Research Article

Quantitative estimation of total phenolics content, total flavonoids content, and total antioxidant potential in various promising extracts of Triphala powder and its fruit constituents

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ABSTRACT

Triphala is a well-known Ayurvedic formulation, used against a number of ailments since ancient times. It is an equiproportional mixture of fruits of three medicinal herbs, Amla (*Emblica officinalis*), Bahera (*Terminalia belerica*), and Harad (*Terminalia chebula*). **Aim:** The proximate composition: Moisture, crude fat, ash, crude protein, crude fiber, and total carbohydrates was determined from the seeds of Amla, Bahera, and Harad. The antioxidant potential of promising extract of Triphala and its fruit constituents in various solvents was evaluated using 2,2′-Diphenyl-1-picrylhydrazyl (DPPH) and phosphomolybdate assay. A comparative study for total phenolic, total flavonoids content, and total antioxidant activity was done using these extracts in various solvents. **Materials and Methods:** Triphala as a whole and its three individual constituents show specific antimicrobial activity against certain bacteria and fungi. Many herbal and patent drugs have been formulated by these fruit constituents. These fruit constituents primarily contain tannins, flavonoids, phenolic compounds, saponins, terpenoids, ascorbic acids, carbohydrates, and many other compounds. **Results:** The result shows that the total antioxidant capacity follows the order as Amla >Triphala >Bahera > Harad and in aqueous medium being the highest and in case of DPPH; the order is as follows: Bahera>Amla >Harad>Triphala and in methanol being the highest. The synthetic antioxidants such as butylated hydroxyl anisole and butylated hydroxyl toluene have restricted use in food because of their carcinogenic properties. **Conclusion:** Therefore, the search for effective, nontoxic natural compounds with antioxidant activity has been intensified in recent years. These medicinal plants contain substantial amounts phytochemicals with capacity to scavenge the free radical and can serve as an alternate potential source of natural antioxidants.

Keywords: Antioxidants, flavonoids, phenolics, phytochemicals

Introduction

The search for new cures has always been dependent on the traditional knowledge of medicinal plants. In spite of the introduction of modern high-tech drug discovery and screening techniques, traditional knowledge systems have given evidence to the discovery of valuable drugs.[1] Due to local availability, easy consumption as raw or simple medicinal preparations and in-expensiveness of conventional medicinal plants, the traditional medicinal practices are forming an integral part of complementary medicine.

The researchers have channeled their interest in isolating natural antioxidants while keeping the adverse effects of synthetic antioxidants in mind[2] being very effective in controlling oxidative stress and thus preventing the initiation of disease propagation. Therefore, an alternative is the consumption of natural antioxidants from various food supplements and traditional medicines.[3] Researchers have formulated several modes of alienating health issues and one such option is the facile and cost-effective herbal medicinal practices. A number of fresh and non-drug substances were innovated as a result of herbal medicinal practice. Hence, it is of prime importance to formulate novel medicines by eradicating restraints in the herbal medicinal practices.

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The use of plants as antioxidants in processed foods is becoming of increasing importance in the food industry as an alternative to synthetic antioxidants. Antioxidant activity of plant materials is well correlated with the content of their phenolic compounds.[4] Extraction of antioxidants from plant samples most frequently involves the method of solvent extraction. The choice of solvent has been shown to have a significant influence on the concentration of antioxidants extracted.[5,6] Phenolic compounds contribute to the overall antioxidant activities of plant foods. However, owing to the differing antioxidant potential of compounds with different polarities in complex whole foods, all methods for assessing the antioxidant capacity of food samples are strongly affected by the solvents used during extraction.[7]

In Indian medicinal preparation, Triphala (tri "three"; phala "fruits") comprises of equiproportional herbal fruits: namely, *Emblica officinalis, Terminalia belerica*, and *Terminalia chebula*. The "*Tridoshic rasayana*"; three constituent elements are characterized with balancing and rejuvenation effects which govern the human life. Triphala constituents are rich in anti-oxidants and reported to poses antimicrobial properties. This formulation has been in extensive use in Ayurveda for treating several disorders such as gastrointestinal, cardiovascular, and visual systems.[8] The essential bioactive phytochemicals of Triphala include alkaloids, essential oils, flavonoids, saponins tannins, terpenoids, and phenolic compounds.[9] The plants that are rich in phenolic compounds serve as a resource of antimicrobials and antioxidants.[10-13]

