

Sensory evaluation of *Zingiber Officinale* and *Tinospora Cordifolia* mix powder with enhanced synergetic antioxidant efficacy by fuzzy logic and general mathematical formula

Abstract

The *Zingiber officinale* (ginger) and *Tinospora cordifolia* (giloy) are rich sources of antioxidant compounds and have been used for several decades to improve human health with relatively low side effects. The present research attempted to carry out the sensory and antioxidant activity evaluation of dried mixed paste powder of *Z. officinale* and *T. cordifolia* by establishing fuzzy logic, the newly proposed General Mathematical Formula (GMF) and the radical scavenging assay. Antioxidant activity was higher in the powder obtained in combinatorial process than the powders obtained in the separate processing of ginger or giloy. The sample containing powder, sugar and salt at 5, 5 and 1 % were more acceptable (good) and attributes generally fell under 'important' by both methods. Fuzzy logic and GMF results were matched perfectly for the category of samples, and quality attributes in general, and GMF method is simple and accurate for sensory evaluation.

Keywords: *zingiber officinale*, *tinospora cordifolia*, fuzzy logic, antioxidant, sensory evaluation

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Introduction

Medicinal plant extracts have become increasingly popular in the pharmaceutical and food industries in recent years because of their relatively low side effects, easy availability, and affordable cost, making them an excellent source of medicines. Oxidative stress is caused due to the elevated concentrations of free radicals or reactive oxygen species in the body, which can damage the body.¹ Antioxidants in medicinal plants, are present due to the availability of flavonoids, phenolics, vitamins, and secondary metabolites.² Hence, the use of antioxidants is increasing in the human diet to protect against scavenging free radicals. *Z. officinale* and *T. cordifolia* are important plants rich in several ethno-medicinal and nutritional values and are extensively used worldwide as herbal remedies. Sufficient evidence is present to consider these plants for antioxidant activity, antidiabetic activity, hepatoprotective activity, anti-inflammatory activity, and immune modulator activities.^{3,4} Different phytochemical compounds are responsible for these beneficial activities and ultimate effect of these herbal compound reactions can be antagonistic, additive or synergetic.⁵ Herbs, spices, fruits, and vegetables are considered for their high synergetic effects, leading to promising antioxidant activity.⁶ Current work was carried out to evaluate the sensory attributes and the synergistic interactions on antioxidant efficacy. This approach may enhance their antioxidant properties with advantage of synergistic interactions, which may reduce their adverse reactions and negative organoleptic effects in food and increase its use in food industries.

Ginger (*Z. officinale*) and Giloy (*T. cordifolia*) are the most widely marketed spice and herbals due to their stimulating effects on health and medicinal and nutritional values. However, no sensory studies are reported to improve the quality of attributes and check the acceptability of ginger and Giloy drinks. The present study

attempted to enhance synergetic antioxidant efficacy and improve powder drinks attributes and sensory quality. The sensory evaluations of four different formulations were done by fuzzy logic and General Mathematical formulae (Algebraic Equations).

Materials and methods

Materials

Quantitative analysis was carried out using analytical grade reagents, chemicals, and solvents purchased from SD Fine Chemicals. With expert assistance, we obtained Giloy (*T. cordifolia*) stems and leaves from the nearest village in Bengaluru, washed them with hot water to remove impurities, and cut them into tiny pieces. Initially, chopped giloy leaves and stems were subjected to 80°C (10 min) to obtain paste. They were then mixed with ginger-settled solids to make the paste (Figure 1). Fresh ginger root (*Z. officinale*) was obtained from the local market in Bengaluru. The plant materials were cleaned with distilled water and juice extracted by the mechanical press and mixed with giloy paste as per the procedure in Figure 1.

Antioxidant activity determination

Preparation of ginger and Giloy extracts powder solutions

About 100g of powder was extensively extracted with ethanol (95%) with Soxhlet extraction (15 h). The extract was concentrated with a Rota-vacuum evaporator so that there was no solvent in the residue, and it was stored in the refrigerator until its usage.

