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Antioxidant Activity and Total Phenolic Content of *Triphala churna*

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Abstract – Triphala churna is a widely used herbal formulation that contains equal proportion of dried fruit powder of *Emblica officinalis*, *Terminalia chebula* and *Terminalia belirica*. In the Indian system of medicine, it is used in cleaning wounds, urinary disorders, diabetes mellitus, leprosy, constipation, eyesight promotion, piles, and as a rejuvenator. In the present study, the methanolic extract of 5 commercial Triphala was evaluated for antioxidant activity by 1,1-diphenyl-2-picryl-hydrazyl free radical scavenging method, total phenolic content by Folin-Ciocalteu method and gallic acid equivalents (GAE) by high performance thin layer chromatographic (HPTLC) method. All extracts exhibited antioxidant activity significantly. The IC_{50} of the extracts ranged between 7.16 to 12.96 μg/ml. The total phenolic content of the extracts was found to be 195.3-296.4 mg of GAE/gm dw. The HPTLC chromatographic data reveal that the content of GAE present in the extract was found to be 7.17-4.11 μg/ml. The study reveals that out of the churnas analysed, **C** was found to exhibit the most potent antioxidant activity. A clear correlation between IC_{50} and content of GAE nor the total phenolic content could be observed. The study reveals that the consumption of Triphala would exert several beneficial effects by virtue of its antioxidant activity.
Keywords – Triphala, antioxidant, total phenolic content, gallic acid equivalents, HPTLC

Introduction

Triphala churna is a widely used herbal formulation that contains equal proportion of dried fruit powder of *Emblica officinalis*, *Terminalia chebula* and *Terminalia belirica*. In the Indian traditional medicine (Dash and Kashyap, 1987; Sivarajan and Balachandran, 1994), it is used in cleaning wounds, urinary disorders, diabetes mellitus, leprosy, constipation, eyesight promotion, piles and as a rejuvenator.

E. officinalis and its active constituents were reported to possess antioxidant (Chaudhuri, 2002), hypolipidemic (Anila and Vijayalakshmi, 2002), adjunct to cancer chemotherapy (Haque *et al.*, 2001), anti-inflammatory (Thorat *et al.*, 1995), cytoprotective (Biswas *et al.*, 1999), immuno-modulatory (Sairam *et al.*, 2002), antitumor (Khan *et al.*, 2002), hepatoprotective (Bhattacharya *et al.*, 2000), CVS (Bhattacharya *et al.*, 2002), and hypoglycemic activities (Anila and Vijayalakshmi, 2000). *T. chebula* and its principle constituents were reported to possess cardiac (Shin *et al.*, 2001), anticancer (Saleem *et al.*, 2002), antioxidant (Saleem *et al.*, 2001), anti-inflammatory (Moeslinger *et al.*, 2000), hypolipidemic (Shaila *et al.*, 1998), anti-HIV (Ahn *et al.*, 2002), immuno-modulatory (Hamada *et al.*, 1997), and dermal wound healing (Suguna *et al.*, 2002). *T. chebula* extracts were reported to possess anti-HIV (Kusumoto *et al.*, 1992), hypoglycemic (Kar *et al.*, 2003), hypolipidemic (Shaila *et al.*, 1998) and antioxidant (Saleem *et al.*, 2001) activity.

The antioxidant activity (Sabu and Kuttan, 2002) of Triphala was reported to be evaluated by lipid peroxidation and hydroxyl radical scavenging properties *in-vivo*. The antidiabetic (Sabu and Kuttan, 2002) and antimutagenic (Kaur *et al.*, 2002) properties of Triphala were also reported. Many claimed and reported activities of Triphala ingredients are related to antioxidant property. Therefore, in the present study, the methanolic extracts of 5 commercial Triphala churna were evaluated for antioxidant activity (*in-vitro*) by 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) scavenging method. The total phenolic content of the extracts were determined using Folin-Ciocalteu's reagent. The gallic acid equivalents (GAE) of the extracts were determined by high performance thin layer chromatographic (HPTLC) method.

Materials and Methods

Chemicals and Instruments – Triphala churnas (A, B, C, D and E) were procured commercially from Chennai, India. DPPH and gallic acid were purchased from Sigma, USA. All the other chemicals used were of analytical quality

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and procured from E. Merck India. Triple glass distilled water was used. Centrifugation was performed in Sigma 3K30. The spectral measurement was recorded in Shimadzu UV-Visible Spectrophotometer 1601. HPTLC was performed in Camag HPTLC Linomat IV sampler using precoated silicagel plate ($60F_{254}$).

Extraction – Triphala churna (25 g) was extracted by cold maceration with aqueous methanol (100 ml-50% v/v) for 72 h with occasional shaking. The extract was filtered and vacuum dried by rotary vacuum film evaporator and was stored at 4°C.

Quantitative evaluation of antioxidant activity – Antioxidant activity was evaluated by using DPPH free radical scavenging method (Hatano *et al.*, 1989). Triphala churna extract (5 g) was dissolved in minimum quantity of aqueous methanol (50% v/v) and the volume was made upto 50 ml with the same solvent to obtain a concentration of 100 mg/ml. The stock solution was further diluted to obtain different concentrations. DPPH free radical solution (0.5 ml-0.022% in 80% v/v aqueous methanol) was added to the test extract. The resultant solution was incubated at room temperature for 30 min and absorbance was recorded at 517 nm. A control was performed excluding the extract. Ascorbic acid was used as standard. IC_{50} value was obtained by linear regression method using percentage activity and concentration.