The fruits of *T. chebula* Retz, *T. belerica* Roxb, and *E. officinalis* Gaertn are widely used in the Indian traditional system of medicine.[14] The half-ripe fruit of *T. belerica* and the pericarp of *T. chebula* fruit was reported to be purgative.[14] The fruit of *T. chebula* was traditionally used to cure asthma, urinary disorders, and heart disease, and it has cardiotonic activity.[15,16] In Ayurveda, the fruit of *E. officinalis* is used as a cardiotonic, cerebral, and intestinal tonic,[17] and it is also reported to have anticancer properties.[17,18] The fruit of *E. officinalis* is a rich source of Vitamin C, a well-known antioxidant.[19] The crude extract of Amla was found to counteract the hepatotoxic and nephrotoxic effects of metals[20] due to its antioxidant properties.

These constituents have also been reported in ayurvedic medicine for its higher anti-microbial activity against a wider spectrum of pathogenic and non-pathogenic bacterial strains.[10,11,13] The present study is aimed to assess the antioxidant and anti-microbial efficacy of the botanical species that constituent Triphala all along the phytochemical, proximate composition, and nutritional parameters as well.

Materials and Methods

Plant materials

The fruits of *E. officinalis*, *T. belerica,* and *T. chebula* were acquired from Chaudhary Charan Singh Haryana Agricultural University, Hisar. After the commercial harvest time, each sample was picked and selected for the same uniformity of maturity by evaluating the color and taste. The fruits were stored under freeze-drying conditions till further analysis.

Chemicals

Commercially available and highest purity chemicals were used for various experimental procedures. Folin and Ciocalteu's phenol reagent, ammonium molybdate, sodium phosphate, oxalic acid, sodium hydroxide, anthrone, and methanol were procured from SISCO Research Laboratories Private Limited (SRL). Nitric acid 69%, sulfuric acid 98%, sodium nitrite, aluminum chloride, perchloric acid (about 70%), and phenol were sourced from Merck Specialties Private Limited. Sodium carbonate, sodium sulfate, sodium bicarbonate, and petroleum spirit (60–80°C) (petroleum ether) were supplied by Qualigens Fine Chemicals. 2,2°C-Diphenyl-1-picrylhydrazyl (DPPH) and catechin were obtained from Sigma-Aldrich. All other chemicals and reagents used were of analytical grade.

Preparation of extracts

The powdered samples (seed, aerial parts, and roots) of Amla, Bahera, and Harad (~ 8 g each) were kept in a thimble to be kept in a classical Soxhlet assembly present along with a 250 mL RB flask. A variety of solvents (distilled water, methanol, ethanol, and acetone) were added up to one and a half siphons that are approximately 150 mL. At boiling temperature of the solvent, the solvent vapor travels up a distillation arm and floods into the chamber housing the thimble filled with seed, aerial parts, and roots of Amla, Bahera, and Harad samples. The condenser ensures that any solvent vapor cools and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound dissolves in the warm solvent. When the Soxhlet chamber is almost full, the chamber is emptied by the siphon. The solvent is returned to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the still pot. Process was continued for 5 h with completion of up to seven to eight cycles through siphon mechanism in case of volatile solvents as methanol, ethanol, and acetone. In the case of water as solvent, one cycle was completed lately. Hence, in the case of aqueous extracts, the extraction was carried out for a longer time with the completion of 7–8 cycles through siphon mechanism. After the completion of the first extraction cycle, the extraction is repeated twice (each extraction time 2 h) with a suitable amount of the solvent. Filtrates of the solvent from three extraction steps were collected and their volumes were noted. The extracts prepared were used for estimation of total phenolics, total flavonoids, for the evaluation of DPPH free radical scavenging activity and antioxidant potential using phosphomolybdate assay and synthesis of metallic/bimetallic nanoparticles along with their antimicrobial activity against the available pathogens.

Determination of total phenolics and flavonoids

Singleton and Rossi's Folin–Ciocalteu method using gallic acid as standard was followed for the determination of total phenols. The total phenols in the promising extracts of seeds, roots, and aerial parts (only aqueous extracts) of Amla, Bahera, and Harad were estimated. Absorbance was adjusted within calibration limits by diluting 0.2 mL of each extract with the respective

solvent. 1.0 mL of 1 mol/L Folin–Ciocalteu reagent and 2.0 mL of Na_2CO_3 (20%, w/v) were added and mixed into the diluted extract, and the volume was made up to 10.0 mL with water. The mixture was centrifuged at 6000 rpm for 10 min after waiting for some time. Then, the absorbance at 730 nm of the supernatant solution was measured using ultraviolet (UV)-VIS double beam spectrophotometer against a blank containing respective solvent instead of extracts prepared similarly. The total phenolics contents (TPC) in the extracts were calculated using the standard curve and these results were to be expressed as mg GAE/g.

