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Research Article

EVALUATING THE NUTRITIVE PROPERTIES OF MIXED PLANT DERIVED PRODUCTS WITH AND WITHOUT SOYAMILK FOR PHARMACOLOGICAL USAGE

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ABSTRACT

Objective: Normally this day's nutritive products are available, but most of them are synthetic made materials which have low quality ingredients and for storage purpose it is coated with phenolic compounds. It is not at all harmful, though it is not fully absorbed by our body receptors in intestine and way too costly the products are these days. To compensate this issue we have evaluated the nutritive properties of mixed plant derived products with and without soyamilk.
Methods: Natural existing products like *Moringa olifera* leaves, spinach leaves, Colocasia fruit part were obtained from the concerned source and they were powdered and crude extract are used to mix along methanol extract. This extract is used for estimation of antioxidants, phenols, vitamins and carbohydrate. Later extract is dissolved in soyamilk, the studies of interaction and various biochemical methods are carried out.
Results: These studies showed the presence of phenols were higher in the soya milk which makes it contiguous and stagnant.
Conclusion: Comparison studies are made between soyamilk after mixing the extract in multiple standards.

Keywords: Nutritive products, Soyamilk, Moringa Olifera, Carbohydrate.

INTRODUCTION

The lack of proper nutrition in the country which is mainly the deficiency of the protein and nutrients in the diet of the individuals is due to the financial crisis. The rich source of the nutrient is the plant and animal source have very high costs so it is difficult to afford them therefore the multiple micronutrient deficiencies are much more probable then the singular nutrient deficiencies[1].Soymilk has a creamy white and aqueous appearance and its extract is prepared from soybean and is very much similar in consistency and appearance to cow milk[2].It is extremely nutritious and is a rich source of a large number of essential nutrients which are crucial for our diet such as carbohydrates, protein, fat, vitamins and minerals[3]. Because of affordable price and this nutritional value, soymilk is considered as an active ingredient in the diet of the people in most of the developing countries.

Due to decreased financial status states recently the use of soymilk has considerably increased and as it an excellent alternative to cow milk for lactose intolerant people and is strongly recommended for them as it is deficient in lactose [4].In addition to this it has other advantages over cow milk as it prevents a lot of fatal diseases like osteoporosis, protein energy malnutrition in children and kidney disorders. These advantages has let to its widespread use in Africa than the advantages soymilk also has certain disadvantages as well the lack of proper processing techniques could make the soymilk susceptible to the attack by spoilage causing organisms[5]. These microbes mainly includes the mesophillic aerobic bacteria, coli forms, yeasts and moulds which causes undesirable changes in the color and flavor of the milk making it unfit for human consumption [6].In African countries and Nigeria where the soya milk is prepared at home the soymilk can be readily spoiled by contaminations from the raw materials and the micro flora invading the utensils [7].The toxic metabolites secreted from these organisms can cause acute health problems in the consumers.The importance in the use of soya milk has been realized over the years is an excellent source of nutrient to the children who has food allergies against cow's milk and is a nutritional supplement for the individuals lacking the enzyme lactase in their intestine [8]. It is also a healthy nutritional supplement as it is protein rich, is free from cholesterol and is containing saturated fatty acids in traces.Soya milk has also a role in curing a large no of diseases like atherosclerosis, cancer, osteoporosis, obesity, menopausal disorder and diabetes [9].The consumption and variety of the soy based products is restricted due to its chalky taste and flavour which is quite undesirable. It can be assumed that in the future products like flavored soya milk and foods rich in soy would have an emerging market and potential. An increasing market to the soymilk could encourage other industries to process and develop certain products whose sensory and nutritional properties are alike to cow's milk [10]. There has been an increasing awareness among consumers in the fields of health and marketing to create certain probiotic foods using the soy and soy based products as the starting material [11].Today many supermarkets in the emerging countries like Japan, UK andUS have a lot of dairy products which are essentially rich in probiotics, prebiotics, omega-3, plant sterols [12].

