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Phytochemical Variations in Commercially Available Triphala Powder: A Well Known Dietary Supplement of Indian System of Medicine

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ABSTRACT

This study was aimed to evaluate the phytochemical profile and variability among marketed Triphala powder samples. The results showed that Triphala powder is laden with high amount of phenolics, tannins, ascorbic acid along with total sugar and starch. A remarkable variation was observed among phytoconstituents of the top ten most popular brands. Total phenolics were recorded in the range of 21.15±1.13 to 44.80±0.50% while tannin was in the range of 18.83±0.61 to 40.59±0.61%. A good amount of ascorbic acid (0.041±0.002 to 0.164±0.002%) was also present in the Triphala powder. Nutritional analysis revealed remarkable variations in sugar (3.22±0.88 to 13.65±0.50%) and starch (3.29±0.20 8.24±0.12%) content. Some phytoconstituents that were recorded in higher concentration in the first year of study were in lesser amount during the second year of the study or vice versa.

Key words: Triphala powder, dietary supplements, marketed, phytochemicals, variability

INTRODUCTION

Triphala powder or *Triphala churna* (Admixture of three fruits) is a widely used polyherbal formulation of Indian system of medicine (Ayurveda), easily available in the global market as a dietary supplement. It is a powdered mixture of dried fruits of three important myrobalans i.e., *Emblica officinalis* Gaertn., *Terminalia belerica* Roxb. and *Terminalia chebula* Retz. in equal proportion (API, 2007). The recipe for this Ayurvedic formulation is described in the ancient books on Ayurveda, the "Charak Samhita" and "Susruta Samhita" which date back to 1500 B.C. (Gupta, 2010). Triphala is mild, non-habit forming, safest and most strengthening laxative and purgative formulation hence recommended for all. Triphala is also regarded as a secret formula for the maintenance of a healthy digestive system which is a key to overall health. The power behind Triphala's benefits comes from the ingredients that make up the formula. It encompasses the medicinal properties of all the three ingredients. Phenolic acids and tannins are the most commonly found polyphenolic compounds. The major chemical compounds reported from this formulation are phenolics (25-38%), comprising mainly of tannin (35%), gallic acid (3-7%), elagic acid (~2%), chebulagic acid (~ 5%) and chebulinic acid (~5%) along with good amount of ascorbic acid (0.050-0.33%) flavonoids and saponin (Mukherjee *et al*., 2006; Naik *et al*., 2006; Sharma *et al*.,

2012). Presence of good amount of phenolics and ascorbic acid is believed to be major chemical components of Triphala powder having therapeutic potential against age related disorders. Studies have also proved that Triphala powder provide protection against harmful γ-rays (Jagetia *et al.,* 2002) it was also found to possess hypoglycemic activity when tested in animal models (Ghosh *et al.*, 2004). This wonder formulation is also found highly beneficial in the management of hemorrhoids and anorectal pile (Rao *et al.*, 2004; Singh *et al.*, 2005). Triphala is also beneficial in gouty arthritis as it possesses strong anti-inflammatory activity (Sabina and Rasool, 2008). It is also found to cure dermal wounds by reducing bacterial activity and improving the level of collagen, hexosamine, uronic acid and superoxide dismutase (Kumar *et al.*, 2008). Triphala powder is also reported as an effective medication for dental care as it inhibits the formation of dental biofilm, protect gum cells and also act against dental plaque, gingival inflammation and microbial growth (Bajaj and Tandon, 2012; Jagadish *et al.*, 2009). High amount of therapeutically active phytoconstituents like polyphenols and ascorbic acid are belived to be responsible for the antioxidant potential of Triphala as it was found to prevent the cold induced oxidative stress and elevation of LPO and corticosterone level (Dhanalakshmi *et al.*, 2006). Triphla have also shown the cytochrome P450 inhibitory potential and cytotoxic effects on breast cancer cell lines (Ponnusankar *et al.*, 2011; Sandhya and Mishra, 2006). It is also used as rejuvenating agent and widely recommended by herbal practitioners for various ailments of the human body therefore rightly called elixir of life.