As per the description laid by Marinova *et al*. (2005), aluminum chloride colorimetric assay was used for the determination of total flavonoids using catechin as standard, the total flavonoids in promising extracts of seeds, roots, and aerial parts (only aqueous extracts) of Amla, Bahera, and Harad were estimated. To the test tubes, 1.0 mL of each extract along with 4.0 mL of double distilled water and 0.3 mL of $NaNO_2$ (5%, w/v) was added. After 5 min, it was followed by the addition of 0.3 mL of 10%, w/v $AlCl_3$, and 2.0 mL of 1M NaOH immediately. The total volume was made up to 10.0 mL with double distilled water with thorough mixing. The absorbance at 510 nm was measured with the help of UV-VIS double beam spectrophotometer against blank containing respective solvent instead of extracts prepared under identical conditions. The total flavonoids contents (TFC) in extracts were calculated using the standard curve and these results are expressed as mg CE/g.

DPPH free radical scavenging activity

Hatano *et al*. (1988) provided a method to evaluate the antioxidant potential of the extract by DPPH free radical scavenging assay.

DPPH free radical scavenging method is an antioxidant assay based on electron-transfer that produces a violet solution in the solvents at an absorption maximum at 517 nm. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule or a proton donor, giving rise to colorless solution (i.e., disappearance of the maxima). Using this principle, the radical scavenging effect of each fraction was measured.

The completely dried up promising extracts of Amla, Bahera, and Harad and Triphala powder were taken and their dry mass weight was noted. In the case of aqueous extracts, the dry mass was redissolved in 50% (v/v) methanol:water to make the stock solution. Using the stock solution, different concentrations (100 μg/mL–5000 μg/mL) were made by appropriate dilutions with the solvents (i.e., with methanol:water for aqueous extracts). The experimental procedure for evaluation of antioxidant activity involves the addition of 0.2 mL of extracts (different concentrations), 3.0 mL of DPPH (0.1 mM in 100% methanol) was added, and mixing for 5 min. A control comprised of 0.2 mL of each solvent instead of extract. After 30 min of incubation in dark, the absorbance of the sample as well as control at 517 nm was measured using the UV-VIS double beam spectrophotometer Model 2203 (Systronics Co.) against a blank containing respective solvent. The evaluation is performed in triplicates. A plot of percent DPPH free radical scavenging activity versus extract concentration (x-axis) is drawn. A quadratic regression equation ($y = ax^2+bx+c$) was obtained. IC_{50} was calculated from the equation $ax^2+bx+c = 0$ using the formula:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

Where, $x = IC_{50}$ (μg/mL)

Calculation

The percentage of DPPH scavenged (% $DPPH^*_{sc}$) was calculated using:

$$\%DPPH^*_{sc} = \frac{A_c - A_s}{A_c} \times 100$$

Where, A_c is the absorbance of control and A_s is the absorbance of the sample.

Total antioxidant potential using phosphomolybdate assay

The phosphomolybdenum method of Prieto *et al*. (1999) with slight modifications was adopted for the determination of the total antioxidant capacity (TAC) of the extracts.

PM assay is based on the reduction of Phosphate-Mo (VI) to Phosphate Mo (V) by the antioxidants present in the sample and subsequent formation of a bluish-green colored phosphate/Mo (V) complex at acidic pH. The phosphomolybdenum method is routinely applied in the laboratory to evaluate the TAC of plant extracts spectrophotometrically at lmax. PM assay measures the reduction degree of Mo (VI) to Mo (V). PM assay is a quantitative method to investigate the reduction reaction rate among antioxidant, oxidant, and molybdenum ligand. It involves in thermally generating auto-oxidation during prolonged incubation period at a higher temperature. It gives a direct estimation of the reducing capacity of antioxidant.

Reagents involved

One milliliter each of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate were added in 20 ml of distilled water and made up volume to 50 ml by adding distilled water.

