Optimization of total polyphenol content and antioxidant activity on prepration of novel bittergourd sweet

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ABSTRACT: The present study on "Optimization of total Polyphenol Content and antioxidant activity on prepration of novel bittergourd sweet." The burfi was prepared form khoa with tha addition of bitter gourd in different ratios and artificial sweeteners. Before preparation of burfi, proximate composition of bitter gourd and khoa was analysed. It was observed that the bitter gourd contains of 92.63% moisture, 0.99% ash, 0.38% protein and 23.87% carbohydrates. Then four type of burfi were prepared with different proportions of bitter gourd and khoa such as: 100% KH (control) 10% BG+90%KH, 15% BG+85%KH, 20% BG+80% KH. On the basis of sensory evaluation, the accepted sample was than analyzed for their proximate composition, total phenolic content and antioxidant activity.

The importance of natural antioxidants for medical and food application is commonly performed through extraction procedures to provide maximum yield of substances and of the highest yield quality by optimization of some process parameters, sample/solvent ratio and type of solvent, time and temperature.

The present study was aimed at to evaluate the antioxidant activity of bitter gourd. The extract of the samples were prepared by maceration process using different organic solvents viz., acetone, ethyl acetate and methanol.

The antioxidant potential of various extracts of test sample was evaluated by1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay. The percent inhibition of various extracts was compared with standard antioxidant used.

In DPPH radical scavenging method, the antioxidants react with the stable free radical DPPH (deep violet colour) and convert it to 1, 1-diphenyl-2-picryl hydrazine with discoloration. The scavenging effects of extract increased with their concentrations to similar extents.

In this assay, it was observe that methanol extract of raw bitter gourd at concentration of 100ug/ml exbibited maximum inhibition (44.38%). So methanol extract of raw bitter gourd is quite effective as an antioxidant agent. Similarly acetone extract of raw bitter gourd significantly reduced the stable DPPH radical with an inhibition of 28.44% at the dose of 100ug/ml.

In bitter gourd burfi, it was observed that ethyl acetate extract at concentration of 100ug/ml exhibited maximum inhibition (60.15%) while acetone extract of bitter gourd burfi significantly reduced the stable DPPH radical with an inhibition of 19.52% at the doses of 100ug/ml. The methanol extract of bitter gourd burfi with 100 ug/ml concentration showed 28.70% inhibition. Hence the results concluded that

ethyl acetate extract of bitter gourd burfi is quite effective as an antioxidant agent.

The total phenolic content was found to be highest in acetone extract of raw bitter gourd (i.e.103.3mg) at a concentration 100ug/ml and acetone extract (109.7 mg at same concentration) of bitter gourd burfi with increasing solvent polarity. The results suggest that extraction by acetone could give higher phenolic content as compared to methanol and ethyl acetate.

The results of the present study support the incorporation of bitter gourd in sweet to make novel food and can commercialize the product. The product is useful for the prevention of cardiovascular and age-related diseases.

Keywords: Bittergourd, polyphenol content and antioxidant activity.

INTRODUCTION

The Bitter gourd is a common vegetable that have medicinal uses also. Its common name is bitter melon, bitter gourd, balsam apple, balsam pear, karela etc. The bitter gourd is cultivated all over India. Bitter gourd grows in tropical and subtropical areas. The family iof bitter gourd is cucurbitaceae and its botanical name is *Momordica* M. *charantia.* The seeds are shite in raw fruits and become red when they are ripe. There are two varieties of this vegetable: the large kind is long, oblong and pale green in colour. The other kind is small, little oval and dark green. Both the types are bitter in taste. They turn reddish orange when ripe. The fruit has a distinct variety looking exterior and an oblong shape. It is hollow in cross-section, with a relatively thin layer of flesh surrounding a central seed cavity filled with large flat seeds and pith. Seeds and pith appear white in unripe fruits, ripening to red; they are not intensely bitter and can be removed before cooking. The pith will become sweet when the fruit is fully ripe, and the pith's color will turn or green bell pepper. The skin is tender and edible. Bitter melon comes in a variety of shapes and sizes. The typical Chinese phenotype is 20-30 cm long, oblong with bluntly tapering ends and pale green in color, with a gently undulating, warty surface. The bitter melon of India has a narrower shape with pointed ends, and a surface covered with jagged, triangular "teeth" and ridges. Bitter melon contains a bitter compound called momordicin that is said to have a stomachic effect.

Bitter gourd is a medicinal plant also having many nutritional components. The proximate composition of bitter gourd is : moisture- 92.4%. It is a good source of minerals and vitamins such as calcium-20 mg, phosphorus-70 mg, iron-1.8 mg, vit.c-88 mg, small amount of vit.B comples. Its calorific value is 25.

The Bitter gourds have many health benefits and medicinal properties. These are such as kills bacteria, reduce inflammation, kill viruses, fights free radicals, kills cancer cells, kills leukemia cells, prevents tumors, cleanses blood, reduces blood sugar and balance hormones. (Leslie Taylor, 2005).

Beside these stem and leaf of bitter gourd is used in cancer treatment, in vital infections (HIV, herpes, Epstein Barr, hepatitis, influenza, and measles), in bacterial infections (Staphylococcus, Streptococcus, and Salmonella), as a bitter digestive aid (for dyspepsia and sluggish digestion) and in diabetes.

Bitter melon contains an array of biologically active plant chemicals including triterpenes, proteins and steroids. Alkaloids, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, genticis acid, goyaglycosides, goyasaponins, guanylate cyclase inhibitors, gypsogenin, hydroxytryptamines, karounidiols, lanosterol, lauric acid, linoleic acid, linolenic acid, momorcharasides, momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordicosides, momordin, multiflorenol, myristic acid, nerolidol, oleanolic acid, oleic acid, oxalic acid, pentadecans, peptides, petroselinc acid, polypeptides, proteins, ribosome-glycosides, stigmasta-diols, stigmasterol, taraxerol, trehalose, trypsin inhibitors, uracil, vaccine, v-insulin, verbascodide, vicine, zeatin, zeatin riboside, aezxanthin, and zeinoxanthin are all found in bitter melon. (Leslie Taylor, 2005).

