ORIGINAL RESEARCH

HEALTH ENHANCING PROPERTIES OF AN EDIBLE FLOWER *Sesbania grandiflora* (L) Poir (AGATHI) BASED PROCESSED PRODUCT.

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ABSTRACT: Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases. Current research is now directed towards natural antioxidants originated from plants due to safe therapeutics. *Sesbania grandiflora* commonly known as Agathi is used in Indian traditional medicine for a wide range of various ailments. To understand the mechanism of pharmacological actions, antioxidant properties of edible Agathi flowers were tested using standard *in vitro* models. The successive methanolic extract exhibited strong scavenging effect on α, α-diphenyl-β-picryl hydrazyl (DPPH) free radical, and reducing power have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage. The antioxidant activity has been reported to be concomitant with reducing power of the extracts. Aqueous extracts of Agathi flowers were utilized in the formulation ofjelly and subjected to sensorial evaluation. Overall acceptability, texture and taste of control and experimental jellies were comparable. A significant ($p \leq 0.05$) difference in the color and flavor of sample was observed with increase in addition of Agathi flower extract. Thus jellies containing Agathi flower extract can be a potent source of exhibiting antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage. Formulating a jelly with aqueous extract of *Sesbania grandiflora* resulted in the development of a functional food.

KEYWORDS: Antioxidants, Free radicals, Scavenging activity, functional food

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1. INTRODUCTION

An integral part of normal human physiology reveals the presence of free radicals, in the form of reactive oxygen and nitrogen species. Free radical reactions occur in the human body and food systems. Oxidative stress results in over production of these reactive oxygen and nitrogen species and cause imbalance of the bodily antioxidant defense system and free radical formation. These reactive species can react with bio molecules, causing cellular injury and death. Oxidant by-products of normal metabolism have been demonstrated to cause cancer, ageing, cardiovascular disease, and immune system decline and brain dysfunction. When the human defence system is overwhelmed by an excessive generation of oxidants, the nucleic acids, lipids and proteins can suffer oxidative damage, resulting in tissue injury. Increased intakes of dietary antioxidants may keep the balance between antioxidants and oxidants in living organisms (1). Dietary antioxidants from the plant sources (fruits and vegetables) have been demonstrated to protect the human body from the damage of oxidant by-products of human normal metabolism (2).

Dietary antioxidants can augment cellular defenses and help to prevent oxidative damage to cellular components (3). In addition, these naturally-occurring antioxidants can be formulated to give nutraceuticals that can help to prevent oxidative damage from occurring in the body. Many of the phenolic compounds represent a particularly rich family of phytochemicals, playing an important role as antioxidants, effectively reducing the oxidative stress and acting as chemo-preventive agents (4). Polyphenol-rich diets arise from the consumption of fruits and vegetables have been found to be associated with the decrease of cancer incidences (5).

Natural antioxidants occur in all parts of the plant (wood, bark, stems, pods, leaves,fruit, roots, flowers, pollen, and seeds) (6). Flower is an important part of a plant which contains a great variety of natural antioxidants such as phenolic acids, flavonoids, anthocyanin and many other phenolic compounds (7,8). Plant foods play an important role in the diet as they provide nutrition .Among the various parts of plant, flowers play an important role. Edible flowers contribute to the aesthetic appearance of food as they are used during the cooking preparation. In many parts of the world, the feedings of people continues with old traditions and the assortment of foodstuffs produced begins markedly extended with edible flowers.

Sesbania grandiflora (L.) Poir, flowers are also commonly known as Agati, Agastya etc. in ayurvedic system of medicine and reputed in the indigenous medicine in India. It is an open branching tree tall up to 15m and 39cm in diameter belongs to family Fabaceae. It is native to India, Indonesia, Malaysia, Myanmar, Philippines.It is also known as ''humming bird tree" in English. All parts of this unique plant are useful and have a wide spectrum of medicinal properties. The plant has various uses in folk and traditional medicine which includes headache, swellings, anemia, bronchitis, pains, liver disorders, and tumors (9). The bark is astringent, cooling, bitter, tonic, anthelmintic activity. The flowers are cooked and eaten as vegetable.