MATERIALS AND METHODS

Sampling

Natural products samples like *Moringa* olifera leaves, banana florescence, spinach leaves, *Colocasia* fruit and soya beans were collected from the local vegetable market of Vellore. Samples were used along methanol as extraction buffer and soxhlet apparatus was used as extracting instrument for differentiating solid particles and soluble particles present in the samples. The soya bean was soaked overnight and removed outer layer of the beans were grinded. Perhaps the soya milk and samples were processed through various biochemical testing to check its performance and various compositions present in it.

Nutritional and nutraceutical composition

Carbohydrate: By using Standard Anthrone reagent at 630nm, total carbohydrate content was determined by Anthrone method.

CALCULATION

Amount of carbohydrate was determined in 100 mg of the sample:

$$=\times 100 \quad \frac{\text{mg of glucose}}{\text{Volume of test sample}}$$

Proteins: By using Bradford Reagent Method having BSA (Bovine Serum Albumin) as Standard at 595nm absorbance was estimated the proteins present in the different samples.

Vitamins: Ascorbic acid: At 520nm Estimation of ascorbic acid was done. Take only 0.5ml after TCA is added and filter whole solution [13, 14]. Procedure is given below in table-1 format:

Table 1: Ascorbic acid (Vitamin C) Estimation

S.No.	Blank	1	2	3	4	5	Test1	Test2	Test3	Test4
Vit. C(ml)	0	1	2	3	4	5	-	-	-	-
Sample(ml)	-	-	-	-	-	-	3	3	3	3
Phosphate Buffer(ml)	5	4	3	2	1	-	2	2	2	2
Pectin(mg)	1	1	1	1	1	1	1	1	1	1
Substrate(whole)	Incubate for 30mins at 50°C									
TCA(ml)	1	1	1	1	1	1	1	1	1	1
Ninhydrin(ml)	1	1	1	1	1	1	1	1	1	1
O.D(520nm)	Optical Density was measured by using UV/Visible Spectrophotometer, Ultraspec 1100 pro, Amersham Biosciences at 520 nm.									

Antioxidants: The antioxidant activity methods have been usedwhich has been clearly given in table-2, tocompare and monitoring the antioxidant activity of foods and the absorbance was measured at 700 nm [15, 16].

Table 2: Antioxidants Estimation Assay

Ingredient	Blank(ml)	Test(ml)	Standard (ml)
Phosphate buffer	2.5	2.5	2.5
Potassium ferricyanide	2.5	2.5	2.5
Trichloro acetic acid	2.5	2.5	2.5
Distilled water	2.5	2.5	2.5
Ferric chloride	0.5	0.5	0.5
Sample (Test)	——	1	——
Vitamin C	——	——	1

Phenols: Anti-oxidants like phenols act and scavenge the hydroxyl radicals so that strain which has maximum phenol concentration exhibited the best antioxidant activity[17, 18, 19]. This test reveal that different culture strain have different phenol concentration. The concentration of phenol in test sample was given by Gallic acid stock solution standard 0.1 ml of Gallic acid = 10 µg/ml phenol.

All extracts were measured the total phenols at 765 nm by using FolinCiocalteu reagent.

Statistical Analysis

By various Statistical tools in MS excel 2010, for each of the sample analytical procedure, four different samples results were analyzed.

RESULTS AND DISCUSSION

Sample Collection

Four different samples were dried and extracted from methanol. Soya milk was prepared by overnight soaking of soya beans in water. Later both sample of plant extract and soya milk were mixed to produce a new drink.

Estimation and Characterization of different Nutrient Contents of Sample

Total Carbohydrate Estimation

Determination of total carbohydrate content was estimated by Anthrone Reagent at 630nm.

