

DEVELOPMENT OF AN HERBAL SYRUP USING PROSOPIS CINERARIA BARK

Aarti Sipani¹ and Daisy Sharma²

¹M.Sc Food Nutrition and Dietetics, Assam down town University, Guwahati (Assam)

²Assistant Professor, Dept. of Food Nutrition and Dietetics, Assam down town University

E-mail : aartisipani27@gmail.com

Phone no. : 7399338930

ABSTRACT

The present study was undertaken with an aim of developing an herbal syrup using *Prosopis cineraria* (L.) Druce stem bark. The plant is considered important medicinal plant and is used traditionally to cure various ailments and possess several pharmacological properties. Two formulations were developed from the bark of *Prosopis cineraria*, one consisted only the juice extracted from the bark as the main ingredient while the other consisted equal amount of bark juice as well as juice of *Garcinia pedunculata*. Acceptability trials of both the samples were conducted by semi – trained panels and both the samples were accepted for further analysis. Storage of both the samples were done by keeping the syrups in airtight plastic and glass containers at room temperature. Assessment of moisture content, DPPH free radical scavenging activity, total phenolic content were carried out and the result obtained was 6.8 % w/w, 76.53 ± 6.16 IC₅₀ value, 0.3616 mg GAE/g, respectively. The phytochemical screening of the methanolic extract revealed the presence of alkaloids, tannin, flavonoids and phenols.

Keywords – *Prosopis cineraria*, Stem bark, Antioxidant, Medicinal plant, *Garcinia pedunculata*

INTRODUCTION

Plants are considered to be one of the best source of medicine across the world. Presence of various antioxidants is the main reason for the therapeutic potential of the herbal plants. (Pourmorad *et.al*, 2006). Ayurveda is considered as the traditional herbal medical system of India with years of experience and good support. In ancient literature ayurveda mentioned a plant *Prosopis cineraria* as an important indigenous plant which possess various clinical properties (M.D Ukani *et.al*, 2000). The plant, *Prosopis cineraria* is commonly known by the name, Khejri and generally grows in dry and arid areas such as regions of Arabia and some dry states of India chiefly Rajasthan, Gujarat, Haryana, Punjab, Western Uttar Pradesh and in drier regions of Deccan. It is known by different names in different areas such as Janti and Chonksa (Delhi), Jhind, Jhand (Punjab and Haryana), Banni (Karnataka), Sumri (Gujarat), Kandi (Sindh) and Khejri (sanskrit) (Soni L.K *et.al*, 2018). Further it is also called as the ‘wonder tree’ or the ‘golden tree’ of the Indian deserts (Liu Y, *et.al*, 2012).

Prosopis cineraria species plays an important role as folk medicine in the indigenous system of medicine for several health problems. It possess various pharmacological properties such as analgesic and antipyretic action, antibacterial, antihyperglycemic,

antioxidant activity, antidepressant, antihyperglycemic activity, antitumour, anticonvulsant, vasodilatory, etc activities (Pareek A.K *et.al*, 2015). According to Burkart (1976), Sweetish bark of *Prosopis cineraria* was used as food to spare many lives during India's Rajputana famine (1868 – 69). It was grounded into flour and made into cakes. Bark is useful in the treatment of Rheumatism, cough, common cold, anthelmintic disorder, dysentery, bronchitis, asthma, leucoderm piles and tremors of the muscles (Pareek A.K *et.al*, 2015)

Syrups are considered as the best of all liquid oral formulation. Syrups are defined as aqueous concentrated preparation of a sugar or sugar substitutes. In addition flavouring agent and medicinal substance can also be added in its preparation (Balaji P, 2013). Due to their sweet taste they provides a good introduction to the world (Gladstar R, 1993). There are number of medicinal plants in traditional system of medicine which are useful for number of ailment and a number of remedial preparations can be prepared from. Hence a study was conducted on “Development of an herbal syrup using *Prosopis cineraria* bark” and the herbal syrup is developed using dried powder decoction of bark of *Prosopis cineraria* to validate its folkloric uses.

MATERIALS AND METHODS

Selection of samples

Bark of *Prosopis cineraria* and *Garcinia pedunculata* (thekera) were selected for the present study due to their easy availability, accessibility and large content of antioxidants and most importantly high therapeutic properties. Bark of *Prosopis cineraria* were procured from Bikaner district of Rajasthan and *Garcinia pedunculata* were collected from the local markets of Guwahati.