Oxidative stress has been associated with inflammation whish has been implicated in various diseases such as cancer, diabetes, asthma, and autoimmune diseases. The development strategies for reducing inflammation and oxidation status could lead to effective treatments of these diseases. Thus in this manner natural products containing biologically active molecules could participate to the prevention or the treatment of some of these diseases. In this regards, the present investigation involves an *in vitro* study of Agathi flowers for certain health enhancing properties.The objectives of this investigation are to determine the total polyphenol contents and characterize the free radical scavenging activity of the plants by the methods used and to develop a jelly with aqueous extract of *Sesbania grandiflora* resulting in development of valueadded foods and nutraceuticals. In this investigation, water was used as the extraction solvent to extract the hydrophilic antioxidants present in the plants. For use in foods, plant extracts made with water are nutritionally more relevant and would have obvious advantages in relation to certification and safety (10). The use of a crude extract as an additive needs to be considered so that the sensory properties of the food product are not adversely affected.

2. MATERIALS AND METHODS

Chemicals: Folin and Ciocalteu's phenol reagent, DPPH (α,α-diphenyl-β-picrylhydrazyl) methanol, acetone, ethanol, petroleum ether, tannic acid, linoleic acid ,absolute ethanol, potassium ferricyanide, sodium carbonate, trichloro acetic acid, ferric chloride, ammonium thiocyanate were procurred from Merck, SRL / S.D.fine chem / Sigma, India.

Jelly materials Sucralose, sodium benzoate (Eagle products *fssai)*, potable water and agar were commercial grade and were procured from local market. All ingredients are generally recognized as safe (GRAS).

2.1. Procurement of sample

Sesbania grandiflora flowers were acquired from the gardens of Sri Sathya Sai Institute of Higher Learning, Anantapur, located in Andhra Pradesh,

India in the months of November and December. They were identified and authenticated by the Department of Botany, Sri Krishna Devaraya University, Anantapur, Andhra Pradesh, India and lodged in university Herbarium. The specimen was assigned Herbarium No. SKU (No.45767) and taxonomically identified *as Sesbania grandiflora* (L.) Poir as per plant list 2010. Upon arrival at the laboratory, samples were washed with water to remove debris and damaged portions. The flower samples were shade dried until there was no change in weight. The dried flowers were stored in sealed polyethylene bags with silica gel included as a dessicant.

2.2 Chemical analysis of shade dried Agathi flower (SDA) powder

The moisture, protein, total ash, crude fiber contents, total lipids **(**1) were analysed in flower samples. The total carbohydrate content was also estimated (by difference) method Estimation of vitamin C (11). The total flavonoids in Agathi flower powder were extracted in absolute methanol according to the procedure of Kosalec *et al*., (11). The flavonoid content was expressed as mg quercetin equivalents (QE) in 100g of sample. Extraction for the estimation of total phenols was carried out using a modified method of Banerjee *et al*., (12). Total polyphenols were determined according to the Folin-Ciocalteau procedure (13). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per 100 grams of sample. All analyses were carried out in triplicate and expressed as the mean value and standard deviation was calculated.

2.2.1 Preparation of *Sesbania grandiflora* aqueous extract (SAE) for jelly preparation

Decoction is the most widely used and popular traditional method for the preparation of aqueous extracts of medicinal plants. It is made by boiling the sample in water for a period of fixed time duration (14). Dried flowers were ground using a domestic blender and0.5 g of this material was extracted using 25 ml of deionised water. The mixture was allowed to stand at room temperature for 1 h in the dark, with occasional agitation. The aqueous extract was obtained by filtering the mixture through Whatman No. 1 filter paper and used for analysis without further treatment.

2.2.2 Jelly Selection The final concentration (1%) of *S. grandiflora* aqueous extract (SAE) determined by a preliminary study, corresponded to a biologically active dose (25mg/kg) in healthy and streptozotocin induced diabetic male mice. The SAE was taken at different concentrations. All data were compared with control.