DPPH radical scavenging activity

1,1-diphenyl-2-picrylhydrazil (DPPH) was utilized to estimate the radical scavenging activity of powder. To prepare samples, 3 ml of

powder (25 -100 µg) extract in methanol and 1 ml of DPPH (0.1Mm) in methanol were mixed and shaken vigorously for 30 min at 25 °C. Absorbance was measured at 517 nm by using a spectrophotometer. In the reaction mixture, there was a decrease in absorbance, suggesting a more remarkable ability to scavenge free radicals. The following formula was used to estimate the concentration of DPPH radicals.^{8,9}

$$\text{DPPH scavenging activity (\%)} = \frac{A_c - A_t}{A_c} \times 100$$

(Ac = absorbance of the control reaction mixture, At = absorbance of the sample reaction mixture)

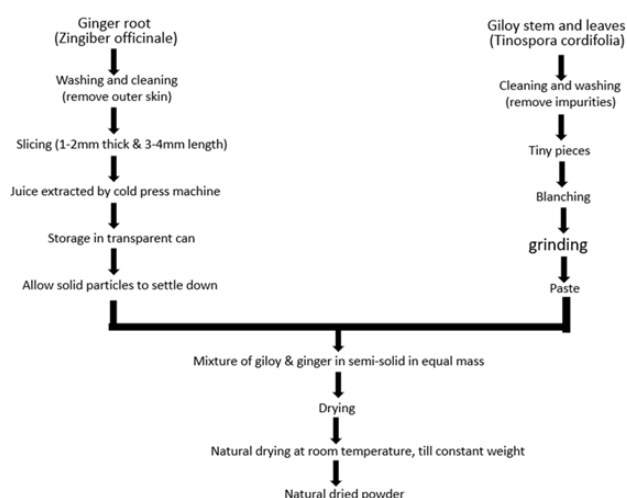


Figure 1 Procedure to make Natural Dried powder of Giloy and Ginger paste. Ginger and giloy powder were placed in an air-tight container and stored in the refrigerator till further analyses.

Determination of metal chelating activity

Metal chelating activity was assessed by adding 0.1 mM FeSO₄ (0.2 mL) and 0.25 mM ferrozine (0.4 mL) subsequently into 0.2 mL of plant extract (Chew Y et al). After incubating at room temperature for 10 min, absorbance of the mixture was recorded at 562 nm. Chelating activity was measured using the following formula:

$$\text{Metal chelating activity} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

Where A_{control} is the absorbance of control reaction (without plant extract), and A_{sample} is the absorbance in the presence of a plant extract

Preparation of drink samples

The powder was mixed in a drink to make four samples for sensory evaluation. The proportion of different samples is shown in Table 1. The composition level of mixed powder was decided by primary sensory evaluation, and the most acceptable powder composition was found 5% in the drink. Powder, salt, and sugar are dispersed in pure drinking water in proportions mentioned in Table 1 and allowed to boil water for 5 minutes to make the drink safe for human consumption and free from all kinds of impurities

Table 1 Composition of different samples

Samples	Ginger and Giloy powder (%)	Sugar (%)	Roasted salt (%)	Water (%)
S1	5	5	1	89
S2	5	0	0	89
S3	5	5	0	89
S4	5	0	1	89

The four different samples (S1, S2, S3, and S4) were evaluated by judges for sensory properties of the drink; prepared samples (mixing the powder of ginger and giloy, sugar, salt, and water) kept at a cool and dry place to attain the room temperature before starting the sensory evaluation. No additives or preservatives were added to the samples.

