

Studies on utilization of Adulasa (*Adhatoda vasica*) dried extract based carbonated RTS beverage

A.M. GEDAM, R.B. KSHIRSAGAR, A.R. SAWATE AND B.M. PATIL

Efforts have been made to prepare dried extracts (solid extract) and extract (aqueous extract) from adulasa leaf. Physico-chemical properties of prepared extracts were evaluated. On the basis of their extractable values and alkaloid content (0.30 %) adulasa dried extracts were added at 0.25, 0.35 and 0.45 per cent for preparation of adulasa dried extracts based carbonated RTS beverage. Adulasa was found to be rich in alkaloids. The acceptability of prepared beverage was organoleptically evaluated. The test score indicated that among the carbonated beverages, the beverage with 0.35 per cent adulasa dried extracts with sucralose (0.2 %) as sweeteners was accepted by panel members. TSS of the carbonated beverage was decreased with addition of sucralose further increases with increase in proportion of adulasa dried extracts. Selected carbonated beverage found to be rich alkaloid content (210mg/200ml) and was organoleptically accepted.

Key Words : Adulasa, Dried extract, Alkaloid content, Carbonated RTS beverage

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INTRODUCTION

In India diabetic patient are increasing day by day and according to world diabetic foundation it has the world's largest diabetes population, followed by China with 43.2 million and it has major concern among health experts and national and international healthcare. World health organization (WHO) has identified diabetes as an epidemic condition and one of the major killers of the decade. Estimation by WHO, there will be about 250 million cases of diabetes mellitus throughout the world by 2025 (Mishra, 2011). Diabetes mellitus is a complex disorder that characterized by hyperglycemia resulting

from malfunction in insulin secretion and/or insulin action both causing by impaired metabolism of glucose, lipids and protein. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs. (Shanmugasundaram *et al.*, 2011).

Adulasa (*Adhatoda vasica*) is an evergreen shrub of 1-3 feet in height with many long opposite branches. Leaves are large and lance-shaped. Leaves opposite and exstipulate (Kumar *et al.*, 2014). The efficiency of crude extract of Adulsa (*Adhatoda vasica*) on blood sugar level of 20 diabetic patients (male and female). It was observed that sugar level in blood of patients was significantly decreased from 290 to 220 and 395 to 310 mg per litre in both fasting and random condition in female patients. Whereas results indicates more effect in male patients 280 to 185 and 386 to 180 mg per litre, respectively, for before and after intake of diet (Talib *et al.*, 2002). The extract exhibited α -amylase and α -glucosidase inhibitory activity with (15.25-43.22%) and (20-58%), when

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compared with Acarbose (99%). *Adhatoda vasica* leaf extract given to wistar rats via oral route at 50 mg/kg b.w. produced significant inhibition of postprandial hyperglycemia (Agawane *et al.*, 2015).

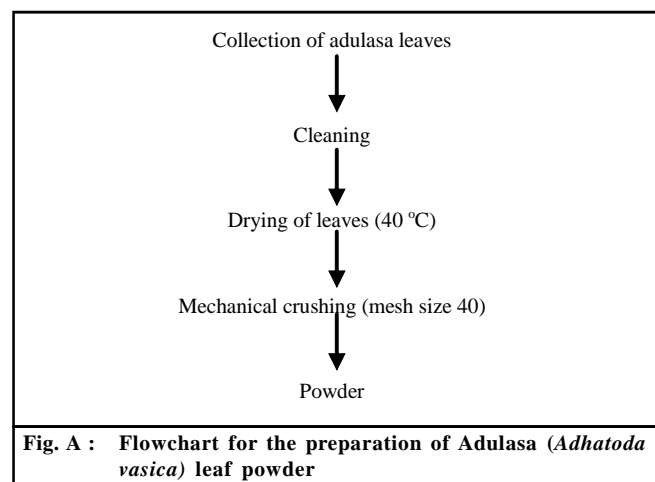
The *Ahatoda vasica* plant has been included in the WHO manual the use of Traditional Medicine in Primary Health Care, which aims to profit health workers in South East Asia to keep them informed of the therapeutic utility of their surrounding flora (Singh *et al.*, 2011).