2.2.3 Preparation of jellies

The jellies were prepared by the standard procedure (15), by mixing water, sodium benzoate, extract and sugar. The jellies were prepared by initially mixing agar while heating the water followed by the addition of sucralose, sodium benzoate and *Sesbania grandiflora* aqueous extract (SAE) upon cooling. Table1.contains the composition of jellies with different levels(G1-25ml,G2-50ml,G2-75ml) of incorporation of *Sesbania grandiflora* aqueous extract (SAE).This combination was maintained by continuous stirring to avoid formation of lumps. Then the mixture was poured immediately into cylindrical glass containers and capped. The jars were completely submerged in boiling water for 5 min, left at room temperature and finally were refrigerated at $4^{\circ h}$C .Samples were warmed at room temperature before analysis. Fig.1 represents Technological scheme of formulating Agathi flower extract based jellies

Table1. Formulation of jelly

Ingredients	G	G1	G2	G3
Sugar(g)	50	50	50	50
Water(ml)	250	225	200	175
Agar agar(g)	2	2	2	2
Vanilla essence(ml)	1	1	1	1
Sodium benzoate(g)	0.25	0.25	0.25	0.25
Agathi flower extract(ml)	—	25	50	75

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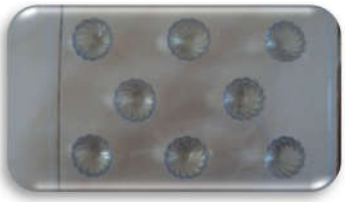

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Fig 1. Technological scheme of formulating Agathi flower extract based jellies

1. Plucking and cleaning of agathi flower 2. Preparation of extract 3. Preparation of jelly-melting of agar, 4&5 Mixing of sugar extract and flavouring agent, 6. Fltering and Filling jelly in large containers, 7. Pouring of jelly in moulds 8 & 9. Unmoulded Agathi jelly.

2.3 Sensory Analysis

Sensory analysis was carried out by panel members, in the laboratory of Department of Food and Nutritional Sciences, Sri Sathya Sai Institute of Higher Learning, Ananatapuramu. A panel of 10 judges experienced in sensory evaluation constituted for sensory analysis. During sensory analysis , Sensory attributes were estimated using 9 point–hedonic scale(14) ranging from 1–9 was; A score of 9 represented: 9 Like extremely; 8 Like very much; 7 Like moderately; 6 Like slightly; 5 Neither like nor dislike; 4 Dislike slightly; 3 Dislike moderately; 2 Dislike very much; 1 Dislike extremely.

2.4 Physio Chemical analysis

The appearance of the formulation was observed which included clarity, transparency was determined visually and *pH* was measured using *pH* meter. NEB (Non enzymatic browning),Total soluble solids, expressed as $^{\circ}$Brix, were determined according to the method given by Ranganna (17) using Abbes digital hand refractometer at 28ºC. The surface colour characteristics of jelly samples were measured by a colour reader (Konica MINOLTA, CR–10) in terms of 'L', 'a' and 'b' values. 'L' values express the whiteness of the sample with 100 as perfect white and '0' as black. Values of 'a' and 'b' indicated red–green and yellow–blue chromaticity

2.5 Gelling capacity

The gelling capacity was measured by visual method. 100µl sample was placed in a vial 2ml and equilibrated at 35^{0} C and then visually assessing the gel formation and noting the time taken for gel formation (16).

2.6 **Gelation temperature**

10 ml of the sample solution and a magnetic bar were put into a transparent vial that was placed in a low-temperature water bath. A thermometer with accuracy of 0.1 °C was immersed in the sample solution. The solution was heated at the rate of 2°C/min with the continuous stirring. The temperature was determined as GT, at which the magnetic bar stopped moving due to gelation. Each sample was measured at least in triplicate (16).

2.3 *In vitro* antioxidant activity

Extraction

Extractions of samples were carried out using a modified method of Banerjee *et al*., (2).The weighed samples (2g of dried materials) were extracted twice with aqueous methanol (50% v/v) for 18 hours at room temperature, centrifuged at 3000 rpm for 10 minutes. The supernatants were combined and volumes were made up to 25ml for dried samples respectively.

Measurement of DPPH radical scavenging activity

The free radical scavenging activity of the extracts and ascorbic acid used as positive control was determined using stable radical DPPH (α, α-diphenyl -β-picrylhydrazyl) as per the method given by Blois (3). Aliquots of the tested samples and ascorbic acid were placed in test tubes and freshly prepared DPPH solution (25mg/L) in methanol was added in each test tube and mixed. After 30 minutes, the absorbance of reaction mixture was measured at 517 nm.