Panelist's selection and sensory evaluation of drink samples

The triangle test was performed for screening of judges at 60% succession.¹⁰ After screening 185 members, one hundred healthy and non-smoking judges were selected. In equal proportions (50 from each group), females and males were selected (aged between 20 and 60 years). The necessary instructions were given to judges about the sensory attributes of ginger and Giloy drinks and the scoring of attributes.^{11,12} The sensory evaluation was completed as per the given regulations.¹² Four-drink samples prepared by mixing ginger and giloy powder were given to the judges for sensory evaluation. The judges were powerfully told to clean their hands, and swill their mouths with water each time before judging another quality attribute. The order of judging of attributes was the first aroma, second color, third taste, and last Mouthfeel.¹¹ The prepared samples were kept in a cool and dry place to attain room temperature before the start of sensory evaluations. A 5-point fuzzy scale was utilized to assess the quality of ginger and giloy drink samples on a fuzzy scale (not satisfactory, fair, medium, good, or excellent) as well as for the general quality attributes (not important at all, somewhat important, important, highly important, or extremely important).

Sensory evaluation of drink by fuzzy logic

The response from judges was analyzed by fuzzy logic.¹³⁻¹⁷ The following steps were used while doing a sensory evaluation with the fuzzy logic method: (i) Estimation of a sensory score of the triplet of the sample (ii) Computation of quality attributes sensory score triplets (iii) Calculation of relative weightage triplets, for quality attributes (iv) Triplets value for an overall sensory score of the sample (v) Calculating overall membership function for sensory scores (vi) Overall ranking after obtaining similarity values for drinks samples (vii) Ranking of quality attributes of drinks in general.

Sensory evaluation by general mathematical formula (GMF)

In the General Mathematical Formula (GMF) sensory evaluation of ginger and giloy drinks, the score given by judges was used, and important steps involved are (i) quality attributes average score (ii) estimation of Average scores in general for quality attributes (iii) estimation of weightage for samples' quality attributes in general (iv) overall score value for samples' acceptability and (v) calculation of overall scores of samples.

For each samples judge preferences were recorded to calculate the average score for all quality attributes on five-point sensory scale factor (Table 2). If 'T' is a number of samples, 'P' is a quality attribute. m,n,q, h, and k number of judges gave excellent, good, medium, fair, and not satisfactory, respectively. The average score for 'T' sample under-quality attribute 'P' was calculated as described below:

$$[(k \times 0) + (h \times 2.5) + (q \times 5) + (n \times 7.5) + (m \times 10)] / (k + h + q + n + m)$$

After simplification, the above eq. can be written below

$$S_{TP} = [(k \times 0) + (h \times 2.5) + (q \times 5) + (n \times 7.5) + (m \times 10)] / Z \dots\dots\dots (1)$$

Where S stands for sample, T represents sample number, P is quality attributes, and Z represents the total number of judges. The average score of quality attributes, in general, was calculated by score or preference given by judges on a 5 scale with given numerical value factors on the same scale (5-point sensory scale) (Table 3).

Table 2 Five-point sensory scale factors, and the numerical value of each factor with respect to sample quality attributes

Sensory scale factors	The numerical value of factors
Not satisfactory	0
Fair	2.5
Medium	5
Good	7.5
Excellent	10

Table 3 Five-point sensory scale factors and the numerical value of each factor concerning quality attributes in general

Sensory scale factors	The numerical value of factors
Not at all important	0
Somewhat important	2.5
Important	5
Highly important	7.5
Extremely important	10

To calculate the average score of quality attributes (P), equation..2 is used, in which ‘m’ represents the number of judges opted ‘extremely important’, ‘n’ represents the number of judges marked quality attributes ‘highly important’, ‘q’ represents number of judge in favor of ‘important’, ‘h’ represents number of judges lined with ‘somewhat important’ and ‘k’ represents number of judges marked ‘not at all important’

$$Q_p = [(k \times 0) + (h \times 2.5) + (q \times 5) + (n \times 7.5) + (m \times 10)] / Z \dots\dots\dots (2)$$

Where, Q stands for quality, P represents quality attributes, and Z is the total no. of judges

To calculate the weightage for each sample quality in general, values obtained from equation 2 were used in Eq.3

If P1, P2, P3, and P4 are four quality attributes of sample, then quality attribute weightage with respect to P1 was calculated by eq.3. Similarly, the values for P2, P3, and P4 were calculated

$$P_{1W} = Q_{P1} / (Q_{P1} + Q_{P2} + Q_{P3} + Q_{P4}) \dots\dots\dots (3)$$

Q represents the attributes’ quality, and W represents the quality attribute’s weightage.