Formulation of syrup using the bark of *Prosopis cineraria*

The barks of *Prosopis cineraria* and *Garcinia pedunculata* were first cleaned, washed and then the barks were dried in oven and turned into powder using grinder. To extract the juice of bark powder, the powder was soaked in water for 24 hours. Two formulations of syrup were developed by using the bark of *Prosopis cineraria* to enhance the quality, taste and flavor. First formulation consisted of only the bark of *Prosopis cineraria* as main ingredient and ginger juice was added to enhance the flavor where as the second formulation consisted of equal amount of juice of bark of *Prosopis cineraria* as well as *Garcinia pedunculata* juice. Both the syrup were formulated using sugar syrup, prepared by the application of heat using 1:1 ratio of sugar and water.

The preparations of two formulations are discussed along with the given table:

SAMPLE CODE	INGREDIENT USED				ADDITIONAL INGREDIENT
	SUGAR (g)	WATER (ml)	BARK JUICE (ml)	GARCINIA PEDUNCULATA JUICE (ml)	GINGER
S1	100	100	100	-	10 ml

S2	100	100	50	50	-
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Table – 1 : Formulations of different syrups

Sensory evaluation

For evaluating the acceptability of the developed formulations score card method were selected. A score card was made consisting a table utilizing the Hedonic ratings of nine point scale (Peryam and Pilgrim, 1957) from like extremely to dislike extremely. The hedonic rating test was used to measure the consumer acceptability of food products where two samples were served to the panelist at one session and asked to rate the product based on the qualities taken into consideration such as colour, appearance, taste, flavor, consistency and overall acceptability.

Physico chemical analysis

- (a) Determination of moisture - Moisture content of the samples was determined following the A.O.A.C (2000) method. Five grams of samples in triplicates were placed in pre – dried and weighed in aluminium dish spreading as thinly as possible over the base of dish and oven dried at 105° C for 1 hour, cooled in a dessicator and weighed. Continued drying until a constant weight has been reached and the moisture content was calculated from the weight loss of the sample.

$$\text{Moisture} = \frac{\text{Difference in weight}}{\text{Weight of the sample}} \times 100$$

- (b) Determination of DPPH free radical scavenging assay - Radical scavenging activity of plant extracts were determined by colorimetric assay using DPPH (2,2diphenyl + picrylhydazyl) radicals source of free radical on the basis of method of Blois. A solution of 0.1 mM of DPPH radical solution was prepared in Methanol. In a clear 96 well plate 100µl of standard (ascorbic acid) or sample in various concentration (1-100µg/ml) and 100µl methanol/water was transferred and then 200µl of DPPH solution was added. The reaction mixture were left for 30 minute at room temperature in dark. The absorbance of each 96 well was measured at 517nm in Thermo Multiskon reader. The activity is stated as percent DPPH scavenging and calculated as

$$\% \text{ DPPH scavenging} = \frac{\text{control abs} - \text{sample abs}}{\text{control abs}} \times 100$$

where, Abs = Absorbance
 (Blois MS, 1958)

- (c) Preliminary phytochemical screening - Phytochemical screening was carried out for all the extracts to confirm the presence of phytoconstituents for alkaloids, saponins, phenols, flavonoids and Tannins.

Test for Alkaloid - Dragendroff's Test: About 0.5 g of the sample was warmed with 2ml of 2% H_2SO_4 solution on water bath for two minutes. After that the solution was filtered and few drops of Dragendroff's reagent were added. Orange red precipitate confirms the presence of alkaloids.

Test for Saponin - Foam test : About 0.2 g of sample was shaken with 4 ml of distilled water and then heated to boil. Appearance of creamy small bubbles or honey comb structure indicates the presence of saponin.

Test for phenol - Ferric chloride test : About 0.5 g of sample was added to 1ml of 10% FeCl_3 solution, a deep bluish green colouration indicated the presence of phenol.

Test for Flavonoid - Lead acetate test : About 2.0 g of sample was taken and dissolved in dilute NaOH solution after that HCL solution was added. A yellow solution turned into colourless which indicated the presence of flavonoid.

