International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Analysis of Total Phenol in Developed Nutraceutical by UV-VIS Spectrophotometry

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Abstract: ***The consumption of fruit plays important roles as a health protecting factor. Grape and mangosteen fruit are structurally diverse, from simple molecule to oligomers and polymers usually designated as tannins. This beneficial effect is mainly associated with the antioxidant activity of the phenolic compounds which are largely present in this fruits. This study was to quantitatively estimate the total phenol content in the developed nutraceutical product with the combination of grape seed extract and mangosteen extract. The developed nutraceutical contains a desirable amount of phenols. Spectrometry in the ultraviolet region is a useful tool for the analysis of polyphenols. So the developed nutraceutical is analyzed by proposed Ultra Violet-Visible Spectrophotometry method. In this study the result shows both grape seed extract and mangosteen extract analysed by Ultra Violet visible spectrophotometer contain some amount of phenolic content.***

Keywords: Polyphenols, Mangosteen, Grape seed, UV- Vis- Spectrophotometry, Developed nutraceutical

1. Introduction

Plants have the ability to synthesize chemical compounds (active principles), their active properties being correlated with the biochemical mechanisms of human metabolism. Polyphenol is the generic name of the compounds with several hydroxyl phenols in a single molecule (Callemiena *et al*, 2010 and Zheng *et al*, 2009).

Polyphenolic compounds are the largest and most abundant secondary metabolites present in the plant kingdom. They possess a common structure comprising an aromatic benzene ring with one or more hydroxyl substituent (Figure 1). They represent large and diverse group of molecules including two main families. The flavonoids based on common C6-C3-C6 skeleton and the non-flavonoids. (Weisshaar *et al*, 1998 and Shirley W, 2002). Polyphenols usually found in plant materials can be divided in three groups: simple phenols, hydroxyl cinnamic acid derivatives and flavonoids (Corciova *et al*, 2013).

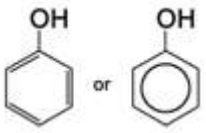

Figure 1: Structure of a Phenol

Plant phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule. Thus, plant phenolics comprise simple phenols, coumarins, lignins, lignans, condensed and hydrolysable tannins, phenolic acids and flavonoids (Vaca, A *et al*, 2012 and Komes *et al*, 2011). Tannins (from a strictly biological point of view) are part of the plant's defense system against bacteria, viruses, etc. *(*Seeram *et al*, 2006). The oxidation process of these phenols results in compounds containing gall oil rings (Beghin A *et al*, 2008), of which polyphenolic antioxidants are the most frequent type of molecule in this category (Young *et al*, 2009 and Hanzlikova K *et al*, 2004).

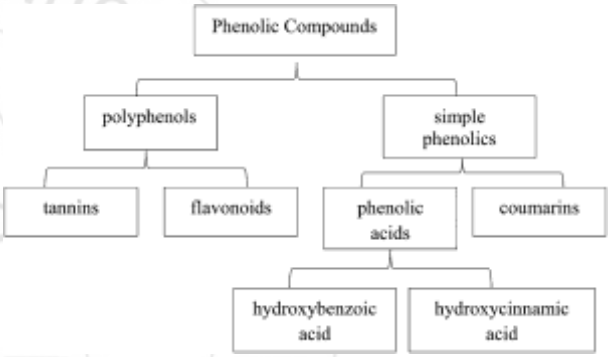

Figure 2: Classification of phenolic compounds

Polyphenolic compounds are widely distributed (Figure 2) in plant species, especially in vegetables and fruits and have been reported to have multiple biological effects, including antioxidant activity (Kahkonen *et al*, 1999). Polyphenols have numerous biological effects, including antioxidant action by the scavenger activity of hydroxyl, peroxyland superoxide radicals. Administration of flavonoids inhibits cardiovascular diseases and cancers risk (Duthie *et al*, 2000), have anti-inflammatory, diuretic, anti-gout, hepato protective and antiviral activity (Waterhouse *et al*, 2001). Polyphenols exhibit beneficial effects on human health thanks to the strong free radical scavenging and antioxidant activity, as well as cardio protective, vaso-dilatatory, anti-carcinogenic, anti-inflammatory, anti-allergic, anti-bacterial, immune-stimulating, anti-viral, and estrogenic properties (Facino *et al*, 1994, Carini *et al*, 2001, Aldini *et al*, 2003, Buelga S *et al*, 2000, King *et al*, 2006). Current evidence strongly supports a contribution of polyphenols to the prevention of cardiovascular diseases, cancers, and osteoporosis and suggests a role in the prevention of neurodegenerative diseases and diabetes mellitus. (Scalbert *et al*, 2000).