It is the quality and quantity of phytoconstituents that actually determine the therapeutic potential of any formulation. Polyphenols and ascorbic acid have already been reported as major phytochemicals responsible for the therapeutic potential. Depending upon the type, quality of raw material and mode of processing the formulation/drugs possess theses active constituents in definite amount, generally lower than the raw materials. Herbal medicines particularly of Indian and Chinese system of medicine have gained popularity in recent years. These medicines are considered as dietary supplements and can be purchased from any part of the world. Availability of various brands of formulations provides better choice to consumers in term of quality and price and simultaneously generates large variation at phytochemical level as there is no set parameters for botanical medicines to maintain the mandatory minimum level of active principles.

In views of the wide concern over the quality and poor efficacy of herbal formulations it was found necessary to quantify the therapeutically important phytoconstituents and nutrients of the marketed Triphala powder samples. The study was carried out only to put on view general scenario of phytochemical variability in marketed Triphala powder samples rather to fortify or criticize any brand.

MATERIALS AND METHODS

Experimental material: On the basis of the availability and popularity of the brands a total twenty samples of Triphala powder (10 samples of selected types in year 2009 and 10 samples of the same types in year 2010) were collected from the market of Gwalior district, Madhya Pradesh India in original packings. Collected samples were categorized into multinational brands (Mult.), regional brands (Reg.), locally manufactured (Loc.) and Triphala prepared by herbal practitioners (HP). Among collected types of Triphala powder, 4 were of multinational brands (Mult. D, Mult. Z, Mult. B and Mult. DI), 3 of regional brands (Reg. B, Reg. U and Reg. S), 2 of local manufacturers (Loc-1 and Loc-2) and one was from Herbal Practitioners (HP). All the ten selected

types were subjected to phytochemical analysis of therapeutically important phytoconstituents like total phenolics, tannin, ascorbic acid along with total soluble sugars and starch in two consecutive years.

Estimation of phytochemicals: Therapeutically important phytoconstituents (total phenolics, tannin, ascorbic acid) along with some nutritional components (total sugar and starch) were analyzed during the study. Analysis of total phenolics and tannin was carried out in accordance with the Makkar *et al.* (1993) whereas ascorbic acid was estimated by a titrimetric method as suggested by Roe (1954). Total sugar and starch was estimated by the standard method as proposed by Dubois *et al.* (1951) and Clegg (1956), respectively. All the results were expressed in percentage on dry matter basis.

Statistical analysis: One way ANOVA at $p<0.05$ followed by Tukey HSD test was used to determine the significance level using the Minitab-16 software.

RESULTS

Quantitative estimation of total phenolics, tannins, ascorbic acid, total soluble sugar and starch was carried out to assess the phytochemical variability among marketed Triphala powder samples. The results of analysis of variance ($p<0.05$) showed significant variations among the phytoconstituents of various brands of Triphala powder. The total concentration of phytoconstituents was calculated from the calibration curves made up of glucose (for total soluble sugar and starch) and tannic acid (total phenolics and tannin). All the results are expressed on dry matter basis in percentage (Table 1).

Total phenolics: Phenolics are considered as major phytoconstituents of Triphala powder responsible for the broad spectrum therapeutic values. Therefore, the quantity of phenolics has been considered directly related with the quality and efficacy of the Triphala.

During present investigation a significant variation was observed in the concentration of total phenolics in Triphala powder of different brands. Percentage concentration of total phenolics (tannic acid equivalent) was found in between 21.96±0.45 to 40.99±1.70% and 21.15±1.13 to 44.80±0.50% in Triphala powder samples collected in years 2009 and 2010, respectively. The highest concentration of total phenolics in samples of year 2009 (40.99±1.70%) and 2010 (44.80±0.50%) was recorded from Triphala powder samples of multinational manufacturers 'Mult. Z' and 'Mult. D', respectively. While least concentration was recorded from the samples of herbal practitioners (HP) (21.96±0.45%) and locally manufactured "Loc-1" (21.15±1.13%) collected during the years 2009 and 2010, respectively. In remaining samples the range of phenolics concentration was observed in between 40.38±1.85 to 24.7±1.77% and 43.48±0.52 to 24.72±0.28% in samples of years 2009 and 2010, respectively. A significant difference in the values of phenolics concentration was observed in the samples of the same brands collected during two successive years. During investigation it was observed that the amount of total phenolics was much higher in samples of multinational manufacturers as compared to the sample of regional, local manufacturers and herbal practitioners. Whereas samples of the same brand analyzed in two consecutive years also showed large variations in the values of total phenolics (Table 1).