Determination of reducing power

The reducing power of the extracts was determined according to method of Oyaizu, 1986 (13).

Statistical analysis: The mean and standard deviation values of the parameters analysed were calculated using standard formulae. Students't' test and ANOVA test was applied to find out if significant differences exist between the various samples for the different parameters analyzed.

3. RESULTS AND DISCUSSION

3.1 Nutrient and Phytochemical composition of Agathi (*Sesbania grandiflora)* flowers

The nutrient and phytochemical composition of sample subjected to different methods are represented in Table 2. Moisture content of sample was estimated to be 84.50 %, a value closer to that reported by Gopalan *et al*.,(4) .Drying of flowers ensures availability of the flowers require proper post handling methods. *Sesbania grandiflora.* Flowers are moderate source of protein and fat content of 2.7 and 0.9 g/100g respectively found to be slightly higher values (1.0 and 0.5 g/100g) as reported by Gopalan *et al*., (4). The total mineral content of *Sesbania. grandiflora* was found to be 0.82g/100g closer to value(0.4g/100g) as reported by Gopalan *et al*.,(4). Flowers of *Sesbania. grandiflora* are good sources of natural antioxidants .The ascorbic acid content in agathi flowers was determined to be 28.80 mg/100g.The polyphenol and flavonoid content of agathi flower were estimated to be 49.20 mg GAE/100g and 71.09 mg QE/100g respectively. The methanol extract of agathi flower contains higher levels of phenolics and flavonoids as also reported by Rastogi *et al.,* (18). Phenolic compounds such as flavonoids, phenolic acid, and tannins possess diverse biological activities such as anti-inflammatory, anticarcinogenic and anti-atherosclerotic activities. These activities might be related to their antioxidant activity.

Table 2: Nutrient and bioactive composition of shade dried Agathi flower powder (on dry weight basis).

Component	Content
Moisture(g/100g)	84.50± 0.06
Fat (g/100g)	0.90 ± 0.03
Protein (g/100g)	2.70± 1.50
Total carbohydrate	14.6 ±0.24
Fibre	1.05±0.05
Total minerals (g/100g)	0.82 ± 0.05
Ascorbic Acid (mg/100g)#	28.80±1.25
Total polyphenols (mg GAE/100g)	49.20±0.10
Total Flavonoids (mgQE/100g)	71.09±0.78

Value reported on fresh weight basis and values are Mean±SD of three values

3.2 *In vitro* antioxidant activity of Agathi flowers

Free radical scavenging potential of methanolic extracts of Agathi leaf flowers at different concentrations were tested by DPPH method and expressed as percentage inhibition are depicted in Fig.2. The essence of DPPH method is that antioxidants react with DPPH (α,α–diphenyl–β–picrylhydrazyl) and the radical is converted to α,α–diphenyl–β–picrylhydrazine with discolouration indicating the scavenging potential of the antioxidant The DPPH radical scavenging activities of methanolic extracts of Agathi flower were found to range from 16.3 to 61.7% for different concentrations tested. The values were found to be dose dependant.