METHODOLOGY

The present investigation was carried out in Department of Food Engineering, College of Food Technology, VNMKV, Parbhani during year 2016-17. Fresh adulasa leaves were obtained from the Dept of Botany, College of Agriculture, VNMKV, Parbhani. Other raw materials like sugar, artificial sweeteners, citric acid and artificial green colour were procured from the local market.

Preparations of adulasa powder :

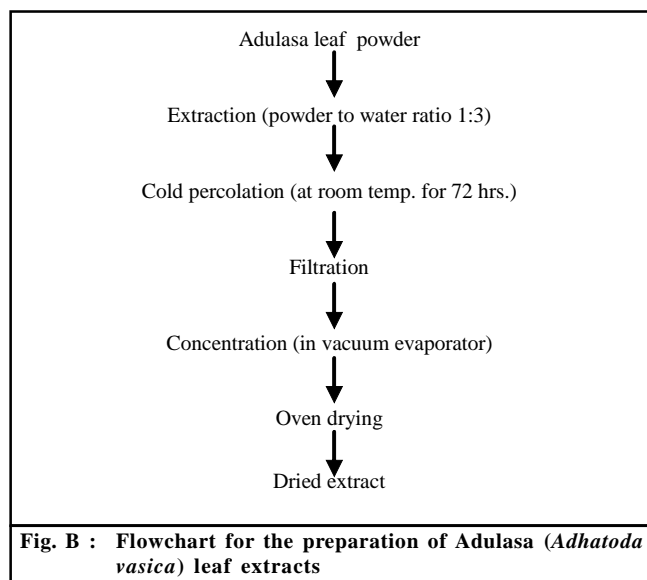
Fresh leaves samples of adulasa (*Adhatoda vasica*) were washed and subjected to sun dried, followed by oven dried. Finally the samples were crushed and converted into powdered form and saved for further analysis (Gulfaz *et al.*, 2005).



Preparations of Adulasa dried extract :

The leaf were mechanically crushed (mesh size 40) (Fig. A) and extracted with water through cold percolation at room temperature upto 72 h. The extract was filtered and concentrated in rotary evaporator under reduced pressure to obtain semisolid material to get final extract.

It was further dried by oven and dried extract obtained (Srinivasan and Kumaravel, 2015).

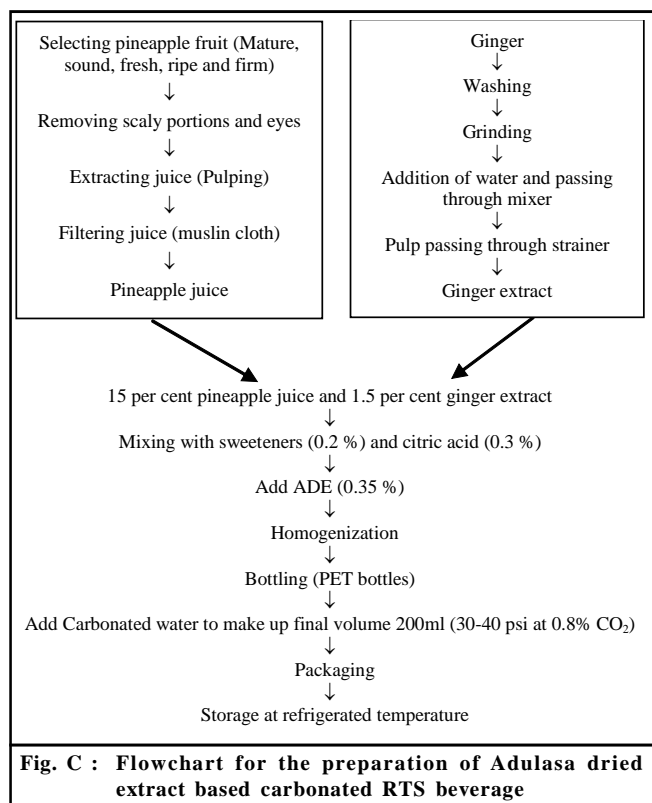


Physico chemical properties of adulasa dried extract:

Moisture, fat, protein, crude fibre, and ash content of adulasa leaf powder was evaluated as per A.O.A.C. (2000) (Fig. B). Carbohydrates by differential method. Mayer reagent test for alkaloids test Kanthale and Panchal (2015). Total alkaloids content estimated as per the method given by Harborne (1973).