The antioxidant can reduce the risk of many diseases. Fruits are one of the most important sources of antioxidant such as vitamins and phenolic phytochemicals. The antioxidant activity of dietary polyphenols is considered to be much

greater than that of the essential vitamins, therefore contributing significantly to the health benefits of fruits (Taso *et al*, 2003, Revilla and Rayan, 2000)

The consumption of fruits plays important roles as a health protecting factor. This beneficial effect is mainly associated with the antioxidant activity of the phenolic compounds which are largely present in fruits. These effects are due to the properties of antioxidants to act as reducing agents as chelators and by trapping free radicals. these highly reactive molecules are present in biological systems and may oxidize nucleic acids, proteins, lipids, which may initiate degenerative diseases such as cancer, heart disease, dermal disorders and aging (Cook *et al*, 1996, Harborne *et al*, 2000 and Heim *et al*, 2002).

Now-a-days nutraceutical products manufactured from fruits, contain polyphenols in significant amounts (Pandey *et al*, 2009). Evaluation of polyphenols is important to determine the quality of the products (Fecka *et al*, 2009). Although quantitative determination of polyphenols is hampered by their structural complexity and diversity, several methods have used to determine polyphenols in plant extracts (Lopes *et al*, 2009, Moller *et al*, 2009, Lopes *et al*, 2010, Pieroni *et al*, 2011). Spectrophotometry in the ultraviolet region may be a useful tool to help resolve this problem (Ossipova et al, 2001).

Colorimetric reaction are widely used in the UV-Visible spectrophotometry method (Pelozo *et al*, 2008).This method measures the total concentration of phenolic hydroxyl group in the plant extract.

Polyphenol in plant extract reacts with specific redox reagent to form a blue complex that can be quantified by visible-light spectrophotometry where the maximum absorption of the chromophores depends on the alkaline solution and the concentration of phenolic compounds (Schofield P *et al*, 2001). The reaction is generally provides accurate and specific data for several groups of phenolic compounds, because many compounds change color differently due to differences in unit mass and reaction kinetics (Folin *et al*, 1927). Therefore the aim of this study was to quantitatively estimate the total phenol content in the developed nutraceutical product with the combination of grape seed extract and mangosteen extract.

2. Material and Methods

Chemical and reagent
Gallic acid 99.5% was purchased from Chroma Dex. The other chemicals and solvent used in this experiment were analytical grade which were purchased from Fischer scientific (Mumbai, India).

Back ground information of the product
A Nutraceutical product with a combination of mangosteen extract & grape seed extract was developed. The Developed Nutraceutical contains a desirable amount of phenols and it will be analyzed by proposed UV-Visible Spectrophotometry method.

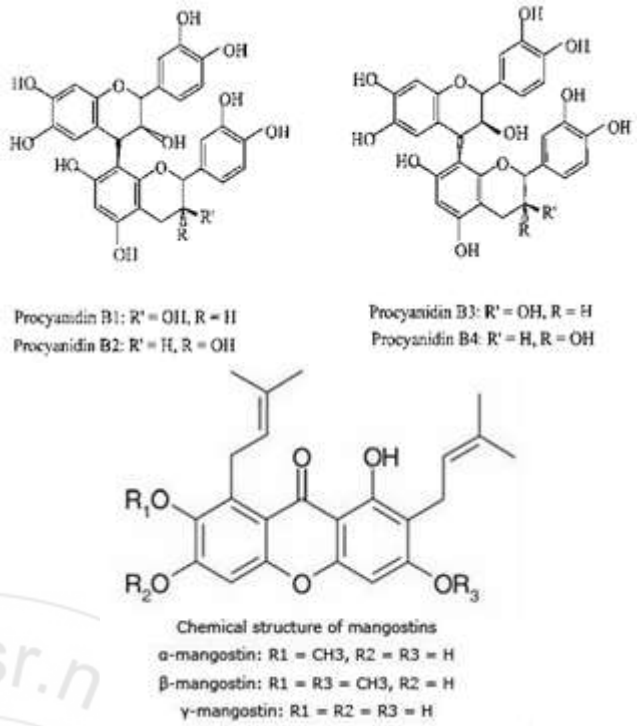

Figure 3: Structure of Polyphenols

Instrumentation
UV spectrophotometric analysis was carried out on a Techcomp8500 spectrophotometer. In the spectrum analysis the wavelength 700nm was defined for the quantitation of polyphenol in mangosteen and grape seed extract.