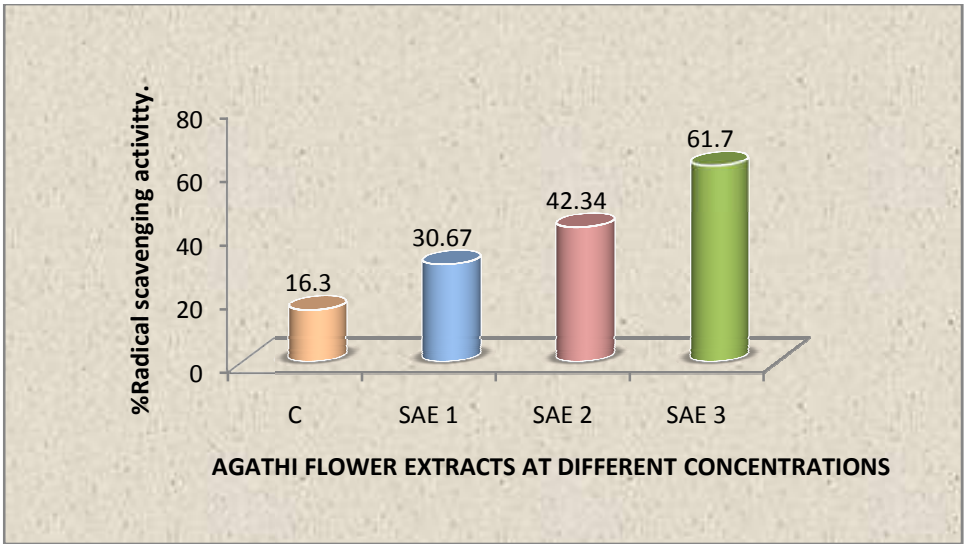


Fig 2. Ferric reducing antioxidant power of Agathi flowers

The EC_{50} values for the flower extracts were calculated , which is the concentration of the extract required to scavenge 50% of DPPH radical is depicted in Fig .The EC_{50} for methanolic extracts of Agathi flowers were found to be 1.06mg/ml, 3.07mg/ml,6.65 mg/ml and 9.58mg/ml.

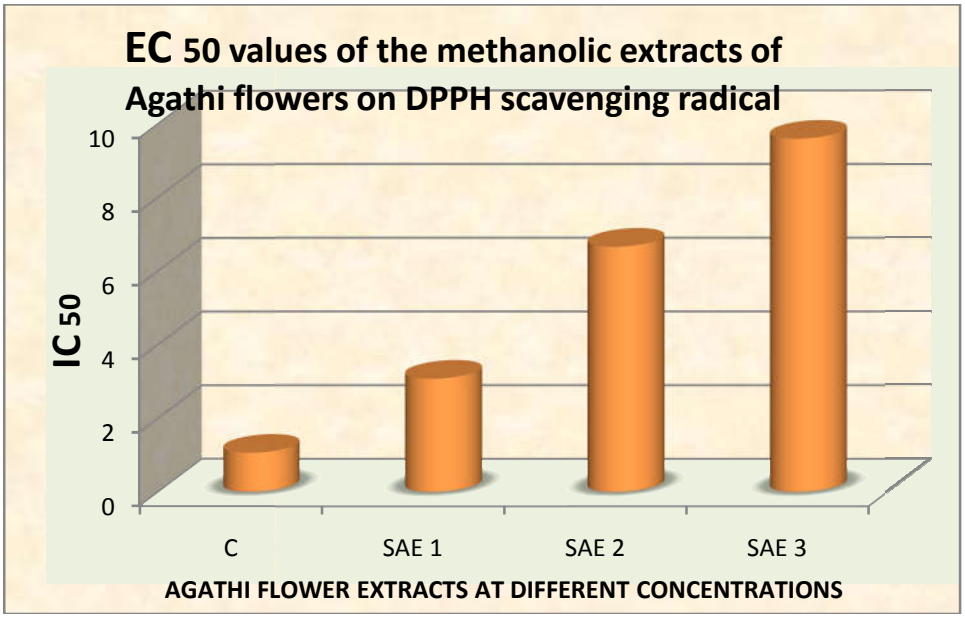


Fig.3. EC_{50} values for the Agathi flower extracts

3.3 Reducing power effects

The reducing power of the extracts of the extracts of the flower extracts of the flower samples were tested by potassium ferricyanide reduction method expressed in terms of OD values (nm) (Fig.3). The reducing power effects of methanolic extracts (50µl-250µl, 2g/25 ml) of dried flower samples were found in the range of 0.09 to 0.87%.The reducing power of various extracts is directly reflected by absorbance value. Antioxidant activity of each extract is proportional to the increase in the absorbance of the reaction mixture. The antioxidant activity has been reported to be concomitant with reducing power of the extracts. Thus the formulated jellies containing the aqueous Agathi flower extract would exhibit antioxidant activity fulfilling as promising source of nutraceutical.