Overall score of T sample was calculated after putting the value obtained from Eq.1 and Eq.3 in Eq.4

$$S_{OT} = (S_T P_1 P_{1W}) + (S_T P_2 P_{2W}) + (S_T P_3 P_{3W}) + (S_T P_4 P_{4W}) \dots\dots\dots (4)$$

(S_O indicates samples’ overall score).

To compute the value of acceptability score or score for quality attributes, the result obtained from eq (1), (2), and (4) were used. The required values were obtained by using Eq.1(similarity value of quality attributes), Eq.2 (samples’ quality attributes in general), and Eq.4 (similarity value of overall ranking of the sample) (Table 4).

Results and discussion

Antioxidant activity

DPPH radical scavenging activity:

Free radical scavenging was used to estimate the antioxidant activity of powder in combination. An evaluation of the DPPH radical scavenging properties of powder was conducted. DPPH radicals were scavenged synergistically by methanolic extracts of powder.^{18,19} *Z. officinale* and *T. cordifolia* had antioxidant activity against free radicals. Alcoholic extract of a mixture of dried powder showed DPPH radical scavenging activity of 38.6, 62.7, 81.6, and 93.4% in 25, 50, 75, and 100 µg of the sample, respectively. They are higher than the values reported earlier.²⁰

Table 4 Linguistic scale for five- to six-point scale conversion

Score (s)	Importance / acceptability
0-1	Not at all necessary / Not satisfactory
1 < s ≤ 3	Somewhat necessary/ Fair
3 < s ≤ 5	Necessary / Satisfactory
5 < s ≤ 7	Important / Good
7 < s ≤ 9	Highly important/ Very good
9 < s ≤ 10	Extremely important/ Excellent

The required values were obtained by using Eq.1(similarity value of quality attributes), Eq.2 (samples’ quality attributes in general), and Eq.4 (similarity value of overall ranking of the sample).

Determination of metal chelating activity

We put our medicinal plant extracts through a metal chelating assay because too much free iron has been linked to the production and stimulation of free radicals in biological systems. The dry powder mixture tested showed substantial chelating activity in the concentration range of 1 to 10 mg/mL (Figure 2). The powder’s methanolic extract effectively scavenged the metals. The metal chelating activity of the dried powder extract was 22.3, 44.9, 70.5, and 81.4% in 1, 2, and 5 mg/L of the sample, respectively.²¹

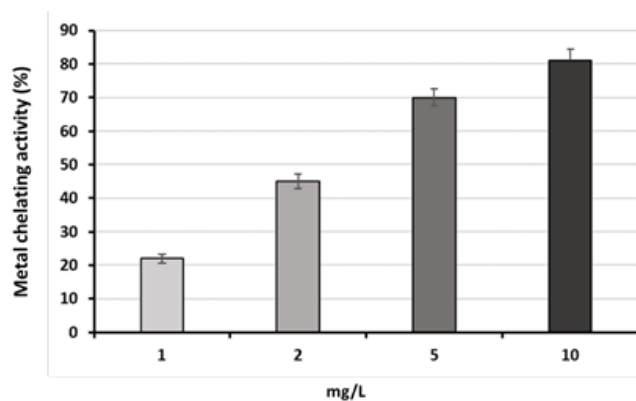


Figure 2 Metal chelating activities of powder at different concentrations. Data are reported as mean ± SE values (n=3).

Sensory evaluations

Sample2 was control (no added sugar and salt), sample3 made with dried powder and sugar only, sample4 were made by mixing of dried powder and salt (no added sugar), and sample1 was made with sugar (5%), powder (5%) and roasted salt (1%) to enhance the properties of sensory attributes of drinks and the formulation was found good and sample2, 3 and 4 were found satisfactory. Table 5 & Table 6 show the responses of judge’s responses on the quality attributes of the drink

sample, in general. The highest similarity value of sample1 evaluated by fuzzy logic Table 7 was 0.7165 (Good) and for samples 2, 3, and 4 were 0.7059, 0.7205 and 0.7194 respectively, which fell under satisfactory and overall samples ranking is S1>S3>S4>S2.