Table 3: Standard Concentration Reading at 630nm of Carbohydrate

Test Tube Blank	Concentration(µg/ml) 0	O.D.(630nm) 0.0
S1	20	0.121
S2	40	0.138
S3	60	0.175
S4	80	0.184
S5	100	0.203

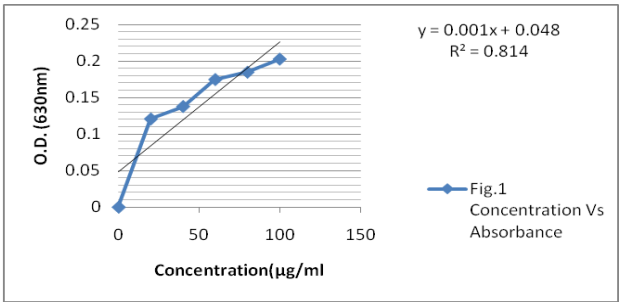

Fig. 1: Graph of Standard Curve of Total Carbohydrate Estimation

Table 4: Concentration of different unknown sample where T1(0.2ml) and T2(0.4ml) are Plant extract of four samples. U1 (0.2ml) and U2(0.4ml) of soyamilk and M1 and M2 are Mixed of plant extract with soyamilk.

Samples in Test tubes	T1	T2	U1	U2	M1	M2
Absorbance	0.075	0.185	0.059	0.103	0.098	0.196
Unknown Concentration	21.5709547	72.00958	14.23443	34.40988	32.11721	77.05345

All unknown sample concentration was determined by MS excel 2010 with help of particular code for different nutritional content.

Protein Estimation : Estimation of protein was determined by Bradford's Reagent:

Table 5: Standard Concentration BSA.

Test Tube Blank	Concentration(µg/ml) 0	O.D.(595nm) 0.0
S1	40	0.030
S2	80	0.043
S3	120	0.054
S4	160	0.087
S5	200	0.158

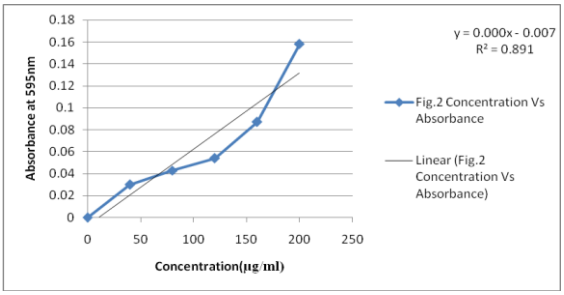

Fig.2: Standard Concentration Graph of BSA at 595nm

Table 6: Concentration of different unknown sample where T1(0.2ml) and T2(0.4ml) are Plant extract of four sample. U1 (0.2ml) and U2(0.4ml) of soyamilk and M1(0.2ml) and M2(0.4ml) are Mixed of plant extract with soyamilk.

Test tube	T1	T2	U1	U2	M1	M2
Absorbance	0.025	0.084	0.074	0.083	0.079	0.089
Unknown Concentration	52.47 258	128.2 595	115.4 143	126. 975	121.8 369	134.6 822

Vitamin Estimation: Estimation of ascorbic acid was done at 520nm using Vitamin C table of 1mg/ml for standard concentration and Ninhydrin Reagent is used.

Table 7: Standard Concentration of Vit. C

Test Tube	Concentration(µg/ml)	O.D.(520nm)
Blank	0	0.0
S1	20	0.031
S2	40	0.065
S3	60	0.103
S4	80	0.274
S5	100	0.581

Fig. 3: Standard Graph of estimation for Ascorbic Acid Estimation at 520nm.

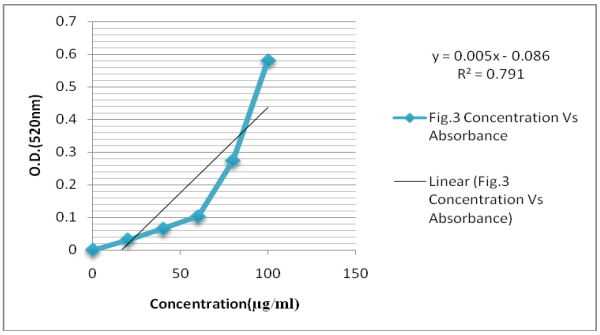

Table8: Concentration of different unknown sample where T1(3 ml) and T2(5 ml) are Plant extract of four sample. U1 (3 ml) and U2(5 ml) of soyamilk and M1 (3 ml) and M2(5 ml) are Mixed of plant extract with soyamilk.