Different concentrations (100 μg/mL–5000 μg/mL) were made by appropriate dilutions with the solvents (i.e., with methanol:water for aqueous extracts) which were earlier made for the estimation of DPPH free radical scavenging activity. For evaluation of antioxidant activity, 1 ml of the extracts prepared (having different concentrations) was added to each test tube individually containing 3 ml of distilled water and 1 ml of molybdate reagent solution. These tubes were kept incubated at 95°C for 90 min. After incubation, these tubes were normalized to room temperature for 20–30 min and the absorbance of the reaction mixture was measured at 695 nm. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as a positive reference

standard. Three replications were carried out for each sample. The graphs were as drawn by plotting percent TAC (y-axis) against extract concentration (x-axis). Then using the Microsoft Excel Software, a quadratic regression equation ($y = ax^2+bx+c$) was obtained. By putting $y = 50\%$ in the equation $y = ax^2+bx+c$; it was converted to the form $ax^2+bx+c = 0$. IC_{50} was calculated from the equation $ax^2+bx+c = 0$ using the formula:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

Where, $x = IC_{50}$ (μg/mL).

Calculation

The percentage of TAC (%TAC) was calculated using the same formula as that $\%DPPH^*_{sc}$.

Results and Discussion

Extract yield

The data of extract yield (g/100 g) of aqueous, methanol, ethanol, and acetone extracts of fruits of Amla, Bahera, and Harad along with Triphala are given in Table 1. It was observed that the extract yield of acetone extract ranged from 1.71 to 8.30 g/100 g and Harad has the highest extract yield (g/100g), that is, 8.30, followed by Amla (4.38), Triphala (4.30), and Bahera (1.71). In ethanol extracts, extract yield ranged from 4.92 to 6.66 g/100 g. It was found maximum in Triphala (6.66), followed by Harad (5.52), Amla (4.99), and Bahera (4.92). In methanol extracts, extract yield ranged from 1.82 to 9.62 g/100 g. It was maximum in Harad (9.62), followed by Triphala (8.57), Amla (4.43), and Bahera (1.82). In aqueous extracts, extract yield ranged from 5.53 to 10.84 g/100 g. It was maximum in Bahera (10.84), followed by Triphala (7.84), Amla (6.89), and Harad (5.53).

Medicinal plants have been extremely studied for their antioxidant assay. Extraction solvent significantly alters the antioxidant estimation; therefore, selection of extracting solvent of matching polarity is one of the most important factors in the extraction of antioxidants.

Recovery of antioxidant phytochemicals from plant materials is controlled by their chemical properties and distribution in plant matrix. Because of the presence of different groups of antioxidants with differing chemical behavior in plant matrix, extract yield and resulting antioxidant activities are strongly on the nature of extracting solvent as a polarity of the solvent used for extraction purposes may or may not be matching. Plants extracts made with water are nutritionally more relevant and also required for clinical usages. In the present studies, the extract yield was found to be highest in aqueous media, followed by methanol, ethanol, and acetone as per the order of solvent polarity.

Table 1: Extract yield (g/100g) of acetone, methanol, ethanol, and aqueous extracts of Amla, Bahera, and Harad along with Triphala

Samples ↓ Solvents →	Extract yield (g/100g)				
	Aqueous	Methanol	Ethanol	Acetone	Mean
Amla	6.89±0.02	4.43±0.01	4.99±0.01	4.38±0.01	5.17
Bahera	10.84±0.02	1.82±0.09	4.92±0.01	1.71±0.01	4.82
Harad	5.53±0.03	9.62±0.01	5.52±0.02	8.30±0.02	7.24
Triphala	7.84±0.01	8.57±0.02	6.66±0.02	4.30±0.01	6.84
Range	5.53–10.84	1.82–9.62	4.92–6.66	1.71–8.30	
Mean	7.78	6.11	5.52	4.67	

Total phenolics

The TPC in promising extracts (acetone, ethanolic, methanolic, and aqueous) of the fruit of Amla, Bahera, and Harad was estimated with the help of a standard curve using gallic acid as a standard. The regression equation obtained between absorbance (y) and amount of gallic acid (x, in μg) shows absorbance and amount of gallic acid are directly related linearly. In various herbal extracts of aerial parts and roots of Amla, Bahera, and Harad, the TPC (mg GAE/g) was estimated [Table 2]. The TPC in aerial parts and roots of Amla was 36.16 mg GAE/g and 16.84 mg GAE/g, respectively. The TPC in aerial parts and roots of Bahera was 31.34 mg GAE/g and 10.69 mg GAE/g, respectively. The corresponding values of TPC in aerial parts and roots of Harad were 56.76 mg GAE/g and 19.16 mg GAE/g, respectively.