The bitter gourd has natural benefits and curative properties. It has excellent medicinal value. It is antidotal, antipyretic tonic, appetizing, stomachic, antibilious and laxative. Bitter gourd being rich in all

essential vitamins and minerals, especially vit.A, B1, B2, C and Iron, its regular use prevents many complications such as hypertension, eye complications, neuritis and defective metabolism of carbohydrates. It increases body's resistance against infection. But the main problem in its consumption is its bitter taste. By making a sweet product from bitter gourd, it can be possible to take advantage of its health properties by all age group. Further with the replacement of natural sugar with artificial sugar, calorie content of the product can also be decreased.

Till date, as such no easily consumable, sweet product was prepared from bitter gourd, so our present investigation was carried out with the following objectives:

1. Proximate analysis of bitter gourd.

2. Preparation of a novel sweet by incorporating bitter gourd and artificial sweetener.

3. To study the nutritional value of bitter gourd novel sweet.

4. To study the sensory characteristics of bitter gourd novel sweet to assess their acceptability.

5. To study and compare the total polyphenolic content and antioxidant activity in raw bitter gourd novel sweet.

MATERIALS & METHODS

Fresh whole bitter guard fruit were collected from local markets. The bitter gourd was stored at refrigerated temperature (10+2°C) until required for analysis/burfi preparation. Other raw materials such as artificial sugar (made from malto dextrin and sucralose, these are the low calorie sugar substitute i.e. a diet sugar) and khoa were purchased from local market.

3.1 Materials :

3.1.1 Raw materials :

A list of raw materials utilized is given below.

- Bitter gourds
- Artificial sweeteners
- Khoa
- Sodium chloride (table salt)

3.1.2 Chemicals:

The organic solvents (methanol, acetone, ethyl acetate) were used for extraction. All other chemicals used like DPPH, Gallic acid, Folin reagent, Ascorbic acid etc, in this study were of analytical grade.

3.1.3 Glassware:

Glassware's used for various purpose in this study were of Borosilicate. Before use, all the glassware's were washed with laboratory detergent and rinsed in distilled water.

3.1.4 Instrumets:

- Muffle furnance (Narang Instruments)
- Air oven (Yorko, York Instruments)
- Kjeldahl apparatus (Yorko, Yorko Instruments)
- Soxhlet solvent apparatus
- Crude fiber apparatus
- Electronic weighing balance
- Heating plate
- Refrigerator
- Aluminum trays
- Gas cylinder
- Glass ware and utensils
- Double beam spectrophotometer
- Centrifuge

3.2 Methods:

3.2.1 Processing methods:

3.2.1 (A) Preparation of khoa (KH) burfi (control)

To prepare 100% KH burfi, simple method was followed. Khoa was taken into karahi and heated on slow fire to melt and mix it properly. Then after cooling to a little extent, artificial sweeteners were added and mixed thoroughly. After mixing, sheeting was done and then cutting into pieces was carried out. The burfi was then ready for further analysis.

3.2.1(B) Preparation of Bitter gourd khoa burfi (control)

Simple method was followed for burfi preparation with slight modifications.

Burfi making procedure: Salt Treatment

Salt treatment was given to the bitter gourd to remove out its bitterness. First of all, the bitter gourd was peeled and cut into the small pieces. After this, seeds were removed out from it. Then 10% salt solution was prepared and bitter gourd pieces were soaked in it for 24 hrs. After thorough washing with cold water, blanching was done for 10 min to inactivate the enzymes present in it. The pieces were then again dipped into sugar solution to remove out the remaining bitterness. After this, the pieces were crushed in a mixer.

The khoa was then taken in a kadhai and heated slowly, after some time the khoa melts and the crushed bitter gourd was added to the melted khoa, mixed and cooked for some time. Then after some cooling, artificial sugar was added in it, sheeting was done and placed under refrigeration for cooling. After cooling, cut into the pieces and it is ready for further use.

3.2.2 Chemical Analysis:

The Bitter gourd burfi were analyzed for their chemical compositions. Chemical compositions are determined by AOCAC (1995) standard method.

Moisture

Moisture content was determined by using hot air oven method.

Ash

Ash content was determined by taking 5g of sample in a muffle furnace maintained at 550°C temperature.

Crude fat

Crude fat was determined by using soxhlet extraction method using petroleum ether (60-80°C) as organic solvent.

Crude protein

Crude protein was estimated by Kjeldahl nitrogen estimation method using a factor of 6.31 for conversion of nitrogen into protein.

Crude Fiber analysis

A fat free sample is treated with boiling sulfuric acid subsequently with sodium hydroxide. The residue, after subtraction of ash is regarded as crude fiber. The method for analysis is given below:

Reagents: HCI 1% (v/v), H_2SO_4 stock solution, 10% (w/v), H_2SO_4 working solution, NaOH stock solution 10%, NaOH working solution, Antifoam: 2% silicon, CCl_4, ethanol.
Apparatus: Tall beakers, 1.0L, Crude fiber apparatus, Buncher funnel, Sintered glass crucible, oven, Muffle furnace, desiccators.
Procedure: Weigh 2g fat free dried sample in one liter tall beaker. Add 200ml of hot 1.25% H_2SO_4 and few drops of antifoam. Heat the mixture to boil within on minute on the crude fiber apparatus. Keep the mixtures boiling exactly for 30 minutes under bulb condenser. Filter the contents of beaker through buncher funnel.Wash the sample back into the tall beaker with 200ml of 1.25% NaOH. Bring to boiling point.Boil exactly for 30 minutes. Transfer the matter to sintered glass crucible and wash it with boiling water.Wash with ethanol, two/three times.Dry at 100°C in an air oven to constant weight. Cool and record the weight.Transfer the sintered glass crucible to muffle furnace at 550°C for 1 hrs for ashing.Cool crucible in a desiccators and reweight.