Quantitative evaluation of total phenolic content – Total phenolic content of Triphala churna extract was determined by Folin-Ciocalteu method (Kumazama *et al.*, 2002). Triphala churna extract (5 g) was dissolved in distilled water (25 ml), centrifuged (10,000 rpm - 20 min) and the supernatant (stock solution) separated. The stock solution was further diluted with water to obtain different concentrations. Folin-Ciocalteu's phenol reagent (0.5 ml) was added followed by aqueous sodium carbonate (0.5 ml of 10% w/v). The solution was mixed thoroughly and the absorbance was measured at 760 nm after incubation (1 h) at room temperature. Gallic acid was used as standard. The total phenolic content in the extract was expressed as mg/gm of gallic acid.

Quantitative evaluation of gallic acid equivalents – Quantitative evaluation of gallic acid equivalents of *Triphala churna* was performed by HPTLC method. The evaluation was performed using gallic acid as reference standard, ambient temperature, methanol as mobile phase, sample volume of 10 µl and UV-detection (densitometry) at 254 nm.

Statistical analysis – Unpaired student-t-test was performed (Freund and Walpore, 1980) between the antioxidant activity of Triphala churnas to ascertain the significant difference of the activity. One way analysis of variance (ANOVA) followed by Dunnet's-t-test was applied to determine the antioxidant potency of the *Triphala churnas*.

Results and Discussion

The extractive values of Triphala churnas-A, B, C, D and E were found to be 47.88, 49.01, 37.18, 44.59 and 30.16% w/w respectively. The antioxidant activity data are presented in Table 1. All extracts exhibited antioxidant activity significantly. The IC_{50} values were calculated by linear regression method. The extracts were screened for antioxidant activity from 2-14 µg/ml depending upon the predicted IC_{50} values (r = 0.9719 to 0.9999). The antioxidant activity of the extracts of A, B and C were not significant at 2 µg/ml but extracts of D and E exhibited significant activity from 2 µg/ml onwards. The IC_{50} data of the extracts reveal that extract C had the lowest IC_{50} value which shows that it was the most active *Triphala churna*. The IC_{50} values were found to exhibit an excellent correlation with the Dunnet's-t-values.

The total phenolic content data are presented in Table 2. The total phenolic contents of the extracts were found to be in the order of A>D>B>C>E (195.30-296.4 mg of GAE/gm dw).

Table 1. Antioxidant activity of *Triphala churna*

Sample	Concentration (µg/ml) and antioxidant activity (%)						
	2	4	6	8	10	12	14
A	3.41	23.55[a]	39.25[b]	45.73[b]	53.58[b]	–	-
B	1.84	16.84[a]	19.85[b]	27.57[b]	38.24[b]	44.49[b]	51.1[b]
C	2.7	17.37[b]	35.9[b]	49.81[b]	69.11[b]	–	–
D	8.22[a]	23.01[a]	35.53[b]	49.69[b]	59.53[b]	–	–
E	14.68[a]	20.63[b]	34.27[b]	38.11[b]	49.65[b]	59.79[b]	68.53[b]
	Concentration (µg/ml) and antioxidant activity (%)						
	0.1	0.2	0.3	0.4	0.5	0.6	0.7
Vitamin-C	5.9[a]	13.54[b]	22.57[b]	29.51[b]	37.5[b]	46.22[b]	54.5[b]

Significance level: [a]$p<0.01$, [b]$p<0.001$.

Table 2. IC_{50}, Dunnet's t-value, total phenolic content and GAE of *Triphala churna*

Sample	IC_{50} (μg/ml)	Dunnet's t-value	Total phenolic content (mg of GAE/g dw)	GAE (μg/ml)
A	8.57	20.592	296.4 ± 18.4	4.68
B	12.96	16.285	228.7 ± 38.3	5.93
C	7.16	28.129	211.9 ± 37.5	4.11
D	8.39	22.342	232.6 ± 9.9	4.98
E	10.24	19.515	195.3 ± 6.9	7.17

Table 3. GAE of *Triphala churna* by HPTLC analysis

Sample	Rf Value	% Area	Total Area	GAE (μg/ml)
A	0.39	77.20	16757.5	4.68
B	0.40	81.83	20213.5	5.93
C	0.40	79.46	14315.4	4.11
D	0.40	81.73	16875.9	4.98
E	0.41	60.93	32534.7	7.17

The GAE of the extracts were confirmed from the standard HPTLC chromatogram of gallic acid. The HPTLC chromatographic data reveal that the contents of GAE present in the extract were found to be in the order of E>B>D>A>C (7.17-4.11 μg/ml) (Table 3).

The antioxidant activity (IC_{50}) and the total phenolic content did not exhibit a clear correlation. This may be due to the fact that antioxidant activity is also exhibited by non-phenolic compounds. Thus the antioxidant activity exhibited by the *Triphala churnas* is not entirely due to phenolic compounds and GAE.

The presence and accumilation of free radicals in the body has been attributed to various pathological conditions such as atherosclerosis, hemmorrhagic shock, ischemia (repurfusion injury), cerebral ischemia, lung disease, kidney damage, gene activation, leprosy (Ajitha and Rajnarayana, 2001), cardiac failure (Shin *et al.*, 2001), neurogenarative disorders (Bhattacharya *et al.*, 2002), inflammation (Thorat *et al.*, 1995; Moeslinger *et al.*, 2000), and diabetes mellitus (Anila and Vijayalakshmi, 2000; Kar *et al.*, 2003). Free radicals have also been implicated in the aging process and a considerable interest has been focussed on the search for natural free radical scavengers (Ferrari and Torres 2003; Droge, 2002). The present study shows that the selected herbal formulation Triphala churna exhibited potential antioxidant property and hence the consumption of the medicine might exert several beneficial effects by virtue of its potential antioxidant activity.

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