Ultraviolet visible spectrophotometry (UV-Vis) related to the spectroscopy of photons in the UV-visible region. UV-visible spectroscopy uses light in the visible ranges or its adjacent ranges. The color of the chemicals involved is directly affects the absorption in the visible ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic Spectrum (Gunasekaran *et al*, 2003).

With UV method, changes in the UV-visible spectra of CR-39 with different neutron doses were obtained and the absorbance of the etched samples was measured using UV-Visible spectrophotometer and the absorbance was plotted as a function of neutron dose. Further, it is observed that there is a linear relationship between the optical absorption of these detectors and neutron doses. Hence, a linear graph can be used as a calibration for measuring the unknown dose of neutrons by knowing the optical absorption of the sample. These results are compared with previous work, and in principle, there is a good agreement with their investigations

Preparation of standard solution
Approximately 25 mg of Gallic acid (purity-99.5%) standard was taken in 50 ml standard flask. The standard was prepared by dissolving in 50 ml of water.

Sample preparation
For the determination of Total phenol in the developed nutraceutical with the combination of mangosteen extract & grape seed extract. The capsule was opened and the 25mg of sample was accurately weighed and transferred to a 50ml of

standard flask. The sample was dissolved in Methanol and water in the ratio of 15:35.

Linearity

Linearity was determined by using Gallic acid standard stock solution. Various concentration of standard stock solution 5, 10,20,30,40 and 50µg/ml was taken. All standard solution was injected in triplicate and the calibration curve for concentration versus absorbance (700nm) was plotted and the obtained data were subjected to regression analysis.

Table 1: Gallic acid standard absorbance

Concentration (µg/ml)	Absorbance at 700nm			
	R1	R2	R3	Average
0 (Control)	0	0.0002	0.0002	0.000133
5	0.0438	0.0439	0.0441	0.043933
10	0.086	0.0857	0.0857	0.0858
20	0.2235	0.2234	0.2232	0.223367
30	0.3931	0.3928	0.3936	0.393167
40	0.5687	0.5683	0.5678	0.568267
50	0.7968	0.7976	0.7983	0.797567

Limit of detection and limit of quantification

In conformity with the International conference of Harmonization of technical requirement for the registration of pharmaceuticals for human use" (Baber, N 1994), the approach based on the standard deviation of the response and the slope was applied here in order to assess both the detection and quantitation limits using the following equation

$$LOD = 3.3\ \sigma / S$$
$$LOQ = 10\ \sigma / S$$

Based on the linear equation from the test for linearity, the LOQ was 6.6 µg/ml. According to the Lambert-Beer law, for a given concentration of the sample, the absorbance is not proportional to the concentration (Vogel *et al*, 2002).Based on the linear equation from the linearity test, the LOD was 2.0µg/ml. The LOD & LOQ determined by the linear equation gave different results from those observed experimentally.

3. Results and Discussions

UV-visible spectrophotometry is used to analysis the total phenol in the sample extract. The method was validated for its linearity, LOD and LOQ. The calibration graph for standard gallic acid was within the concentration range of 5-50 µg/ml with a correlation coefficient (r^2) of 0.9823 (Table 1). The LOD & LOQ for gallic acid was found to be 2.0 and 6.6 µg/ml respectively.

The amount of total phenol was determined by UV-VIS Spectrometry method. Gallic acid was used as standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: y = 0.0159x - 0.0493, R2=0.9823, where Y is absorbance at 700 nm and X is the concentration of Gallic acid in µg/ml. Phenolic compounds are a class of antioxidant agents which acts as free radical terminators (Gyamfi, 1999). Table I shows the validation parameter for the quantification of gallic acid by UV-VIS Spectrophometer method. Table 2 shows the standard and sample absorbance at 700nm and the sample in replicates of every concentration.

X is the concentration of Gallic acid in µg/ml
Y is the peak area at 700nm

Table 2: Method validation parameter for the quantification of Gallic acid by the UV-Visible spectrophotometer method

Parameter	Result
Linear range(µg/ml)	May-50
Regression equation (µg/ml)	y = 0.0159x - 0.0493
Correlation coefficient(r2)	0.9823
LOD(µg/ml)	2
LOQ(µg/ml)	6.6

In this analysis the developed nutraceutical is taken for estimation. The developed nutraceutical is a combination of mangosteen extract 400 mg and grape seed extract 100 mg. The total volume of the sample is 500mg. From the Developed nutraceutical 25mg of the sample is taken for UV –visible spectrometry analysis with an absorbance read at 700nm. Table 2 shows the absorbance at 700nm and the sample in replicates of every concentration.