Total flavonoids

TFC in aqueous extracts of aerial parts and roots of Amla, Bahera, and Harad

The TFC in promising extracts (acetone, ethanolic, methanolic, and aqueous) of the fruit of Amla, Bahera, and Harad was estimated with the help of a standard curve using catechin as a standard. The regression equation obtained between absorbance (y) and amount of catechin (x, in μg) shows absorbance and amount of catechin are directly related linearly. In various herbal extracts of aerial parts and roots of Amla, Bahera, and Harad, the TFC (mg CE/g) was estimated [Table 3]. The TFC in aerial parts and roots of Amla was 3.61 mg CE/g and 1.40 mg CE/g, respectively. The TFC in aerial parts and roots of Bahera was 8.76 mg CE/g and 3.56 mg CE/g, respectively. The corresponding values of TPC in aerial parts and roots of Harad were 13.71 mg CE/g and 7.41 mg CE/g, respectively.

TPC in promising extracts of fruits of Amla, Bahera, and Harad along with Triphala powder

The data of total phenolics (mg GAE/g) content in acetone, ethanolic, methanolic, and aqueous extracts of fruits of Amla, Bahera, and Harad along with Triphala are given in Table 4. TPC in acetone extract ranged from 35.04 to 75.75 mg GAE/g and Amla has the highest phenolics

Table 2: Total phenolics content in different parts of Amla, Bahera, and Harad using aqueous extracts

Plants ↓ Parts →	Total phenolics contents (mg/g GAE)	
	Aerial Part	Roots
Amla	36.16±0.02	16.84±0.05
Bahera	31.34±0.03	10.69±0.02
Harad	56.76±0.01	19.16±0.03

content, that is, 75.75 mg GAE/g, followed by Triphala (38.44), Harad (37.19), and Bahera (35.04). In ethanolic extracts, TPC ranged from 37.19 to 114.41 mg GAE/g. It was maximum in Amla (114.41), followed by Triphala (43.36), Bahera (42.39), and Harad (37.19). In methanolic extracts, TPC ranged from 56.24 to 107.53 mg GAE/g. It was maximum in Amla (107.53), followed by Bahera (76.23), Triphala (71.97), and Harad (56.24). In aqueous extract, TPC ranged from 40.15 to 200.93 mg GAE/g with a maximum in Amla (200.93), followed by Triphala (66.24), Bahera (57.93), and Harad (40.15).

TFC in promising extracts of fruits of Amla, Bahera, and Harad along with Triphala powder

The data of total flavonoids (mg CE/g) content in acetone, ethanolic, methanolic, and aqueous extracts in the fruit of Amla, Bahera, and Harad along with Triphala are given in Table 5. TFC in acetone extract ranged from 3.23 to 31.47 mg CE/g and Bahera has the highest flavonoids content, that is, 31.47 mg CE/g, followed by Amla (13.34), Triphala (9.90), and Harad (3.23). In ethanolic extract, TFC ranged from 5.04 to 17.05 mg CE/g. It was maximum in Triphala (17.05), followed by Bahera (11.95), Amla (6.76), and Harad (5.04). In methanolic extract, TFC ranged from 30.94 to 38.55 mg CE/g. It was maximum in Bahera (38.55), followed by Triphala (37.74), Harad (33.64), and Amla (30.94). In aqueous extract, TFC ranged from 5.57 to 22.14 mg CE/g. It was maximum in Bahera (22.14), followed by Harad (12.12), Triphala (7.04), and Amla (5.57).

Various extracts prepared using solvents of varying polarity were screened for their free radical scavenging and reducing capacity by chemical assays. TPC was determined to assess their effect on the antioxidant potential of the extract. The properties of extracting solvents also affected the measured TPC and thus antioxidant activity. Extracts of all the samples, namely, *T. belerica, T. chebula, E. officinalis,* and Triphala powder, exhibited significant antioxidant potential and were proved to be more active. Significant antioxidant potential in the extracts confirmed that these plants can be used as an alternate source of natural antioxidants. Recovery of antioxidants from plant materials is controlled by their chemical properties and distribution in plant matrix.

Because of the presence of different groups of antioxidants with differing chemical behavior in plant matrix, extract yield and resulting antioxidant activities are strongly dependent on the nature of extracting solvent as polarity of the solvent used for extraction purposes may or may not be matching. Plants extracts made with water are nutritionally more relevant, used for the synthesis of metallic/bimetallic nanoparticles and also required for clinical usages.