Carbohydrate

The carbohydrates content was determined by difference method (Kik and Sawyer, 1991).

Total phenolic content and Antioxidant activity:

During this study DPPH (1, 1-Diphenyl-2-picrylhydrazyl) essay (Hsu et al.,2006) is used to determine antioxidant activity of bitter gourd plant and bitter gourd burfi extracts. Total phenolic content of plant extracts by Folin Ciocalteu method (Folin et al., 1927) was also determined. The procedure for determination is given below:

(a)Determination of total phenolic content (Folin-Ciocalteu method):

Total phenolics content was determined according to the Folin-Ciocalteu method, using Gallic acid as standards. Extract powders (1 mg) were dissolved in 1 ml 50% methanol solution. Extract solution (0.5 ml) was mixed with 0.5 ml 0f 50% Folin- Ciocalteu

reagent. After 2-5 min, 1.0 ml of 20% Na_2CO_3 was added to the mixture and incubated for 10 min at room temperature. The mixture was centrifuged at 150 g for 8 min and the absorbance of the supernatant was measured at 730 nm. The total phenolic content was expressed as Gallic acid equivalents (GAE) in milligrams per gram sample.

(b)Determination of antioxidant activity (DPPH radical scavenging assay):

Five mg extract powder was dissolved in 5 ml of method solution to obtain 1000 ug/ml sample solution. This solution was serially diluted into 10, 20, 30, 40, 50, 70, 80, 90, 100 ug/ml with methanol. In each reaction, the solutions were mixed with 1 ml of 0.1 mm,1, 1-Diphenyl-2-picrylhydrazyl (DPPH) and 0.05 ml samples at room temperature for 30 min. Methanol solution was used as negative control. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. DPPH is a purple-colored stable free radical; when reduced it becomes the yellow-colored diphenylpicrylhydrazine. L-Ascorbic acid was used as positive control. The antioxidant activity of test samples was evaluated by calculating the percent inhibition of superoxide anion radical by applying the following formula.

% inhibition= $[(A_0-A_1)/A0] \times 100$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance of the extract.

3.3 Sensory Evalution

The different burfi prepared from bitter gourd and khoa with the addition of artificial sugar were subjected to sensory evaluation by a panel of ten members. The prepared burfi was evaluated for colour, appearance, texture, taste and overall acceptability. The Organoleptic score given by judges on a nine point hedonic rating scale is given as:

Rate	Organoleptic score
Very desirable	9
Desirable	8
Moderately desirable	7
Slightly desirable	6
Neither desirable nor undesirable	5
Slightly undesirable	4
Moderately undesirable	3
Undesirable	2
Very undesirable	1

3.4 Statistical analysis

The data were analyzed statistically in a completely randomized design (CRD) using on factor analysis of variance (ANOVA) with the help of OPSTAT.

.RESULTS AND DISCUSSION

The present investigation was carried out on the topic "Optimization total polyphenol content and antioxidant activity on preparation of novel bittergaurd sweet ". The presentation of results and discussion has been arranged under the following headings:

4.1. Proximate composition of khoa and bitter gourd.

4.2. Sensory evaluation of bitter gourd burfi of different ratio

4.3. Proximate composition of bitter gourd burfi in accepted ratio.

4.4. The antioxidant activity of bitter gourd and bitter gourd burfi.

4.5. Total polyphenolic content of bitter gourd and bitter gourd burfi.

Burfi was prepared by incorporation of bitter gourd and artificial sweeteners in khoa.

4.1 Proximate composition of khoa and bitter gourd:

The khoa and bitter gourd was analyzed for various chemical constituents and the results are presented in **Table 1**. The result of analysis of khoa for its proximate composition shows that khoa contain 23.54% water, 0.97% ash, 19.23% protein, 32.15% fat, and 23.87% carbohydrates.

The result shows that bitter gourd contains 92.63% water and 0.38% fat which differ slightly from the values obtained by Kochhar et al (1996) for moisture (93.43%) and fat (0.49%). This may be due difference in variety of bitter gourd. The protein content value of bitter gourd was found 2.20%. The crude fibre content and carbohydrate content of bitter gourd was 0.95% and 2.84% respectively.

Table 1. Promximate Composition Of Khoa and Bitter Gourd

Proximate Composition		
Chemical Constituents	**Khoa**	**Bitter Gourd**
Moisture	**23.54%**	**92.63%**
Protein	**19.23%**	**2.20%**
Fat	**32.15%**	**0.38%**
carbohydrates	**23.87%**	**2.84%**
Ash	**0.97%**	**0.99%**

The result shows that bitter guard is rich in moisture (92.63%) whereas in khoa moisture content is 23.54%. Ash content in bitter gourd and khoa in similar having 0.97% in khoa and 0.99% in bitter gourd. Protein content in is high in khoa (19.23%) in comparison with bitter gourd (2.20%).

4.2 Sensory evaluation of different bitter gourd burfi

The sensory evaluation of the bitter gourd burfi was done by a panel of ten members using nine point hedonic scales. The results of sensory evaluations are represented in **Fig 1.**