Preparation of adulasa dried extract based carbonated RTS beverage :

The 15 per cent pineapple juice and 1.5 per cent ginger extract was taken to prepare the pineapple carbonated RTS beverage. The sweeteners, citric acid and preservative were added. The blending of adulsa leaf extract was at different concentrations (0.25%, 0.35% and 0.45%) and mixing and homogenization was carried out. Then the prepared syrup was filled in sterilized PET bottles. After the bottling carbonated water was added to make the final volume 200ml. Carbonation was done by using carbonation unit. The pressure of the carbonator gauge varied at 30 to 40 psi and the volume of CO₂ in the prepared pineapple carbonated beverage represented as 0.8 per cent Sample P₁ (Used 0.25), P₂ (Used 0.35), S₃ (Used 0.45) per cent ADE was carbonated (Fig. C). The product was then bottled and sealed immediately for freshness. The sealed bottles was stored at refrigeration



temperature (Jori *et al.*, 2013 and Sandhan *et al.*, 2009).

Physico-chemical properties of adulasa dried extract based carbonated RTS beverage :

TSS was measured by Abbe refractrometer, titratable acidity as per given by Ranganna (1986) and alkaloids content estimated as per the method given by Harborne (1973).

Storage studies :

Beverage was subjected to storage studies at room temperature and refrigeration temperature through specific time interval (15 days) to evaluate organoleptic parameters.

Sensory evaluation of adulasa dried extract based carbonated RTS beverage :

Prepared adulasa dried extract based carbonated RTS beverage was evaluated for organoleptic characteristics like colour, flavour, taste, and overall acceptability by a panel of semi trained judges, comprised of postgraduate students and academic staff members of College of Food Technology, V.N.M.K.V., Parbhani.

Samples were scored based on a nine point hedonic scale. Judges were asked to rate the product on 9 point Hedonic scale with corresponding descriptive terms ranging from 9 'like extremely' to 'dislike extremely' (Meilgaard *et al.*, 1999).

Statistical analysis :

The data obtained was analyzed statistically by Completely Randomized Design (CRD) as per the procedure given by Panse and Sukhatme (1967). The analysis of variance revealed at significance of $P < 0.05$ level, S.E. and C.D. ($P=0.05$) level is mentioned wherever required.

OBSERVATIONS AND ASSESSMENT

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Physico chemical properties of Adulasa dried extract :

The data regarding the physico-chemical properties of adulasa dried extract was tabulated in Table 1.

Results obtained were with respect to physico-chemical properties of extract revealed that the pH of extract and dried was 5.21 and 5.30, respectively. Moisture content of fresh extract and dried was found to be 71% and 13.40%, respectively. Crude fat, protein, carbohydrate and crude fat of dried extract were analysed and result reported that 1.85, 4.36, 73.11 and 4.10 per cent, respectively. The total ash content of extract was (2.13% w/w) and the ash content of dried was (3.18% w/w). The results are found are in support with Sree and Daniel (2015).

Table 1 : Physico-chemical properties of adulasa leaf extract and dried extract

Properties	Extract	Dried extract
pH	5.21	5.30
Moisture (%)	71	13.40
Crude fat (%)	NA	1.85
Protein (%)	NA	4.36
Carbohydrate (%)	NA	73.11
Crude fibre (%)	NA	4.10
Ash (%)	2.13	3.18
Alkaloids test	Positive	Positive
Alkaloids (%)	0.30	30.0

*Each value is average of three determinations

NA- Not analyzed

Effect of addition of different proportion of adulasa dried extract on physico-chemical composition of carbonated RTS beverage :

Results of addition of different proportion of adulasa dried extract on physico-chemical composition of carbonated RTS beverage were presented in Table 2.