Test for Tannin - Ferric chloride test : About 0.5 g of sample was mixed with 2ml of water and heated on water bath. The mixture was filtered and 1 ml of 10% FeCl_3 was added to the filtrate, a dark green solution indicate the presence of tannin.

- (d) Determination of total phenolic assay - Folin- Ciocalteu method was used to determine the total phenolic content (TPC). An aliquot (least amount) (1 mL) of extracts or standard solution of gallic acid (100, 200,300,400 and 500 $\mu\text{g/mL}$) was added to 25 ml of volumetric flask, containing 10 ml of decontaminated water. A blank reagent using distilled water was prepared. 1 mL of Folin-Ciocalteu phenol reagent was taken and added to the mixture and shaken. After 5 mins 10 mL of 7.5% Na_2CO_3 solution was added to the mixture and the volume was then adjusted up to the mark. Upon completion of incubation for 30-45 minutes in room temperature, the absorbance against the reagent blank was determined at 760 nm with an UV- Visible (Shimadzu Japan). The total phenolic content of the extract was expressed as mg gallic acid equivalents (GAE).

Storage study

Shelf life or storage study was done to access the overall hygiene maintained during the process of preparation of formulated product. For that sensory evaluation and microbial study were done. Both the formulations were stored in sterilized glass and plastic bottles for one months and its quality parameters that is colour, appearance, flavor, etc were studied and the viable plate count method was used to determine the colony present in the product.

RESULTS AND DISCUSSION

Acceptability trial

The overall acceptability mean score of sample – 1 which consist of only bark powder was found to be 7.7 whereas the same for the sample – 2 was 8.26 as showed in Table - 2. According to the study of Egbekun *et.al*, 1996 sensory evaluation of taste, flavor and overall acceptability between *V. doniana* syrup and honey showed no significant difference and both were acceptable. Since there is not much difference in the acceptability score of both the formulations in the present study and both are acceptable, therefore based on the acceptability of sensory attributes both the formulations were selected for further analysis.

SAMPLE	COLOUR	APPEARANCE	TASTE	FLAVORS	CONSISTENCY	OVERALL ACCEPTABILITY
1	7.23±0.73	7.76±0.77	7.53±0.89	8.20±0.80	7.50±0.73	7.70±0.65
2	7.83±0.83	7.73±0.944	8.20±0.99	7.46±1.04	7.80±0.84	8.26±0.82

Table – 2 : Mean acceptability score of formulated products

Moisture content

In the present study the loss of drying was found to be 6.8 % w/w. Other researchers also determined the moisture content of the stem bark of *P.cineraria*. Pathak V and Kumar P. (2017) reported 4.0856% of moisture content, Vyas *et.al* (2017) reported 3.20% and Singh S (2019) reported 7.70%. Since the present study data is found to somewhat nearby to other researchers. Hence, the data of the study is considered to be in agreement.

DPPH radical scavenging assay

Radical scavenging activity of methanol extract of the bark of *Prosopis cineraria* was compared with the standard solution of ascorbic acid. As it is shown in fig-1 and fig-2 the IC₅₀ value of ascorbic acid was found to be 8.0202±0.099 and quadratic regression equation was $y = 4.904 \times + 10.47$ $R^2 = 0.986$ whereas the IC₅₀ value of bark of *Prosopis cineraria* was found to be 76.53±6.16 and its quadratic regression equation was $y = 0.157 \times + 38.69$ $R^2 = 0.970$ which revealed the significant free radical scavenging activity of the methanol extract of the bark of *Prosopis cineraria*. According to the study of Singh S (2019) the DPPH radical scavenging activity of methanolic extract of the stem bark of *Prosopis cineraria* was found to be 85.78% at 1000 µg/ml and 74.36% to 4.74% at 500 µg/ml to 10 µg/ml. This result is found to be nearby to the data of present study.