3.4 Physico chemical analysis

The Physico chemical characteristics of formulated Jellies are shown in Table 3.The pH of jellies with SAE were significantly ($p<0.05$) lower than those of jellies without extract. Addition of aqueous extract of agathi flowers decreased pH. The Agathi flowers extract enriched jellies exhibited pH ranged from 5.33 to 5.98. The pH of jellies enriched with Agathi flower extract was significantly ($p< 0.05$) lower than those of jelly (CJ) without extract. A study conducted on quality evaluation of reduced–sugar pomegranate juice jelly with an aqueous extract of pomegranate, revealed a substantial decrease in pH of formulated jelly (8).The °Brix values obtained for the jelly samples were similar and the addition of SAE on °Brix. . Total soluble solids (TSS) content of control jelly was found to be 52.33° B. The TSS values recorded by G1, G2 and G3 were 53.33, 49.66 and 53.33° B, respectively. Though no significant differences were observed between TSS content of G3 and G1, the addition of Agathi flower extract recorded slightly higher value in comparison to the control jelly. Color values are represented as CIE L*a* and b* in Table 1. A reduction in lightness was observed in G in comparision to jellies with extract. A significant decrease in a* was detected with increase in concentration of extr1act.

Table No. 3: Physico Chemical Characteristics of Formulated Jellies

Sample	TSS	Ph	Titrable acidity	NEB	Moisture	*L**	*a**	*b**
G	52.33 ± 1.52	6.56 ± 0.41	8.86 ± 2.19	0.0013 ± 0.0005	61.04 ± 1.79	34.21 ± 0.7	1.02 ± 0.11	0.08 ± 0.41
G1	53.33 ± 1.52	5.98 ± 0.39	8.86 ± 2.19	0.048 ± 0.016	76 ± 2.20	26.06 ± 0.45	-1.32 ± 0.31	0.34 ± 0.25
G2	49.66 ± 1.52	5.45 ± 0.43	9.5 ± 1.9	0.167 ± 0.055	78.30 ± 1.58	23.9 ± 0.1	-0.95 ± 0.50	0.65 ± 0.17
G3	53.33 ± 0.57	5.33 ± 0.62*	12.66 ± 2.19	0.203 ± 0.072	78.33 ± 1.59	24.69 ± 0.15	-0.79 ± 0.65	0.98 ± 0.2

G control, GI25ml, G250ml, G3 75ml of Sesabania flower aqueous extract (SAE). * ($p \leq 0.05$) significant at 1% level, ** ($p \leq 0.01$) significant at 1% level * Values are mean ± SD of 3 replicates

The titrable acidity of the samples was found to be in the range of 8.66 to 12.66%. The significantly higher value obtained for G3 sample could be due to an increase in the concentration of Agathi flower extract. Acidity values recorded a proportionate increase in the concentration of extract. Agathi flower extract formulated jellies scored significantly ($p < 0.05$) higher NEB values. An increase in NEB was observed in all the samples with increased addition of aqueous extract. Control jelly maintained lowest (0.001) NEB.

3.5 Gelation temperature Gelation temperature, the temperature at which the liquid phase changes to a gel, is an important parameter for jelly systems. The ideal sol to gel transition should be between the average ambient temperature 25^0 C and 35^0 C, In order to obtain a gel having firm body, agar was added into the formulation. Gelation temperature of developed jellies is presented in table 2. The effect of % SAE concentration on the gelation was measured. It is found % SAE concentration affected the gelation temperature of formulation significantly ($p<0.05$); in addition it forms the viscous solution.G3 jelly required lower gelation temperature with respect to G and G1