Table 1: Phytochemical profile of Triphala powder samples collected during the year 2009 and 2010

	Total phenolics (%)		Tannin (%)		Total soluble sugar (%)		Starch (%)		Ascorbic acid (%)	
Brand code	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Mult.D	36.68±0.55^{abc}	44.80±0.50^{a}	32.16±0.77^{abc}	40.37±0.57^{a}	9.41±0.87^{bc}	13.65±0.50^{a}	5.05±0.12^{c}	4.79±0.20^{def}	0.084±0.006^{b}	0.164±0.002^{a}
Mult.B	40.38±1.85^{ab}	30.38±0.76^{e}	36.38±1.91^{ab}	26.74±0.83^{c}	3.22±0.88^{d}	8.58±0.49^{e}	3.29±0.20^{e}	6.77±0.20^{b}	0.042±0.004^{d}	0.049±0.001^{e}
Mult.Z	40.99±1.70^{a}	38.57±0.54^{b}	36.86±1.49^{a}	34.17±0.49^{b}	9.10±0.47^{c}	12.11±0.58^{bc}	4.13±0.16^{d}	5.68±0.37^{c}	0.085±0.002^{b}	0.053±0.004^{e}
Mult.DI	39.11±0.84^{ab}	30.71±2.65^{c}	35.29±0.99^{ab}	27.83±2.36^{c}	4.83±0.17^{d}	10.90±0.13^{bcd}	5.30±0.18^{bc}	4.73±0.11^{ef}	0.053±0.003^{c}	0.106±0.005^{b}
Reg.B	36.94±2.32^{abc}	43.48±0.52^{a}	33.09±2.64^{abc}	40.59±0.61^{a}	9.65±2.81^{bc}	8.79±0.08^{e}	4.42±0.22^{d}	4.22±0.34^{f}	0.159±0.005^{a}	0.095±0.002^{bc}
Reg.U	37.33±2.63^{abc}	24.72±0.28^{d}	33.44±2.70^{abc}	20.50±0.37^{ef}	8.53±0.31^{c}	9.70±0.50^{de}	5.28±0.12^{bc}	5.38±0.2^{cd}	0.076±0.005^{b}	0.087±0.002^{c}
Reg.S	33.60±0.50^{c}	25.24±0.58^{d}	29.39±0.38^{c}	22.63±0.60^{de}	3.53±0.53^{d}	10.77±0.82^{cd}	4.39±0.33^{d}	6.65±0.27^{b}	0.058±0.002^{c}	0.072±0.001^{d}
Loc-1	24.70±1.77^{d}	21.15±1.13^{e}	21.00±1.75^{d}	18.82±1.12^{f}	10.75±0.83^{abc}	8.56±0.06^{e}	5.86±0.28^{ab}	8.24±0.12^{a}	0.041±0.002^{d}	0.043±0.005^{e}
Loc.2	35.52±2.65^{bc}	25.95±0.86^{d}	31.45±1.03^{bc}	23.19±0.73^{de}	13.32±0.14^{a}	12.33±0.52^{ab}	6.29±0.13^{a}	5.53±0.32^{c}	0.050±0.001^{cd}	0.101±0.008^{b}
HP	21.96±0.45^{d}	27.66±0.67^{cd}	18.83±0.61^{d}	25.36±0.75^{cd}	12.45±1.04^{a}	10.73±0.62^{cd}	5.82±0.26^{ab}	5.19±0.20^{cde}	0.058±0.002^{c}	0.043±0^{e}

Mean values of three replicates±Standard deviation. Means in columns that do not share a superscript letter are significantly different (One way ANOVA at $p<0.05$ followed by Tukey HSD test)

Tannin: During the analysis a large variation in tannin concentration was observed in Triphala powder samples of two consecutive years ranging between 18.83±0.61 to 36.86±1.49% and 18.82±1.12 to 40.59±0.61%, respectively. In 2009 highest concentration of tannin was 36.86±1.49% (Mult. Z') while it was 40.59±0.61% ('Reg. B') in the samples of year 2010. The least tannin concentration 18.83±0.61 (in 2009) and 18.82±1.12% (in 2010) was recorded from Triphala powder samples "HP" and 'Loc-1', respectively. In remaining sample tannin concentration was observed in the range of 36.38±1.91 to 21±1.75% and 40.37±0.57 to 20.50±0.37% in years 2009 and 2010 respectively (Table 1). A good variation in the concentration of tannin was observed among the samples of the same brands analyzed in two consecutive years (Table 1).