Adulasa dried extract was used as herbal medicine and hence used in preparation of carbonated RTS beverage instead of raw leaf powder to reduce their intake quantity. Adulasa dried extract was rich in phytoconstituent alkaloids responsible for antidiabetic activity and also had good water soluble extractive value indicating good solubility in water. Depending upon Alkaloids content and the solubility of the extract; adulasa dried extract was selected for further study *i.e.* for the preparation of adulasa dried extract based carbonated RTS beverage. The process of addition of adulasa dried extract in carbonated RTS beverage was selected as per the process supported by Arjune *et al.* (2016). The proportion of addition of adulasa dried extract was selected on the basis of doses of adulasa extract given for the treatment of diabetes by Modak and Rao (1996) and Patel and Mishra (2012).

The values in Table 2 indicated that the TSS of the samples were found to be in the range of control (15°Bx), sample S₁ (15.2 °Bx), sample S₂ (15.3 °Bx) and S₃ (15.4 °Bx), respectively. TSS of control sample was recorded lowest due to no addition of adulasa dried extract. TSS of samples S₁ to S₃ had increasing trend due to increase in proportion of adulasa dried extract.

The pH of control sample of carbonated RTS beverage was 3.60. The pH of the sample S₁, S₂ and S₃ were found 3.95, 4.10 and 4.23, respectively. S₃ sample had highest pH due to addition of highest amount of adulasa dried extract while the pH of the control sample was found to be lowest due to no addition of adulasa dried extract. The pH of the samples had increasing trend from S₁ to S₃. It may be due to increase in proportion of adulasa dried extract having pH near to 5.3. Acidity of control sample of carbonated RTS beverage was 0.34%. The acidity of the sample S₁, S₂ and S₃ were 0.29, 0.27 and 0.24, respectively. Control sample had highest acidity while S₃ had lowest acidity. Decreasing trend in acidity of samples S₁ to S₃ was found due to increase in the pH of the samples as increase in proportion of adulasa dried extract. Study was supported by Jori *et al.* (2013).

Table 2 : Effect of addition of different proportion of adulasa dried extract on physico-chemical composition of carbonated RTS beverage

Samples	TSS (°Bx)	pH	Acidity (%)	Alkaloids content (mg/200ml)
Control	15.0	3.60	0.34	0
S ₁	15.2	3.95	0.29	150.0
S ₂	15.3	4.10	0.27	210.0
S ₃	15.4	4.23	0.24	270.0
S.E. ±	0.0430	0.0083	0.0038	1.3608
C.D. (P=0.05)	0.1295	0.0251	0.0116	4.0965

*Each value is an average of three determinations

Control –Without addition of adulasa dried extract

S₁ – With addition of adulasa dried extract 0.25% in beverage

S₂- With addition of adulasa dried extract 0.35% in beverage

S₃- With addition of adulasa dried extract 0.45% in beverage

Table 3 : Organoleptic evaluation of anti-diabetic carbonated RTS beverage with varying adulasa dried extract

Sample	Sensory attributes			
	Colour	Flavour	Taste	Overall acceptability
Control	8.9	8.9	8.8	8.8
S ₁	8.3	8.2	8.2	8.3
S ₂	8.4	8.3	8.3	8.5
S ₃	8.0	7.1	7.0	7.0
S.E.±	0.0373	0.0569	0.0707	0.0167
C.D. (P=0.05)	0.1122	0.1714	0.1505	0.0502

Control – With addition of sugar and without addition of dried extract

S₁ – With addition of dried extract 0.25% in beverage

S₂- With addition of dried extract 0.35% in beverage

S₃- With addition of dried extract 0.45% in beverage

It was cleared from the table that the alkaloids content was varied from 150 to 270 mg per 200 ml. The treatment S_3 showed higher value for alkaloids. It could be attributed to increase in concentration of adulasa dried extract in the formulation. As per the value shown in table it was found that the Alkaloids content of S_1 , S_2 and S_3 were significantly increasing. The Alkaloids contents of sample S_1 was found to be 150 mg/200 ml similarly that of sample S_2 and S_3 were 210 mg/200 ml and 270 mg/200 ml, respectively. Sample S_3 was found statistically superior over all the samples in Alkaloids content.

Organoleptic evaluation of adulasa (*Adhatoda vasica*) dried extract based carbonated RTS beverage :

The sensory evaluation of adulasa dried extract based carbonated RTS beverage was carried out by a ten semi trained panel members and the score were given w. r. t. colour and appearance, flavour, taste and overall acceptability which was compared with control sample and results pertaining are tabulated in Table 3.