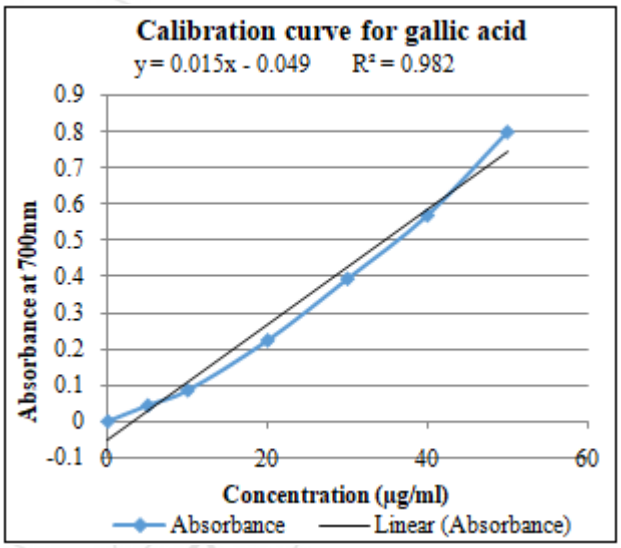

Figure 4: Calibration curve for gallic acid

The Content of Total phenol was analyzed by UV-Visible spectrophotometer and 28.01% of Total phenol was conformed in the sample Green capsule containing Yellow color powder.

Table 3: Absorbance at 700nm

S.No	Volume taken for analysis (ml)	Absorbance at 700nm			
		R1	R2	R3	Average
Standard	2	0.1500	0.1499	0.1499	0.1499
Sample	2	0.0424	0.0422	0.0421	0.0422

Amount of total phenol in sample

The nutraceutical supplement was developed with a special combination of mangosteen extract and grape seed extract by reviewing the research literature. The COA of the grape seed extract from the supplier energies denotes 90% Proanthocyanin in the purchased material. The mangosteen extract = 400 mg. The Grape seed extract = 100 mg. 90% proanthocyanin are present in grape seed extract = 90 mg.

The Developed Nutraceutical supplement with the combination of Mangosteen & Grape seed extract contain = 500 mg25 mg of sample was dissolved in 50 ml of methonol

and water in the ratio of 15:35 and prepared sample solution for UV analysis

500 mg = 90 mg
25 mg =?
25*90 / 500
2250/500 = 4.5 mg

The amount of proanthocyanin in the sample solution will be 4.5 mg. The percentage of proanthocyanin in the sample solution is 18%. The developed nutraceutical were estimated by UV-VIS spectrophometry method to standardize the amount and percentage of polyphenol content.

The Percentage of Total phenol content in the mixture of mangosteen & grape seed extract analyzed by UV is 28.01%. The percentage of proanthocyanin (Phenolic content) is 18%. The remaining 10.01% of Polyphenol is from the other phenolic group of the sample mixture. The amount of Total Phenol in the developed nutraceutical is 7mg.

Table 4: Content of polyphenol

Material	Content	Polyphenol (mg)	Polyphenol (%)
Mangostin Extract	400 mg	--	--
Grape Seed Extract	100 mg	4.5 mg	18%
Developed supplement (Mangostin Extract and Grape Seed Extract)	500 mg	7.0025 mg	28.01%

4. Conclusion

The Developed nutraceutical with the combination of mangosteen extract & grape seed extract is analysis for its total phenolic content shows a remarkable amount of phenolic group in it. The Percentage of Total phenol content in the mixture of mangosteen & grape seed extract analyzed by UV-VIS spectrophotometry is 28.01%

By calculation grape seed extract was estimated with 18 % of proanthocyanin and remaining 10.01% from the other phenolic group of the sample mixture. In this result shows the grape seed extract contain more amount of total phenol group compared to mangosteen extract. In this study the result shows both grape seed extract and mangosteen extract analysed by UV spectrophotometer contain some amount of phenolic content.

5. Acknowledgements

A debt of gratitude is owed to Vijayani Nutraceuticals Private Limited, for their contributions to the development and implementation of this research study.

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