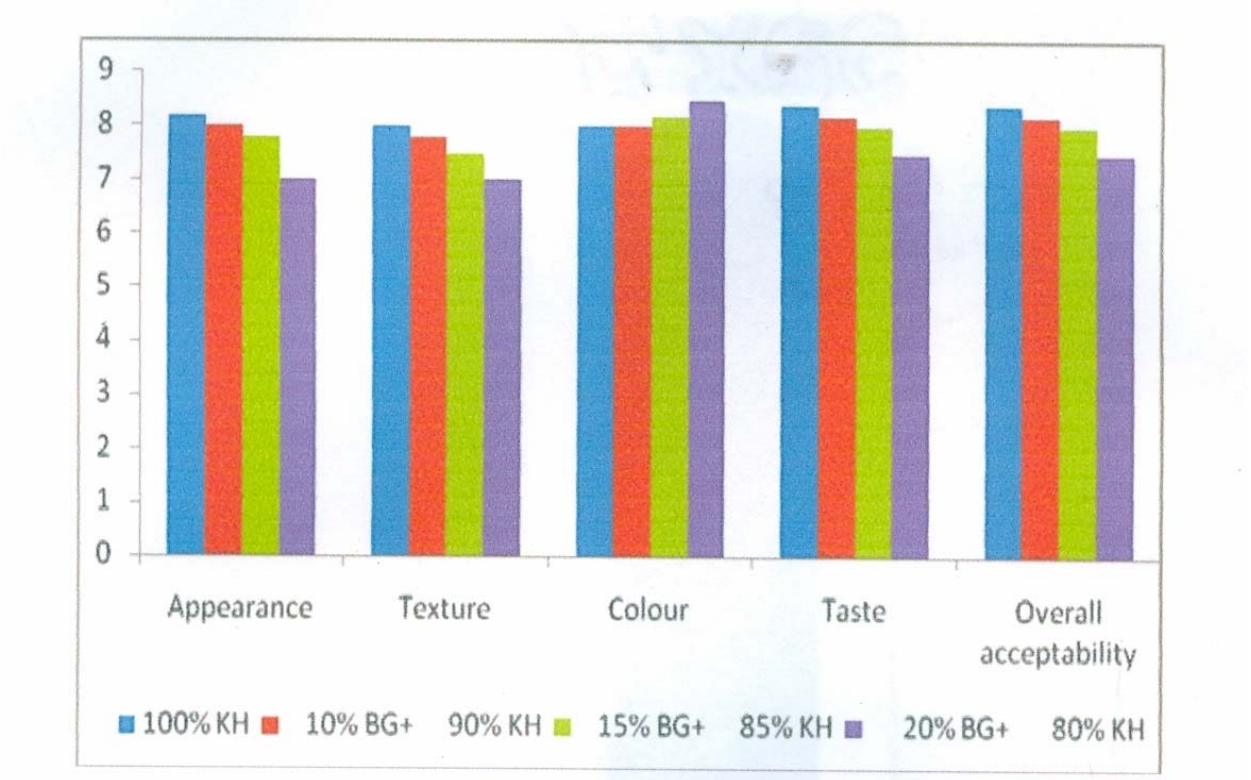

Fig.1 Sensory Evaluation of different bitter gourd burfi in different ratio

The score for appearance of burfi ranges from 7 to 8.2, highest value observed in case of burfi with 100% KH. The appearance of burfi with incorporation of bitter gourd was desirable upto a level of 20%. The score for texture was minimum (7.0) for 20% BG burfi. This can be attributed to the increase in moisture content of burfi with increasing bitter gourd in it, resulting in very soft texture of burfi. The colour of the burfi with 20% (8.5) bitter gourd was good as compare with 15% bitter gourd burfi and 10% bitter gourd burfi. It can be due to presence of bitter gourd in it which imparts a light greenish colour to the the burfi. Taste score value of burfi with 100% khoa burfi and 10% bitter gouard was highest but taste decrease with increasing the concentration of bitter guard was highest but taste decrease with increasing the concentration of bitter gourd. It can be attributed to a little bitter taste of bitter gourd but burfi with 15% bitter guard was 8.0 which is quite good and acceptable.

So, on the basis of sensory evaluation scores, it was concluded that bitter gourd can successfully incorporated in khoa upto a maximum level of 15%. Therefore, the burfi with 15% bitter gourd was found suitable for consumption and carried for further analysis.

4.3 Proximate composition of bitter gourd burfi in accepted ratio

The results of 15% bitter gourd burfi analysis are shown in **Table 2**. The result shows that burfi with 15% bitter gourd contain 26.71% moisture, 0.97% ash, 31.31% fat, 17.62% protein and 22.45% carbohydrates. The composition of 100% khoa (KH) burfi contain 22.90% moisture, 0.97% ash, 31.59% fat, 19.23% protein and 25.79% carbohydrates.

Chemical Constituents	**100% KH**	**15%Bitter gourd**

Moisture	**22.90%**	**26.71%**
Ash	**0.97%**	**0.97%**
Fat	**31.59%**	**31.31%**
Protein	**19.23%**	**17.62%**
Carbohydrate	**25.79%**	**22.45%**

Table 2: Proximate Composition of Khoa Burfi(100%) and Bitter gourd burfi(15% bitter gourd)

Moisture content was increased with addition of bitter gourd due to high moisture content originally present in bitter gourd. Protein content of bitter gourd burfi was decreased with addition of bitter gourd due to low protein content present in bitter gourd.

4.4 Antioxidant activity of bitter gourd and bitter gourd burfi (DPPH assay):

Bitter gourd was extracted with different solvent by maceration method on order of solvent polarity. The different concentrations of all the extracts viz. 10, 20, 30, 40, 50, 60, 70, 80, 90, 100ug/ml were used in the antioxidant assays and all the experiments were performed in triplicate.The present study was carried out to evaluate the antioxidant activities of various extracts of bitter guard and bitter gourd burfi by DPPH scavenging assay. L-ascorbic acid was used as positive control. L-ascorbic acid is a well known antioxidant compound as shown in **Fig.2**