When obtained data was analyzed by GMF method (Eq.4), sample 1 fell under Good quality, and samples 2, 3 and 4 were satisfactory (Table 8), and the results obtained are the same as fuzzy logic result. But the sample ranking was differed from fuzzy logic, GMF's overall samples ranking was observed as S1>S2>S4>S3. The sample's quality attributes in general by GMF (Eq.2), were under an important category with the following order, Taste > Aroma > Mouthfeel > Color

and the same result was also found by the fuzzy logic method (Table 9). The GMF method is simple and easy to understand. The calculations and accurate method to find the rank and category of specific sample attributes. In fuzzy logic, very complex calculations are involved, making it complex and challenging for everyone to reach a final result efficiently. The similarity value of powder drinks was calculated by using both methods (fuzzy logic and GMF). For all samples (S1, S2, S3 and S4), the similarity value of quality attributes obtained after calculation by fuzzy logic (Table 10 & Table 11) and GMF (Table 12) were found to be the same. For sample 1, color, aroma, Mouthfeel, and taste fell under the Good category.

Table 5 Summary of sensory scores concerning quality attributes of drink samples

Sensory quality attributes and drink samples	Sensory scale factors on a 5-point scale				
	Not satisfactory	Fair	Medium	Good	Excellent
Aroma					
Sample1	18	16	23	17	26
Sample2	21	26	26	16	11
Sample3	24	22	24	15	15
Sample4	22	23	26	14	15
Colour					
Sample1	17	17	18	19	29
Sample2	19	21	23	18	19
Sample3	21	21	25	18	15
Sample4	20	23	23	17	17
Mouthfeel					
Sample1	12	14	22	19	33
Sample2	20	22	26	16	16
Sample3	22	20	26	15	17
Sample4	22	21	24	15	18
Taste					
Sample1	12	17	20	22	29
Sample2	12	20	28	22	18
Sample3	14	20	26	22	14
Sample4	14	20	29	21	16

Table 6 Summary of sensory scores with respect to drinks' quality attributes in general

Quality attributes of drinks in general	Sensory scale factors on a 5-point scale				
	Not at all important	Somewhat important	Important	Highly important	Extremely important
Aroma	16	14	22	29	19
Colour	16	17	25	27	15
Mouthfeel	13	21	24	25	17
Taste	16	15	20	22	27

Table 7 Fuzzy logic similarity-values with respect to overall ranking of drink samples

Sensory scale factor	Sample 1	Sample 2	Sample 3	Sample 4
Not satisfactory, F1	0.0178	0.0484	0.0489	0.0504
Fair, F2	0.2093	0.3448	0.3557	0.3568
Satisfactory, F3	0.5524	0.7059	0.7205	0.7194
Good, F4	0.7165	0.6537	0.6498	0.6491
Very good, F5	0.4508	0.2635	0.2506	0.2506
Excellent, F6	0.1071	0.0240	0.0206	0.0207

Table 8 GMF Similarity-values with respect to overall ranking of drink samples

Sample	Sample 1	Sample 2	Sample 3	Sample 4
Scale factor	Good	Satisfactory	Satisfactory	Satisfactory
Score	5.806	4.798	4.726	4.730

Table 9 Ranking of quality attributes concerning drink samples in general by fuzzy logic

Scale factor	Colour	Aroma	Taste	Mouth feel
Not at all necessary F1	0	0	0	0
Somewhat necessary F2	0.0857	0.0548	0.0357	0.0805
Necessary F3	0.7334	0.6095	0.5334	0.7058
Important F4	0.8609	0.9105	0.931	0.8736
Highly important F5	0.1859	0.2617	0.2904	0.2048
Extremely important F6	0	0	0	0

Table 10 Ranking of quality -attributes with respect to drink samples in general by GMF