Solvent polarity plays a key role in increasing phenolic solubility.[21] Phenolic compounds such as butylated hydroxyl toluene (BHT) and propyl gallate are known to be effective antioxidants. Phenolic compounds have been known to act as antioxidants not only because of their ability to donate electrons also because of their stable radical intermediates which can effectively prevent oxidation at cellular and physiological level.[22] Extraction solvent significantly alter the antioxidant estimation; therefore, selection of extracting solvent of matching polarity is one of the most important factors in the extraction of antioxidants. Water, methanol, and ethanol have been widely used for the extraction of antioxidants. In the present study, four solvents with different polarity were used and they can be arranged as following (starting from more non-polar solvent): Acetone <ethanol <methanol <water.

Different phenolic compounds may show different antioxidant activities depending on their structure as well as synergistic or antagonistic effect of other compounds present in the extract. The selection of an appropriate solvent system is one of the most relevant steps in optimizing the recovery of total phenolic content and other antioxidant compounds from a sample.[23] Total phenolics are often extracted in more polar solvents such as aqueous ethanol, acetone, and methanol. Wide range of total phenolics has been reported by various authors in these medicinal plant samples, which might be due to the difference in climacteric conditions, raw material composition, and used solvents of different concentrations for analysis.[11]

Table 3: Total flavonoids content in different parts of Amla, Bahera, and Harad using aqueous extracts

Plants ↓ Parts →	Total flavonoids contents (mg/g CE)	
	Aerial part	Roots
Amla	3.61±0.02	1.40±0.01
Bahera	8.76±0.01	3.56±0.02
Harad	13.71±0.02	7.41±0.02

Table 4: Total phenolics (mg GAE/g) in fruit extracts of Amla, Bahera, and Harad along with Triphala prepared using different solvents

Samples ↓ Solvents →	Total phenolics contents (mg/g GAE)				
	Aqueous	Methanol	Ethanol	Acetone	Mean
Amla	200.93±0.03	107.53±0.18	114.41±0.12	75.75±0.06	124.66
Bahera	57.93±0.01	76.23±0.08	42.39±0.05	35.04±0.02	52.90
Harad	40.15±0.06	56.24±0.04	37.19±0.09	37.19±0.04	42.69
Triphala	66.24±0.09	71.97±0.06	43.36±0.10	38.44±0.12	55.00
Range	40.15–200.93	56.24–107.53	37.19–114.41	35.04–75.75	
Mean	91.31	77.99	59.34	46.61	

Table 5: Total flavonoids (mg CE/g) in fruit extracts of Amla, Bahera, and Harad along with Triphala prepared using different solvents

Samples ↓ Solvents →	Total flavonoids content (mg/g CE)				
	Aqueous	Methanol	Ethanol	Acetone	Mean
Amla	5.57±0.06	30.94±0.02	6.76±0.03	13.34±0.03	14.15
Bahera	22.14±0.03	38.55±0.02	11.95±0.02	31.47±0.03	26.02
Harad	12.12±0.02	33.64±0.02	5.04±0.02	3.23±0.03	13.51
Triphala	7.04±0.02	37.74±0.01	17.05±0.02	9.90±0.03	17.93
Range	5.57–22.14	30.94–38.55	5.04–17.05	3.23–31.47	
Mean	11.72	35.22	10.20	14.49	

The presence of flavonoids and phenolics compounds in the extracts bestows antioxidant efficacy. A cardinal antioxidant property is the ability to scavenge free radicals, species involved in deleterious oxidative reactions, the pathogenesis of various chronic diseases, and premature aging.[24,25] Therefore, it was considered important to assess the free radical scavenging efficacy of the experimental samples.

Evaluation of free radical scavenging activity and TAC of Triphala powder and its fruit constituents: Amla, Bahera, and Harad

DPPH free radical scavenging activity of promising extracts (acetone, methanol, ethanol, and aqueous extracts) of Triphala powder and its fruit constituents: Amla, Bahera, and Harad

Antioxidants prevent the oxidation of essential biological macromolecules by inhibiting the promulgation of the oxidizing chain reaction. Keeping in mind the adverse effects of synthetic antioxidants, researchers have channeled their interest in isolating natural antioxidants which are very effective to control oxidative stress and hence prevent the initiation of disease propagation.[2] Both of these compounds have good antioxidant potential and their effects on human nutrition and health are considerable. The mechanism of action of flavonoids is through scavenging or chelating process. Phenolic contents are also very important plant constituents because of their scavenging ability due to their hydroxyl groups.[26] It is generally recognized that free radicals produced in the body are partly associated with the etiology of cancers and other chronic diseases. Dietary antioxidants, capable of scavenging free radicals, are able to reduce the risk of the disease. Therefore, it is important to determine the radical scavenging effect of antioxidants in fruits. DPPH is a free radical and stable at room temperature, which produces a violet solution in ethanol. Reduction of DPPH by antioxidants results in a loss of absorbance. Thus, the degree of discoloration of the solution indicates the scavenging.