Test tube	T1	T2	U1	U2	M1	M2
Absorbance	0.03	0.061	0.17	0.053	0.051	0.122
Unknown Concentration	28.013 42216	32.692 48792	49.1 446 9	31.4 849 9	31.1 831 1	41.8 996 8

Estimation of Phenol Contents: Phenols act as anti-oxidants and scavenge the hydroxyl radicals so that strain which has maximum phenol concentration exhibited the best antioxidant activity. This test reveal that different culture strain have different phenol concentration. The Gallic acid stock solution standard gives the concentration of phenol in test sample.

0.1 ml of Gallic acid = 10 µg/ml phenol.

Table9: Standard Calibration of Phenol content at 795nm.

Test Tube	Concentration(µg/ml)	O.D.(765nm)
Blank	0	0.0
S1	20	0.108
S2	40	0.197
S3	60	0.275
S4	80	0.343
S5	100	0.422

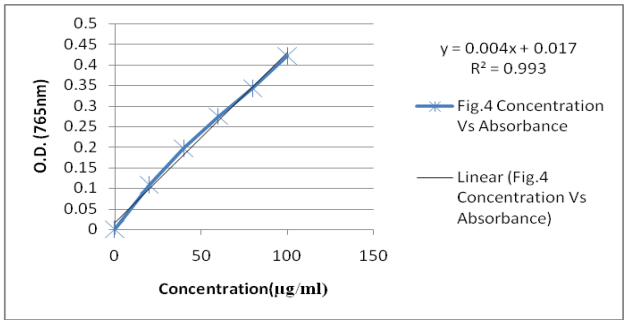

Fig. 4: Standard Curve of Phenol

Table 10: Concentration of different unknown sample where T1(0.2ml) and T2(0.4ml) are Plant extract of four sample. U1 (0.2ml) and U2(0.4ml) of soyamilk and M1(0.2ml) and M2(0.4ml) are Mixed of plant extract with soyamilk.

Test tube	T1	T2	U1	U2	M1	M2
Absorbance	0.035	0.064	0.02	0.026	0.038	0.072
Unknown Concentration	4.518 94	11.49 137	0.912 515	2.355 086	5.240 228	13.41 48

Anti-oxidant activity test: Anti-oxidant activity test showed strain dependent radical scavenging activity.

Table 11: Optical Density at 700nm for Antioxidant activity test

S.N.	Test Tube (ml)	Plant Extract Sample	Soya Milk	Mixed Sample	Standard
1.	S(0.2)	0.002	0.008	0.012	0.100
2.	S(0.4)	0.005	0.018	0.033	0.211
3.	S(0.6)	0.018	0.056	0.076	0.279
4.	S(0.8)	0.036	0.074	0.086	0.340
5.	S(1)	0.088	0.092	0.101	0.785

Scavenging activity:-Scavenging activity can be calculated from formula given below:

Scavenging % activity = $(A_{control}-A_{sample})/(A_{control})\times 100$.

Table 12: Scavenging activity for Antioxidant activity test

S.N.(µg/ml)	Plant Extract Sample	*Soya Milk*	Mixed Sample
1. (20)	98%	92%	88%
2.(40)	97.63%	91.47%	84.36%
3.(60)	93.55%	79.93%	72.76%
4.(80)	89.4%	78.24%	74.71%
5.(100)	88.79%	88.28%	87.13%

CONCLUSION

The various biochemical testing were tested for the presence for nutrient components like carbohydrates, vitamins, protein and phenol content in the sample product and soya milk. These studies showed the presence of phenols were higher in the soya milk which makes it contiguous and stagnant. It is easily get fermented by the microbes present in the environment. But samples used were profoundly benefits the soya milk in its composition and nutrient content point of view. Since soya milk get contaminated easily by microbes a further research study can be pointed out for preservation of soy milk and removal of its pungent smell.

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