounds: alkaloids, tannins, flavonoids, and steroids. The results, as shown in Table 2, indicate a significant enhancement in all tested phytochemical constituents with increasing levels of giloy supplementation. According to table 2 in GLP alkaloid content of control orange juice was 1.13 per cent getting significantly (P<0.05) increased in all three supplemented juice types i.e. in Type-I, Type-II and Type-III at 2.08%, 3.15% and 3.55% respectively (Figure 3). Tannins content of control orange juice was 1.71% getting significantly (P<0.05) increased in all three supplemented juice types i.e. in Type-I, Type-II and Type-III at 1.96%, 3.01% and 3.84% respectively. Flavonoids content of control orange juice was 1.74% getting significantly (P<0.05) increased in all three supplemented juice types i.e. in Type-I, Type-II and Type-III at 2.23%, 3.03% and 3.91% respectively. Steroids content of control orange juice was 0.16% getting significantly (P<0.05) increased in all three supplemented juice types i.e. in Type-I, Type-II and Type-III at 0.33%, 0.45% and 1.18% respectively. These results clearly demonstrate that fortification with giloy enhances the phytochemical content of orange juice, contributing to its potential health benefits. The increase in alkaloids, flavonoids, and tannins—well-known for their antioxidant, anti-inflammatory, and immune-boosting properties—highlights the functional potential of the enriched formulations.

Table 3- Sensory evaluation of orange juice supplemented with giloy leaves powder (%, on dry weight basis)

Treatment	Colour	Appearance	Aroma	Texture	Taste	Overall Acceptability
Control (OJ:100)	8.20±0.13^{a}	8.30±0.15^{a}	8.10±0.31^{a}	8.20±0.20^{a}	8.70±0.15^{a}	8.30±0.11^{a}
Type-I	7.80±0.13^{b}	7.70±0.15^{b}	7.60±0.30^{b}	7.10±0.43^{b}	7.20±0.38^{b}	7.48±0.24^{b}
Type-II	7.50±0.16^{b}	6.60±0.22^{c}	5.50±0.40^{c}	5.30±0.39^{c}	4.40±0.22^{c}	5.86±0.21^{c}
Type-III	6.70±0.21^{c}	5.30±0.30^{c}	4.90±0.23^{c}	4.30±0.21^{c}	4.00±0.00^{c}	5.04±0.14^{c}
C.D. (P<0.05)	0.49	0.59	0.86	0.93	0.86	0.54

Values are Mean ±SE of ten independent observations. NS- Non-significant
OJ: Orange Juice, Giloy Leaves Powder
Type-I: GLP@10%; Type-II: GLP @15%; Type-III: GLP @20%

Table 3 depicts sensory evaluation of prepared orange juice and its comparison with control juice and all 3 prepared juices. And it is clearly evident from table 3 and figure 4 (red trendline across that Type-I) that only low concentration as low as 10% giloy leaves extract supplemented orange juice exhibited all the properties and potential of getting accepted as a fun-cherished beverage. These findings of present study were in concordance with study conducted by Rawat (2020) where the study concluded that acceptability got reduced in giloy stem supplemented orange juice as concentration of giloy stem extract increased in orange juice. In Rawat study most acceptable composition was of 80:20 and in present study it was orange juice supplemented with 10 per cent gioly leaves extract. It was observed in present study that all parameters of color, appearance, aroma, texture, taste considerably decrease as compared to control orange juice thereby reducing its overall acceptability as compared to high acceptability of control orange juice. In control orange juice overall acceptability was 8.3% getting reduced in 3 supplemented types at 7.48% (Type-I, 10% giloy leaves extract); 5.86% (Type-II, 15% giloy leaves extract); 5.04% (Type-III, 20% giloy leaves extract) .

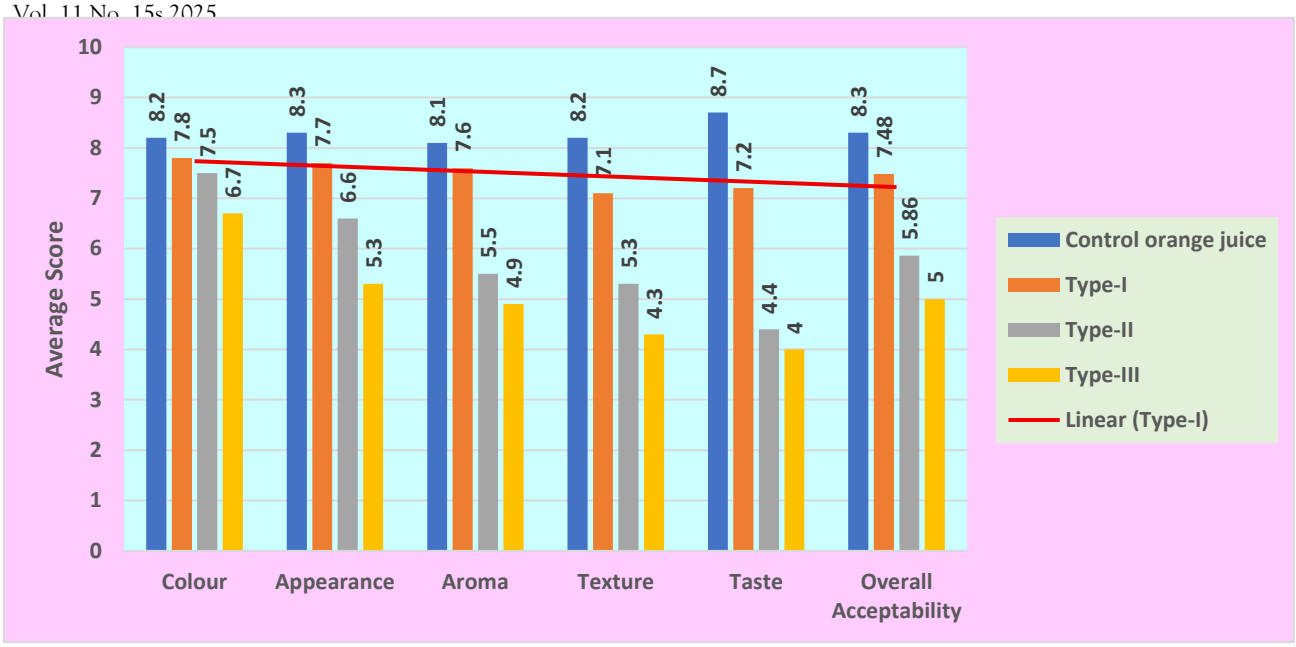

Figure 4: Sensory evaluation of orange juice supplemented with giloy leaves powder

CONCLUSION

Meritorious healthy effects and implications of giloy leaves have been established in Present paper. The observation and findings also indicate that as concentration of giloy leaves extract gets enhanced so gets enhanced the active ingredients rendering these health benefits but the taste, texture, colour, appearance and overall acceptability gets drastically reduced. Products having higher concentration might be more beneficial but useless if there is no potential customer to purchase and consume it. Therefore, looking through the lens of both health/nutrition and marketing trend present paper proposes an acceptable form and formulation of gioly leaf-extract supplemented orange juice which definitely is a win-win situation to the manufacturer and the consumer.

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