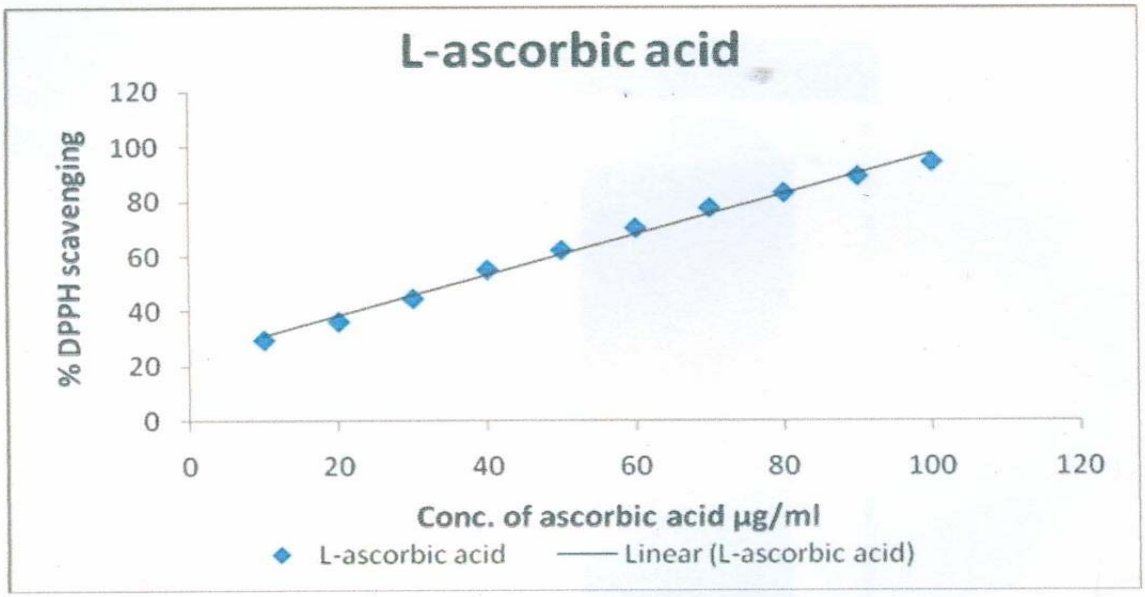

Fig.2 .Standard curve of Ascorbic acid depicting scavenging of DPPH radical.

The results observed in this study showed that the extracts and their refrence chemicals were able to reduce the stable radical purple DPPH to yellow coloured diphenyl picryhydrazine. The degree of discoloration indicates the scavenging potential of the extracts. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Baumann *et al*, 1979).

The different concentrations of methanol, acetone, and ethyl acetate extracts of bitter gourd exhibited different levels of antioxidant activity. Increasing concentration of different extracts of various plant parts lead to the increase in antioxidant activity. In this assay, it was observed that methanol extract of raw bitter gourd at concentration of 100ug/ml exhibited maximum inhibition (44.38%) (**Fig.3**).

Also methanol extract of raw bitter gourd at other concentrations i.e. 10,20,30,40,50,60,70,80,90 ug/ml showed marked DPPH scavenging activity in term of 16.53, 18.25, 19.10, 19.36, 19.45.20.05.21.42, 22.27 and 24.24% inhibition respectively (**Fig.3**). Similarly acetone extract of raw bitter gourd significantly reduced the stable DPPH radical with percentage inhibition of 17.05, 20.41, 21.68, 22.79, 23.82, 24.47, 25.27, 25.53, 26.73 and 28.44 at the doses of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ug/ml respectively. The ethyl acetate extract of raw bitter gourd with same concentrations showed 20.42, 21.68, 21.93, 22.53, 23.05, 23.73, 24.67, 27.33 and 28.07% inhibition respectively (**Fig.3**).

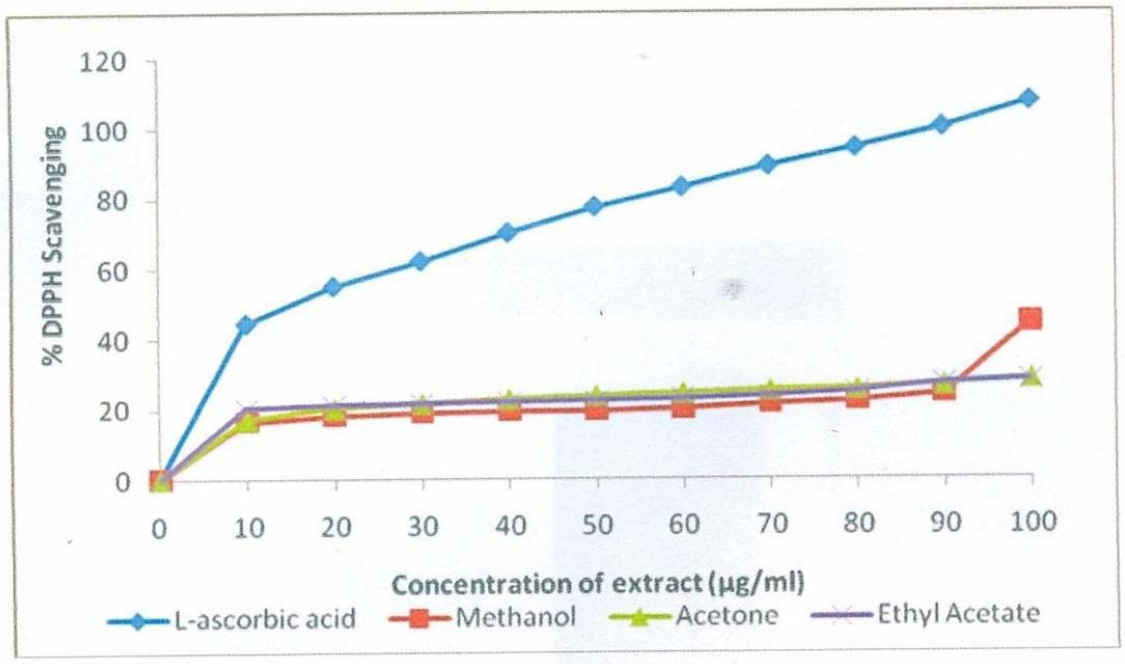

Fig 3 Scavenging of the DPPH radical by different extracts of bitter gourd by *DPPH* scavenging assay.

Hence from the results, it can be concluded that methanol extract of raw bitter gourd is quite effective as an antioxidant agent.