It could be revealed from Table 3 that the maximum score was recorded for control sample (8.8) followed by sample S_2 (8.5) which was higher than samples S_1 and S_3 . Sample S_3 recorded lower score among all the samples, because higher dried extract impart RTS brownish colour to the sample, which forces the panel members to rank lower. The control sample had scored higher for flavour followed by S_2 and S_1 . Sample S_3 was significantly inferior over all, because addition of higher proportion of dried extract affects the flavour of the sample. The taste of the samples significantly affected with addition of dried extract. The sample S_2 containing 0.35% adulasa dried extract was found to be statically significant over sample S_1 containing 0.25 and S_3

containing 0.45 per cent leaf dried extract. The control sample with addition of sugar and without addition of dried extract recorded the highest score in all the organoleptic attributes compared to other sample and also found to be significant over other sample. Considering all the above sensory parameters the sample control found to be statistically significant over the all samples. Sample S_2 liked very much having more alkaloid content which will fulfil the daily requirement for diabetic person was selected for further study.

Effect of fully replaced different sweeteners on sensory characteristics of anti-diabetic carbonated RTS beverages :

The sensory evaluation of adulasa dried extract based carbonated RTS beverage with addition of different artificial sweeteners was carried out using nine point hedonic scale with respect to colour, flavour, taste and overall acceptability and compared with control sample and result recorded are presented in the Table 4.

The data in the below Table 4 showed that the maximum score was recorded for control sample (8.8) followed by sample S_2 (8.5) which was higher than S_1 and S_3 . It was indicated that carbonated RTS beverage prepared with sugar and without adulasa leaf dried record high sensory score in all quality attributes as compared to beverage prepared with addition of aspartame, sucralose and stevia sweeteners.

The lowest score recorded by sample S_3 with added stevia (0.3%) and dried extract (0.45%) due to the bitter taste, sedimentation problems and impart some undesirable flavour was affect the acceptability. The amount of stevia added was more than 0.5%. Stevia also gives negative impact on the total dissolved solids of carbonated RTS beverages as reported by Saniah and

Table 4: Organoleptic evaluation of anti-diabetic carbonated RTS beverages with fully replacement of sugar by different artificial sweeteners

Samples	Sensory attributes				Overall acceptability
	Colour	Flavour	Taste		
Control	8.9	8.5	8.9		8.8
S_1	8.3	8.2	8.0		8.2
S_2	8.4	8.3	8.3		8.5
S_3	8.1	7.9	7.9		8.0
S.E.±	0.050	0.0167	0.0272		0.0567
C.D. (P=0.05)	0.1505	0.0502	0.0819		0.1714

Control – With addition of sugar and without addition of dried extract

S_1 – With addition of Aspartame (0.4%) + 0.35% dried.

S_2 – With addition of Sucralose (0.2%) + 0.35% dried.

S_3 – With addition of Stevia (0.3%) + 0.35% dried

Samsian, (2012).

From the Table 4 it was clear that the sample S_2 (8.5) with added sucralose (0.2%) and dried extract (0.35%) ranked best than S_1 (8.2) sample after control sample. Statistically sample S_2 was significantly superior over samples with added dried extract with aspartame or stevia due to sucralose totally soluble in beverage and does not give bitter after taste also not impart unacceptable flavour these all things improve overall acceptability of carbonated RTS beverage.

Organoleptic evaluation of adulasa dried extract based carbonated RTS beverage stored at ambient temperature (30°C) :

On the basis of results of organoleptic evaluation of adulasa dried extract based carbonated RTS beverage, it was clear that sample S_2 having 0.35 per cent adulasa dried extract and 0.2 per cent sucralose as a sugar substitute was organoleptically acceptable and hence it was selected for further storage study. Sample S_2 was filled in sterilized PET bottle and stored upto 90 days for specific time interval of 15 days kept at ambient

temperature (30°C). The data on changes in organoleptic properties are depicted in Table 5.