Attribute	Colour	Aroma	Taste	Mouth feel
Scale factor	Important	Important	Important	Important
Score	5.20	5.525	5.725	5.30

Table 11 Quality attributes' similarity values are calculated by fuzzy logic concerning drink samples

Sample	Sensory scale	Quality attribute			
		Colour	Aroma	Taste	Mouth feel
Sample 1	Not satisfactory	0.00	0.00	0.00	0.000
	Fair	0.041	0.061	0.0205	0.0023
	satisfactory	0.5566	0.639	0.4636	0.375
	Good	0.9063	0.8639	0.9902	0.9629
	Very good	0.2563	0.1973	0.3662	0.403
	Excellent	0.00	0.00	0.00	0.00
Sample 2	Not satisfactory	0.00	0.00	0.00	0.00
	Fair	0.1284	0.3215	0.0773	0.205
	satisfactory	0.8198	0.9554	0.6909	0.8717
	Good	0.7728	0.5506	0.8804	0.6762
	Very good	0.0938	0.0427	0.2146	0.0714
	Excellent	0.00	0.00	0.00	0.00
Sample 3	Not satisfactory	0.00	0.00	0.00	0.00
	Fair	0.2076	0.271	0.0837	0.2026
	satisfactory	0.8735	0.9175	0.7209	0.8690
	Good	0.6706	0.5765	0.8596	0.6626
	Very good	0.0706	0.0471	0.1853	0.0675
	Excellent	0.00	0.00	0.00	0.00
Sample 4	Not satisfactory	0.00	0.00	0.00	0.00
	Fair	0.19	0.2641	0.100	0.1949
	satisfactory	0.8611	0.9126	0.7698	0.8633
	Good	0.6916	0.5953	0.8431	0.6683
	Very good	0.0747	0.0518	0.1595	0.0683
	Excellent	0.00	0.00	0.00	0.00

*Highest similarity values are in bold of the quality and attributes of the sample.

Table 12 Quality attributes' similarity values calculated by GMF with respect to drink samples

Sample	Color		Aroma		Taste		Mouthfeel	
	Scale factor score	Scale factor score	Scale factor score	Scale factor score	Scale factor score	Scale factor score	Scale factor score	
Sample 1	Good	5.65	Good	5.42	Good	5.975	Good	6.17
Sample 2	Satisfactory	4.92	Satisfactory	4.25	Good	5.35	Satisfactory	4.65
Sample 3	Satisfactory	4.62	Satisfactory	4.37	Good	5.25	Satisfactory	4.62
Sample 4	Satisfactory	4.70	Satisfactory	4.42	Good	5.12	Satisfactory	4.65

In General Mathematical Formula (GMF), attributes rank is only based on a single value score. The same is impossible in fuzzy logic because this method is based on a number of similarity values and the highest value used for deciding the rank or quality of attributes. In fuzzy logic, the similarity value ranges from 0 to 1. At the maximum time, the obtained value may not significantly differ from other values,

so there are chances of statistical error in finding the quality or rank of attributes. In GMF only one value will be there between 0 to 10 to decide the rank or quality of the sample, so it is easy to estimate the quality improvement required to achieve the target quality of products. Finally, both methods (fuzzy logic and GMF) agree that sample 1 is more acceptable than the others.

Conclusion

The present findings could have potential applications in preventing of therapeutic and aging, oxidative stress, and degenerative diseases. The antioxidant activity is majorly due to the polyphenolic compounds of plants due to their redox properties. The synergistic interactions on the antioxidant efficacy of a mixture of *Z. officinale* and *T. cordifolia* under study determine their potential use as natural antioxidants applicable in the pharmaceutical and food industries. The drink sample mixed with 5% powder, 5% sugar, 1% roasted salt, and 89% water was observed as good formulations by both fuzzy logic and General Mathematical Formula methods, and in GMF sensory evaluation methods, all required values were obtained very accurately and based on one value range. The new GMF can be used in sensory evaluation for simple and accurate values.

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Conflicts of interest

The authors declared that there are no conflicts of interest.

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