In bitter gourd burfi it was observed that ethyl acetate extract of bitter gourd burfi at concentration of 100ug/ml exhibited maximum inhibition (60.15 %). Also ethyl acetate extract of bitter gourd burfi at other concentrations i.e. 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ug/ml showed marded DPPH scavenging activity in term of 19.60, 23.73, 26.22, 26.22, 26.73, 26.30, 26.39, 17.90 and 24.07% inhibition respectively. Similarly acetone extract of bitter gourd Burfi significantly reduced the stable DPPH radical with percentage inhibition of 14.31, 16.28, 17.77, 18.33, 19.2, 19.45, 19.02, 19.62, 19.62, and 19.53 at the doses of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ug/ml. The methanol extract of bitter gourd burfi with same concentrations showed 13.79, 16.28, 16.79, 17.30, 18.76, 18.42, 18.49, 19.10, 19.96 and 28.70% inhibition respectively (**Fig.4**). Hence the results concluded that ethyl acetate extract of bitter gourd burfi is quite effective as an antioxidant agent.

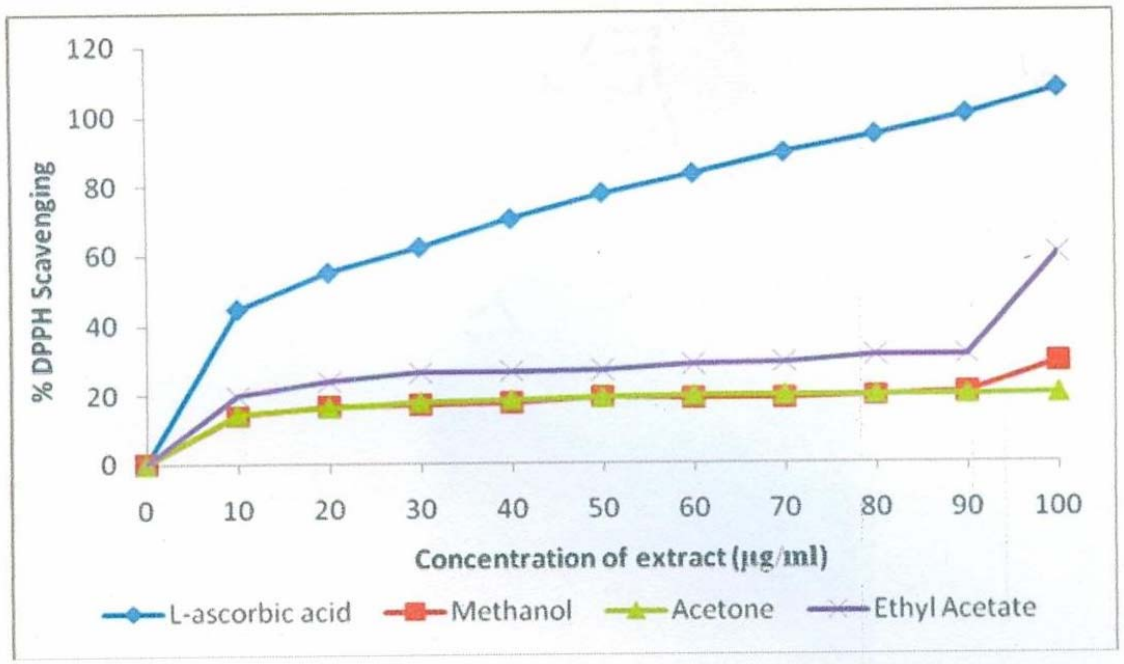

Fig.4 Scavenging of the DPPH radical by different extracts of bitter gourd product *by DPPH* scavenging assay.

Rezaeizadeh et al., (2011) reported that the inhibition percent of methanolic extract of bitter gourd was significantly higher than the inhibition percent of chloroformic extract in the FTC and TBA methods. Methanolic extract contained a significantly higher concentration of tatal phenols and flavonoids when compared with chloroformic extract. Methanolic extract also contained more potent antioxidant and high polyphenol compounds as compared with chloroformic extract. The present study, confirmed that, the type of sovent has an important role in detecting plant compounds. The natural plant antioxidants and phenolics compounds in bitter gourd have the capability of being used in food systems to preserve food quality.

4.5 Determination of total phenolics in bitter gourd and bitter gourd burfi:

Total phenolcompounds, as determined by Folin Ciocalteu method (Mc Donald et al., 2001), were reported as galic acid equivalents by reference to standard curve (y-0.019x, R^2 = 0.056) as shown in **Fig. 5**. Gallic acid being the most important polyphenol in natural products was used to determine the phenolics of tested plant i.e. bitter gourd.