Ascorbic acid: Ascorbic acid is a major phytochemical constituent of *Emblica officinalis*, one of the important ingredients of the Triphala powder. Most of the antioxidant potential of Triphala powder and its capability to fight age related problem is believed due to ascorbic acid. During phytochemical analysis of Triphala powder, concentration of ascorbic acid was found in between 0.041±0.002 to 0.159±0.005% and 0.043±0.005 to 0.164±0.002% in samples collected during the years 2009 and 2010, respectively. In samples of year 2009 and 2010 highest concentration of ascorbic acid was 0.159±0.005% (Reg.B) and 0.164±0.002% (Mult.D), respectively. While the least concentration of ascorbic acid in sample of year 2009 and 2010 was recorded as 0.041±0.002 and 0.043±0.005% (Loc-1), respectively. In remaining samples of year 2009 and 2010 ascorbic acid concentration was in the range of 0.085±0.002 to 0.042±0.004% and 0.106±0.005 to 0.043±0%, respectively. A significant variation in ascorbic acid concentration was observed among different brands and between the samples of the same brands analyzed in two successive years (Table 1).

Total soluble sugar: Being a composite mixture of three dried fruits, Triphala powder is highly rich in sugar content. Results of phytochemical analysis showed the presence of high amount of soluble sugar (in terms of glucose equivalent) in Triphala powder. Concentration of total soluble sugar was found in the range of 3.22±0.88 to 13.32±0.14% and 8.56±0.06 to 13.65±0.50% in samples of years 2009 and 2010, respectively. As compared to 2010 samples a large variation in the range of total soluble sugar concentration was observed in 2009.

A significant variation was observed among various brands as well as samples collected during two consecutive years. Highest 13.32±0.14% (Loc-2) and 13.65±0.50% (Mult.D) and minimum 3.22±0.88% (Mult.B) and 8.56±0.06% (Loc-1) total soluble sugar concentration was recorded in samples of year 2009 and 2010, respectively. In remaining samples of year 2009 and 2010 total soluble sugar was recorded in between 12.45±1.04 to 3.53± 0.53% and 12.33±0.52 to 8.58±0.49%, respectively. During investigation it was recorded that Triphala powder samples of herbal practitioners and local manufacturers were richer in total soluble sugar concentration as compared to the samples of multinational and regional manufacturers. A significant variation was also observed among the samples of the same brand analyzed in two successive years (Table 1). It was also recorded that the concentration of total phenolics and tannins was found to decrease in samples having high concentration of sugars.

Starch: Results of phytochemical analysis of Triphala powder revealed that starch concentration was in the range of 3.29±0.20%to 6.29±0.13% and 4.22±0.34%to 8.24±0.12% in samples of years 2009 and 2010, respectively. In samples of year 2009 highest and minimum concentration of starch was 6.29±0.13 (Loc-2) and 3.29±0.20 (Mult.B); while in the year 2010 it was recorded as

8.24±0.12% (Loc-1) and 4.22±0.34 (Reg. B), respectively. In the rest of the samples of year 2009 and 2010, starch concentration was recorded in the range of 5.86±0.28% to 4.13±0.16% and 6.77±0.20% to 4.73±0.11%, respectively (Table 1).

A significant variation in starch content was observed among brands whereas not much variation was observed in same brands analyzed in two successive years. Only two samples of different brands (Mult. B and Loc-I) were showing maximum variation in 2009 and 2010 (Table 1).

The overall observation revealed that Triphala powder is highly rich in phenolics including tannin. A good amount of ascorbic acid was also recorded. A lot of variation was observed in Triphala powder samples collected during the years 2009 and 2010. Some phytoconstituents that were recorded in higher concentration in samples of year 2009 were found in lesser amount during the year 2010 and vice versa. Triphala powder s amples of multinational (Mult. D, Mult. Z, Mult. B and Mult. DI) and regional manufacturers (Reg. B, Reg. U and Reg. S) were comparatively richer in phytochemicals as compare to the sample of Herbal Practitioner (HP) and local manufacturers (Loc-1 and Loc-2).

Phenolics, the major active constituents of Triphala was recorded in higher concentration in samples of multinational and regional manufacturers whereas more than 40% lesser amount was recorded in samples of local manufacturers and herbal practitioner. Some of the Triphala powder samples showing the higher amount of total soluble sugar and were found poor in total phenolics and tannin concentration. During investigation it was observed that Triphala powder of multinational manufacturers were superior in phytoconstituents followed by regional manufacturers, local manufacturers and herbal practitioners.