The data in the Table 5 revealed that there was significant change in sensorial parameters during 90 days storage period. Changes in organoleptic qualities were observed at 15 days interval. It was observed that fresh beverage scored highest score (8.5) as compare to stored beverage. It was clear that colour recorded lowest score (7.8) on 90th days of storage. During storage of beverage from 0 to 90 days there was decrease in sensory score for overall acceptability (8.5) which was found to be at par with fresh sample. However on 90th day of storage, there was significant decrease in sensory score for flavour, taste and overall acceptability (7.0) was observed but liked moderately by the panel members.

This might be due to increase in acidity and development of sour taste to beverage. It could be concluded that adulasa dried extract based carbonated RTS beverage can be stored upto 90 days at ambient temperature without affecting sensorial parameters. Similar results were observed during storage of low calorie beverage was observed by Gaikwad *et al.* (2013).

Table 5 : Organoleptic evaluation of adulasa dried extract based carbonated RTS beverage stored at ambient temperature (30°C)

Sample	Adulasa dried extract based carbonated RTS beverage (S_2)			
Storage days	Colour and appearance	Flavour	Taste	Overall acceptability
0	8.4	8.3	8.3	8.5
15	8.4	8.3	8.2	8.4
30	8.4	8.2	8.1	8.3
45	8.3	8.0	8.0	8.2
60	8.2	7.8	7.5	8.0
75	8.0	7.0	7.1	7.6
90	7.8	6.6	6.6	7.0
S.E. \pm	0.0356	0.0252	0.0504	0.0371
C.D. (P=0.05)	0.1017	0.0759	0.1517	0.1117

Table 6 : Organoleptic evaluation of adulasa dried extract based anti-diabetic carbonated RTS beverage stored at refrigeration temperature (4°C)

Sample (S_2)	Adulasa dried extract based carbonated RTS beverage			
Storage days	Colour and appearance	Flavour	Taste	Overall acceptability
0	8.4	8.3	8.3	8.5
30	8.4	8.3	8.2	8.4
60	8.3	8.2	8.1	8.3
90	8.1	8.0	7.9	8.1
120	8.0	7.8	7.5	7.7
150	7.9	7.4	7.1	7.4
180	7.8	6.6	6.6	7.0
S.E. \pm	0.0371	0.0504	0.0356	0.0371
C.D. (P=0.05)	0.1117	0.1517	0.1073	0.1117

Organoleptic evaluation of adulasa dried extract based anti-diabetic carbonated RTS beverage stored at refrigeration temperature (4°C) :

The sensory evaluation of selected carbonated beverage (S₂) was further carried out for storage study of 180 days at refrigerated condition. The different sensory attributes like colour and appearance, taste, flavour and overall acceptability were evaluated by panel members.

The data in the Table 6 revealed that there was slight change in sensorial parameters of sample stored at refrigeration temperature (4°C) for 180 days. Changes in organoleptic qualities were observed at 30 days interval. It was observed that fresh beverage scored the highest score (8.5) as compared to stored beverage. From the Table it 6 was clear that there was slight variations in taste of the beverage (8.3 to 6.6) was observed during the storage period of 180 days.

During storage of beverage from 0 to 180 days there was decrease in sensory score for overall acceptability was found from 8.5 to 7.0 on 180th day of storage. There was significant decrease in sensory score for flavour, taste and overall acceptability were reported by the panel members. There was no significant evidence of microbial spoilage. It could be concluded from the table that adulsa dried extract based carbonated RTS beverage can be stored for 180 days at refrigerated temperature (4^o C) without affecting sensorial parameters. However its acceptability score was slightly decreased and liked moderately. Similar results were reported during storage of low calorie beverage by Gaikwad *et al.* (2013).

Conclusion :

It can be concluded that adulasa dried extract is suitable for the preparation of anti-diabetic beverage. The alkaloids content of carbonated RTS beverage increases with increase in proportion of adulasa dried extract. Carbonated RTS beverage with 0.35 per cent adulasa dried extract and sucralose (0.2 %) as artificial sweetener was most organoleptically accepted by panel members having alkaloids content of 210mg/200ml which can fulfil the daily requirement for diabetic persons as per daily-recommended dose of alkaloids content (191.25mg). Prepared beverage can be stored for 90 and 180 days at ambient and refrigeration conditions without affecting organoleptic quality of beverage.

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