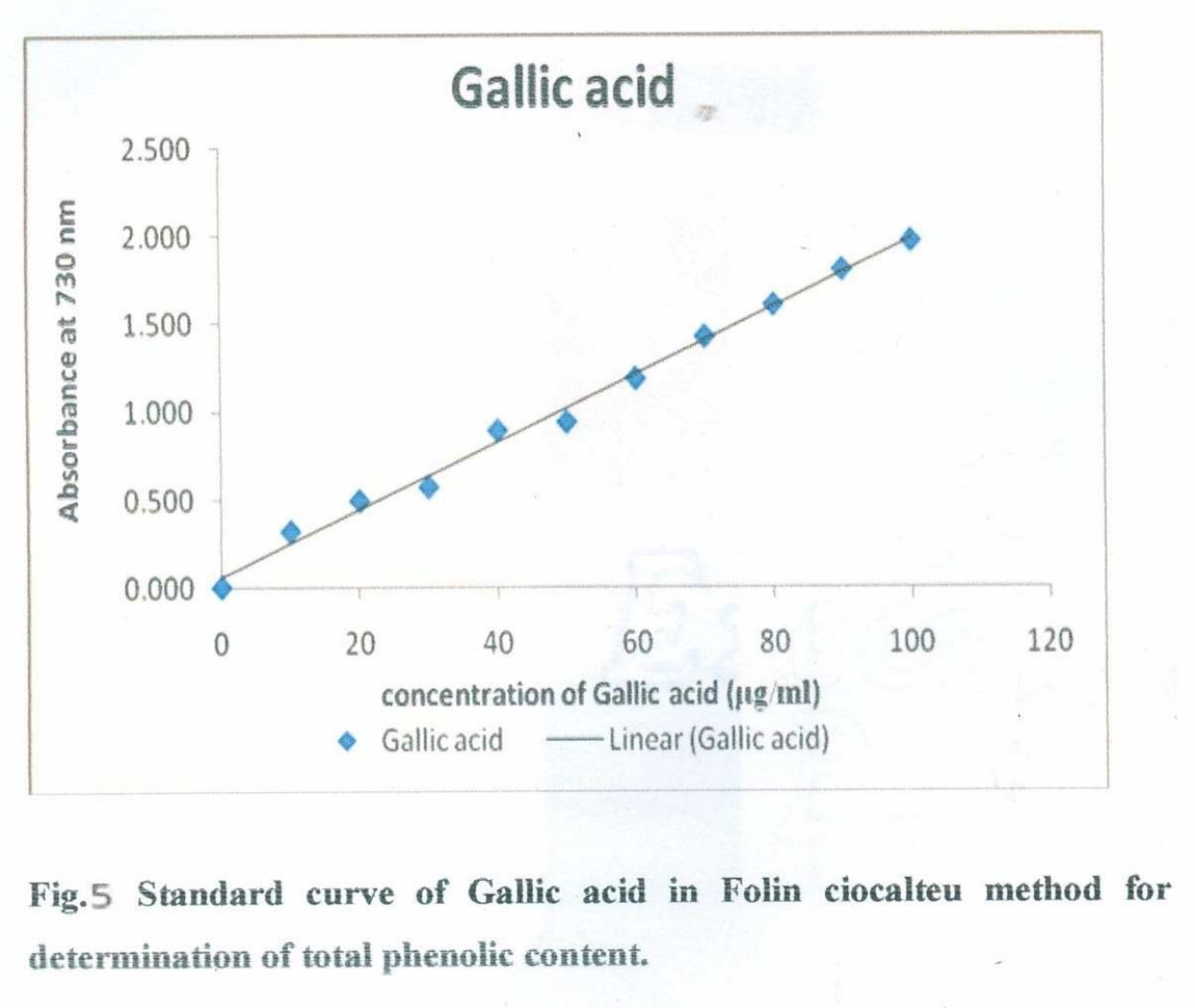

Fig.5 Standard curve of Gallic acid in Folin ciocalteu method for determination of total phenolic content.

Fig 6 and **7** shows the total phenolic contents extracts (i.e. methanol, acetone and ethyl acetate) of bitter gourd and bitter gourd product. In Bitter gourd, the total phenolic content was found to be highest in acetone extract (i.e.103.3 mg) at a concentration of 100ug/ml. Also acetone extract of raw bitter gourd at other concentrations i.e. 10, 20, 30, 40, 50, 60, 70, 80, 90 ug/ml. showed phenolic content in terms of 24.8, 51.6, 66.4, 69.5, 79.1, 84.8, 86.0, 87.5, and 89.2 mg/g, as gallic acid equivalent (GAE) respectively (**Fig.6**). Similarly methanol extract of raw bitter gourd shows significant increase in the total phenolic content such as 21.63, 40.89, 37.89, 38.89, 41.6, 41.6, 43.3, 45.7, 46.3, 47.4 mg/g, as Gallic acid equivalent (GAE) at the doses of 10, 20, 30, 40, 50, 60, 70, 80, 90 ug/ml. respectively. The ethyl acetate extract of raw bitter gourd with same concentrations showed 18.7, 22.1, 23.1, 24.6, 26.4, 26.4, 26.7, 27.5, 29.5, 31.0 mg/g as Gallic acid equivalent (GAE) respectively.**(Fig.6)**

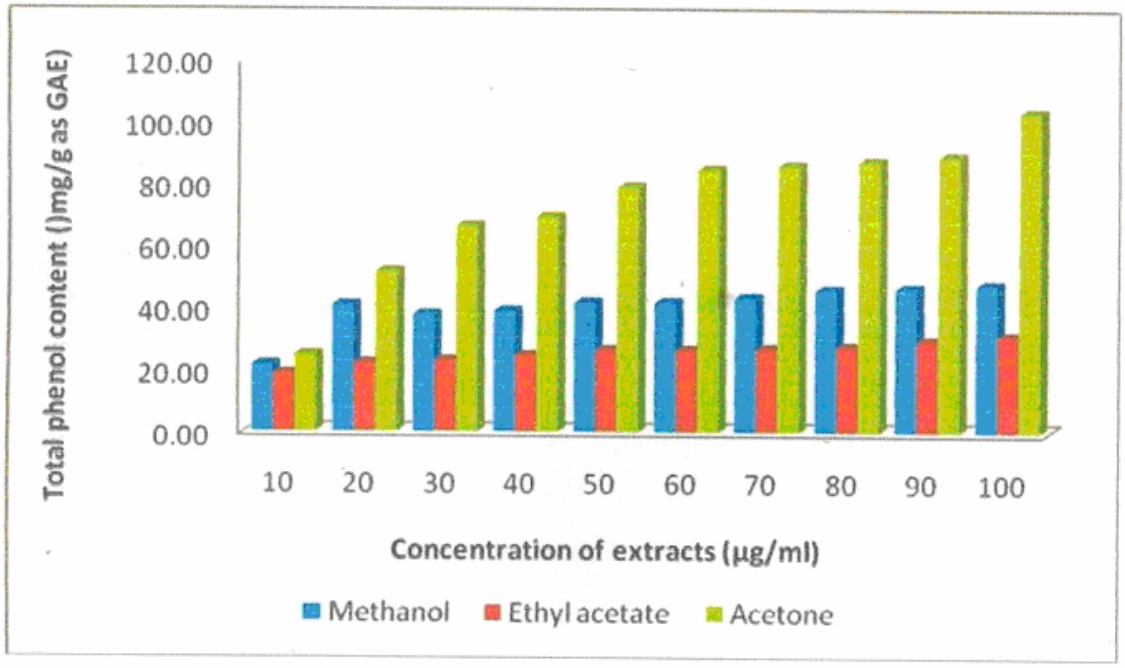

Fig. 6 Total phenol content(TPC) of different extracts of bitter gourd,mg/g, as gallic acid equivalent (GAE).