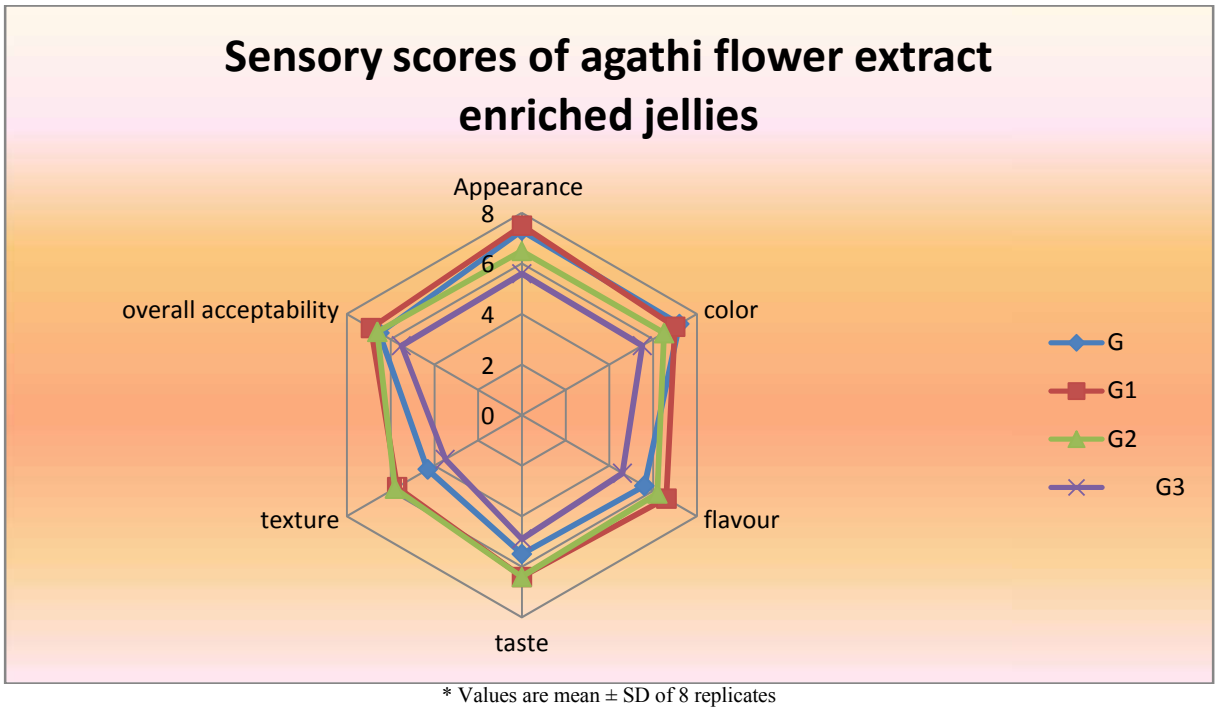
Table 4: Gelation temperature of Developed jellies

Formulation	Gelation temperature (°C) before dilution	Gelation temperature (°C) after dilution
G	35.3 + 0.3	38.2 + 0.4
G1	34.8 + 0.5	35.2 + 0.5
G2	30.7 + 0.3 *	31.6 + 0.6*
G3	27.5 + 0.5 *	28.4 + 0.5*

G control, G1 25ml, G2 50ml, G3 75 mlof *Sesabania* flower aqueous extract (SAE) , * ($p \leq 0.05$) significant at 1% level, * Values are mean ± SD of 3 replicates

3.6 Sensory evaluation

Sensory characteristics perceived by our senses represent the most important criteria quality of edible flowers. Sensorial scores of Agathi flower aqueous extract based jellies are represented in figure4. The G and G1 samples were rated better with respect to appearance and color. Overall acceptability, texture and taste of G1 and G2 were comparable. A significant ($p \leq 0.05$) difference in the color and flavor of sample was observed.



* Values are mean ± SD of 8 replicates

Fig.4. Sensory scores of Agathi based jellies

4. CONCLUSION

Edible flowers of *Sesbania grandiflora* are substantial source of nutrients and chemical compounds on showing antioxidant activity and having marked inhibitory effect on free radicals, thus defining the suitability of flowers for human nutrition and inclusion as promising new source of nutarceuticals. The use of *Sesbania grandiflora* flowers aqueous extract in formulation of jellies could pave the way for the promotion of a possible functional jelly.

5. ABBREVIATIONS

SAE *(Sesabania* flower aqueous extract)
NEB (Non enzymatic browning),
DPPH (α,α-diphenyl-β-picrylhydrazyl)
GAE (gallic acid equivalents)
QE (quercetin equivalents)
G control jelly, G1-jelly containing 25 ml of SAE,G2-jelly containing 50 ml of SAE, G3-jelly containing 75ml of SAE

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CONFLICT OF INTEREST REPORTED: NIL ; SOURCE OF FUNDING: NONE REPORTED