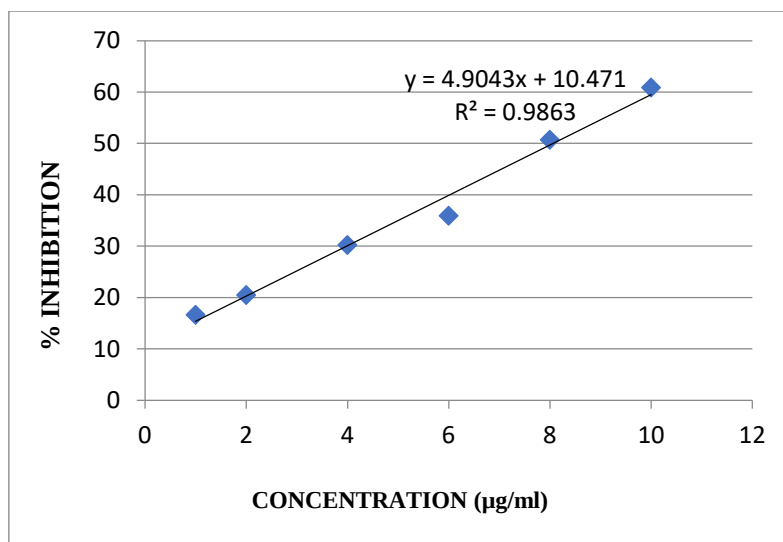


Fig – 1 : Quadratic regression equation for IC₅₀ (µg/ml) values of ascorbic acid

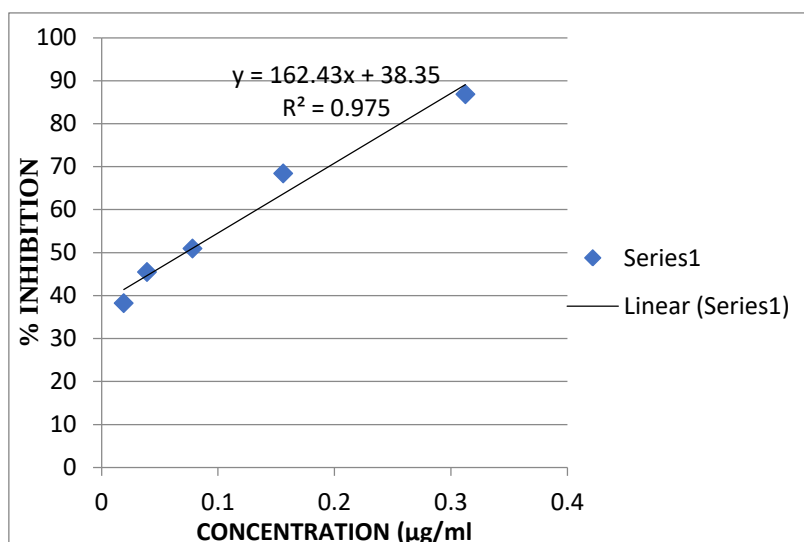


Fig – 2 : Quadratic regression equation for IC₅₀ (µg/ml) value of methanol extract of bark of *Prosopis cineraria*

Preliminary phytochemical screening

Phytochemical screening was carried out for methanolic extracts of stem bark to confirm the presence of phytoconstituents for alkaloids, saponins, phenols, flavonoids and Tannins and the finding of the present study is shown in Table-3 which revealed the presence of Alkaloids, Phenol, Flavonoids and Tannin whereas saponin was absent. A study by Vyas *et.al* (2017) also reveals the presence of alkaloids, flavonoids, and tannins and absence of saponins. Therefore the data found in the present study is in concordance with other studies.

Sl no.	EXPERIMENT	OBSERVATION	RESULT
1	Alkaloids – Dragendroff’s test	Orange red precipitate formed	Present
2	Saponin – Foam test	No honey comb like structure formed	Absent
3	Phenol – Ferric chloride test	Deep – bluish colour was seen	Present
4	Flavonoid – Lead acetate test	Solution turned colourless	Present
5	Tannin – Ferric chloride test	Deep – bluish colour was seen	Present

Table – 3 : Preliminary phytochemical screening of methanol extract of bark of *P cineraria*

Total phenolic content

The total phenol in methanol extract of the stem bark of *Prosopis cineraria* by using Gallic acid as a standard is shown in the figure-3 which depicted the absorbance of Gallic acid by plotting the absorbance against different concentrations ($\mu\text{g/ml}$). The quadratic regression equation of the curve obtained was $y = 0.268x + 0.309$, $R^2 = 0.895$ and the total phenolic (mg GAE/g) was found to be 0.3616 mg GAE/g.