DISCUSSION

Triphala possesses a good amount of therapeutically important phytoconstituents. These phytoconstituents are believed to be responsible for various therapeutic uses of Triphala powder. During present investigation a variation in concentration of phytoconstituents was observed among various brands of Triphala powder. Different source of raw materials, different cultivars, environmental conditions of storage and laxity at the time of manufacturing generate large variation in concentration of phytoconstituents responsible for therapeutic uses.

Phytochemical analysis of various brands of Triphala powder revealed the presence of high amount of phenolics including tannin ranging from 21-44% on dry matter basis. This is in accordance with the findings of Naik *et al.* (2006), Amanullah *et al.* (2011) and Sharma *et al.* (2012), who have reported 38, 42 and 33.5% phenolics in Triphala powder, respectively. *Terminalia chebula* has been documented as a rich source of tannin (Chang and Lin, 2012; Soni *et al.*, 2003). This can be a reason of the high amount of phenolics in Triphala powder. Being a combination of powder of three dried fruits, Triphala powder is rich in nutrients including ascorbic acid, sugars and starch. Pragati *et al.* (2003) reported about 43% sugar contents in dried fruits of *E. officinalis*. This report also justifies our findings where the total soluble sugar concentration in Triphala powder was found in the range of 4.83-13.65% therefore this high sugar content may be attributed to the presence of *E. officinalis* as one of the ingredients. A good amount of ascorbic acid was also recorded from the Triphala powder samples as *E. officinalis* is a rich source of vitamin C (Kondawar *et al.*, 2011; Pragati *et al.*, 2003). During present investigation ascorbic acid was recorded in the range of 0.041-0.164%. Biradar *et al.* (2007) also recorded 0.333% ascorbic acid in freshly prepared Triphala powder.

The study also revealed the large variation in concentration of phytochemicals among the marketed Triphala powder. Gunasekaran and Anita (2010), Patra *et al.* (2011) and Mishra *et al.* (2011) also made similar observations and reported large variations in marketed samples of Ayurvedic formulations and medicinal plants. Variation was also observed within the samples of the same brands analyzed in two different years. Geographical factors may also affect the variation in the diversity of phytoconstituents. Deng *et al.* (2010) have also suggested that this variation may be due to diversity of geographical factors (soil type, sunlight, temperature and precipitation) and post growth factors (harvesting, storage, transportation and manufacturing process) associated with the ingredients of medicinal formulations. Similarly Gao *et al.* (2011) and Kwee and Niemeyer (2011) have pointed out time of harvesting and type of cultivar are also capable of generating variation at the level of phytochemicals. On the other hand, Stuart and Wills (2003), Dubey *et al.* (2004), Kabelitz (2005) and Asekun *et al.* (2007) have given emphasis on storage condition, methods of drying and way of packaging of processed formulation or raw material can also generate variation at active constituent's level. It was also noted that Triphala powder samples of local manufacturers and herbal practitioners were poor in phytochemicals as compared to the Triphala powder samples of regional and multinational manufacturers. This may be due to use of prolonged stored ingredients and high incidence of adulteration of Triphala powder with endocarp of fruits. As stony part of the fruit (endocarp) is devoid of phytoconstituents, therefore the presence of stony parts/seeds results in an overall decline in concentration of phytochemicals. This is also supported by the findings of Bahulikar *et al.* (2002) who reported the variation in tannin concentration of three marketed samples of Triphala powder and predicted that this variation may be due to the presence of seeds of the ingredients that may have been powdered along with the dried flesh of Myrobalans.

CONCLUSION

Results of present investigation showed that Triphala powder is laden with therapeutically important phytochemicals with an abundance of phenolics. Large variation in concentration of phytochemicals exist among Triphala powder brands; this may be due to noncompliance by herbal industry with the quality control guidelines. This variation may result in to alteration in efficacy thereby shatter the beliefs of common people in Ayurveda. Findings of present investigation also showed that Triphala powder of multinational and regional manufacturers were rich in phytochemicals in comparison to Triphala powder of local manufacturers and herbal practitioners. Therefore it is the need of time to formulate for mandatory minimum level of active principles in formulations to reduce the variation in concentration of phytochemicals among brands. So that maximum benefits can be obtained from the formulations.

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