DPPH free radical scavenging activity of promising extracts (acetone, ethanolic, methanolic, and aqueous) of Triphala powder and its fruit constituents: Amla, Bahera, and Harad exhibited wide variation and showed concentration/dosage dependence. The data of DPPH free radical scavenging are given in Table 6.

Antioxidant activity is expressed as IC_{50} value. The more faded color of DPPH solution, the more DPPH is suppressed by the antioxidant compounds of the extract. Antioxidant activity of extract is shown along with IC_{50} value. IC_{50} value of DPPH scavenging activities was contradiction with the percentage of DPPH free radical scavenging activities. It means, the highest antioxidant activity was indicated by the lowest value of IC_{50}.

The environmental conditions[27] such as sunlight condition,[28] the maturity part of plant, and different parts of plant could be a factor to different type and quantity of secondary metabolites.[29,30] The differences and quantity of secondary metabolites of medicinal plant could be the cause of differences in the biological activity of these plant extracts.[31] Antioxidant activity of samples may be suspected of containing the compound capable of donating proton to the free radicals.[32] Flavonoids and phenols were the compounds capable of donating proton to the free radicals.

Table 6: DPPH free radical scavenging activity and IC_{50} of promising extracts of Triphala powder and its fruit constituents: Amla, Bahera, and Harad

Solvent	Fruit	% DPPH free radical scavenging activity						IC_{50} (μg/ml)
		Concentration (μg/ml)						
		5000	2500	1000	500	250	100	
Aqueous	Amla	89.97	74.60	42.24	28.52	16.42	4.36	1325
	Bahera	82.28	78.52	48.36	30.16	16.40	6.42	1152
	Harad	88.79	70.51	40.3	24.16	12.56	2.26	1405
	Triphala	88.25	71.62	42.62	23.64	16.52	3.46	1351
Methanol	Amla	85.62	71.24	44.36	32.16	24.28	14.54	1306
	Bahera	85.61	72.54	48.16	34.22	28.2	18.30	1189
	Harad	86.54	70.16	44.24	32.28	21.16	16.56	1357
	Triphala	84.46	68.62	44.26	32.28	21.16	16.56	1313
Ethanol	Amla	89.90	72.16	44.14	32.46	21.36	11.66	1302
	Bahera	88.56	70.44	46.32	32.18	24.32	16.40	1256
	Harad	82.62	68.36	42.18	30.16	22.68	12.96	1342
	Triphala	88.86	69.22	44.32	33.28	20.26	14.36	1316
Acetone	Amla	89.46	71.36	44.16	22.59	14.25	8.62	1366
	Bahera	88.86	73.30	48.12	34.26	24.42	16.38	1193
	Harad	87.12	68.26	41.62	28.22	20.36	10.28	1393
	Triphala	86.62	69.22	41.40	30.27	21.62	14.98	1373

DPPH: 2,2′-Diphenyl-1-picrylhydrazyl

TAC of promising extracts (acetone, ethanolic, methanolic, and aqueous) of Triphala powder and its fruit constituents: Amla, Bahera, and Harad

IC_{50} is defined as the total antioxidant necessary to decrease the initial DPPH radical by 50% and was calculated for all the extracts based on the % DPPH free radical scavenged. Spectrophotometric quantification of TAC through the formation of phosphomolybdenum complex was performed to evaluate the total antioxidant potential among the experimental samples. TAC of the promising extracts of Triphala and its fruit constituents: Amla, Bahera, and Harad were evaluated. Antioxidants present in the sample reduce the Mo (VI) to Mo (V), which then react with the phosphate group of sodium phosphate to form a green-colored Mo (V)-Phosphate complex (Phosphomolybdenum complex) in an acidic medium. This complex is then spectrophotometrically measured at l_{max}. The reaction is highly time dependent.

TAC of promising extracts (acetone, ethanolic, methanolic, and aqueous) of Triphala powder and its fruit constituents: Amla, Bahera, and Harad exhibited wide variation and showed concentration/dosage dependence. The data of total antioxidant activity are given in Table 7.

The region could lead to a significant difference both in the content of bioactive compounds and their bioactivities. Nevertheless, in work

reported here, the data unequivocally show that DPPH (%) and TAC (%) values are strictly dependent on polyphenolics content. These data are in accordance with that of other authors who have shown that high total polyphenolics content increases antioxidant function and that there is a linear correlation between polyphenolics content and antioxidant function.