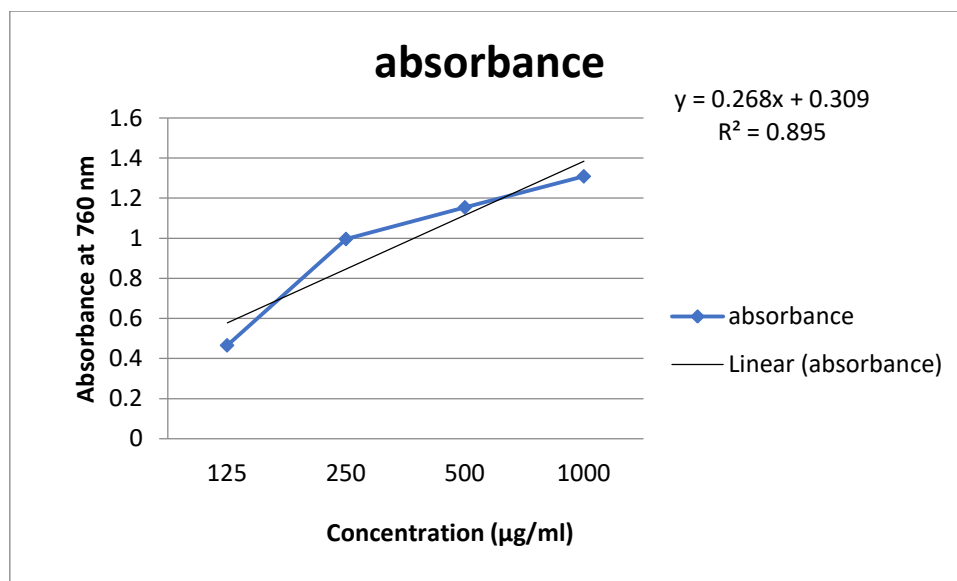


Fig – 3 : Curve of Gallic acid absorbance for determination total phenol content in methanol extract

Storage and microbial colony study

The shelf life of the formulated syrups were studied by storing the products in the air tight glass and plastic containers for a period of 30 days. During storage for 30 days the score of Sample – 1 stored in glass bottle for colour, flavor, taste were decreased through the storage period but not became unacceptable, the mean acceptability score of overall acceptability dropped from 7.70 to 7.36, whereas the other three samples that are Sample -1 stored in plastic bottles, Sample – 2 stored in glass bottle and Sample -2 stored in plastic bottle became unacceptable. According to the study of Thamilselvi *et.al* (2015) overall acceptability of lime based herbal blended RTS beverages such as control, Lime + Tulsi juice extract and Lime + Arugampul juice extract was found to be 8.3, 8.2 and 8.0.

The score of accepted sample that is sample -1 stored in glass bottle is given in Table – 4 :

SAMPLE	COLOUR	APPEARANCE	TASTE	FLAVORS	CONSISTENCY	OVERALL ACCEPTABILITY
1 (GLASS BOTTLE)	7.00±0.86	7.43±0.43	7.3±0.87	7.360±1.03	7.50±1.13	7.36±0.99

Table – 4 : Mean acceptability score of attributes of accepted formulated product

Microbiological quality of any foods is dependent on number of factors such as raw material and sanitization during preparation and storage temperature of products. The variation of pH and total acidity are possible due to various causes. The microbiological

analysis count of the Sample – 1 of present study stored in glass bottle can be seen that though the microbial colonies was formed but the count was not much and was under acceptable limits specified by FSSAI. During the study of storage of myrtle berries syrup for period of three months showed microbial stability (Mobli M *et,al*, 2016).

CONCLUSION

It is concluded that the present study of the development of a herbal syrup ensure food safety for a precise control over public health risk. From the data presented in the current study it is quite clear that the stem bark posses significant antioxidant and phenolic content so possess redox property and claims to have various pharmacological properties. Study shows that the development and utilization of food products from the stem bark of *Prosopis cineraria* can enhance the overall health of the population as well as prevent various life threatening ailments such as diabetes, hypertension, asthma, bronchitis, cancer, depression, convulsion, fever, cough, etc. Hence the result of the present study can be used as a valuable information for development of new products.

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