In bitter gourd burfi, the total phenolic content was found to be highest in acetone extract (i.e.109.7 mg) at a concentration of 100ug/ml **(Fig.7)**. Also acetone extract of raw bitter gourd at other concentrations i.e. 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ug/ml. showed phenolic content in terms of 34.7, 35.5, 36.0, 51.6, 52.4, 60.7, 76.0, 61.7, 81.7 and 81.7 mg/g, as Gallic acid equivalent (GAE) respectively **(Fig.7).** Similarly methanol extract of raw bitter gourd significantly increase the total phenolic content of 17.1, 18.6, 20.6, 21.6, 22.2, 22.6, 24.3, 25.6, 26.5, 29.1 mg/g, as Gallic acid equivalent (GAE) at the doses of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ug/ml. respectively. The ethyl acetate extract of raw bitter gourd with same concentrations showed 16.6, 17.4, 17.4, 19.1, 9.4, 20.0, 20.4, 21.0, 9.4, 20.0, 20.4, 21.0, 21.2,21.9mg/g as gallic acid equivalent (GAE) respectively.

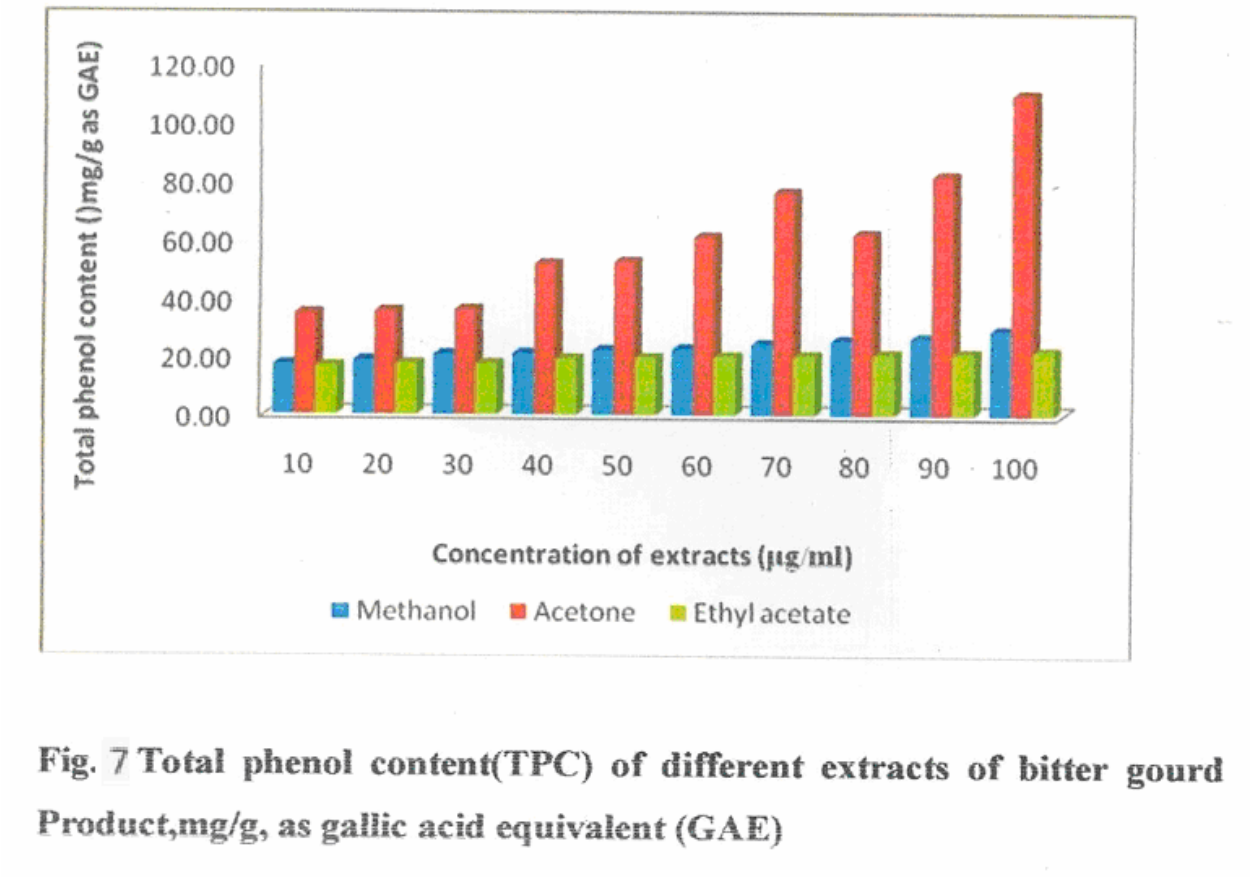

Fig. 7 Total phenol content(TPC) of different extracts of bitter gourd Product,mg/g, as gallic acid equivalent (GAE)

Apart from that, the results also suggest that extraction by acetone could give higher phenolic content as compared to methanol and ethy1 acetate. The findings were likely in agreement with Sun and Ho (2005) who discovered that extracting solvent significantly affected the yield of phenolic content of buckwheat extract. Therefore, this work also shows that different extracting solvents influenced different yields of TPC in present study. High dissolubility of phenolics in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction (Zhou, 2004).

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