Comparison among experimental samples and solvent from IC_{50} values using percent DPPH free radical scavenging activity

From IC_{50} values using percent DPPH free radical scavenging activity [Table 8], we can conclude that among solvents, the mean value of IC_{50} (μg/ml) of methanol extract was found to be lowest (1291), followed by ethanol (1304), aqueous (1308), and acetone (1331). Among samples, Bahera (1198) has the lowest mean value of IC_{50}, followed by Amla (1325), Harad (1374), and Triphala powder (1381).

Table 7: Total antioxidant capacity and IC_{50} of promising extracts of Triphala powder and its fruit constituents: Amla, Bahera, and Harad

Solvent	Fruit	Total antioxidant capacity (%)						IC_{50} (μg/ml)
		Concentration (μg/ml)						
		5000	2500	1000	500	250	100	
Aqueous	Amla	80.24	77.62	45.36	28.23	16.13	3.02	1199
	Bahera	84.27	71.37	44.96	29.43	22.38	19.15	1322
	Harad	82.86	67.34	41.53	30.04	22.38	15.32	1407
	Triphala	80.64	74.60	45.16	28.63	19.96	6.65	1225
Methanol	Amla	82.22	72.22	45.11	30.89	20.44	10.67	1236
	Bahera	82.37	70.11	44.30	29.03	18.71	9.68	1314
	Harad	86.54	70.16	44.24	32.28	21.16	16.56	1357
	Triphala	82.11	70.33	44.67	34.67	25.22	16.22	1284
Ethanol	Amla	85.62	71.24	44.36	32.16	24.28	14.54	1306
	Bahera	84.00	70.00	44.22	28.22	18.89	11.11	1342
	Harad	78.67	70.89	40.44	26.00	16.00	5.33	1391
	Triphala	79.92	69.50	44.15	30.03	20.21	10.32	1309
Acetone	Amla	79.60	71.64	42.79	27.61	18.16	5.72	1333
	Bahera	83.08	68.90	40.80	26.37	13.68	6.22	1408
	Harad	76.37	64.18	39.30	22.14	15.17	9.45	1605
	Triphala	78.86	69.48	41.26	32.08	24.17	15.92	1370

Table 8: IC_{50} values (μg/ml) of different fruits extracts (aqueous, methanol, ethanol, and acetone) of Triphala and its fruit constituents: Amla, Bahera, and Harad using DPPH free radical scavenging activity (%)

Samples ↓ Solvents →	IC_{50} values from DPPH scavenging activity data				
	Aqueous	Methanol	Ethanol	Acetone	Mean
Amla	1325	1306	1302	1366	1325
Bahera	1152	1189	1256	1193	1198
Harad	1405	1357	1342	1392	1374
Triphala	1351	1313	1316	1373	1381
Mean	1308	1291	1304	1331	

DPPH: 2,2′-Diphenyl-1-picrylhydrazyl

Thus, the total antioxidant potential estimation follows the order as follows: Bahera >Amla >Harad >Triphala powder.

Thus, the total antioxidant potential estimation using DPPH free radical scavenging activity (%) follows the order as follows: Bahera > Amla >Harad >Triphala powder.

Comparison among experimental samples and solvent from IC_{50} values using percent TAC

From IC_{50} values using percent total antioxidant activity [Table 9], we can conclude that among solvents, the mean value of IC_{50} (μg/ml) of aqueous extract was found to be lowest (1288), followed by methanol (1298), ethanol (1337), and acetone (1429). Among samples, Amla (1269) has the lowest mean value of IC_{50}, followed by Triphala powder (1297), Bahera (1347), and Harad (1440). Thus, the total antioxidant potential estimation follows the order as follows: Amla>Triphala powder >Bahera>Harad.

Thus, the total antioxidant potential estimation using TAC (%) follows the order as follows: Amla >Triphala powder >Bahera >Harad.

Concluding results

Trends in phenolics and flavonoids contents in different parts of Triphala constituents

Parameters	Order
Total phenolics content (mg GAE/g))	Harad>Amla>Bahera (Aerial parts>Roots)
Total flavonoids content (mg CE/g)	Harad>Bahera>Amla (Aerial parts>Roots)

Trends in phenolics and flavonoids contents in promising extracts of Triphala and its fruit constituents

Parameters	Order
Total phenolics content (mg GAE/g)	Amla >Triphala >Bahera >Harad (Aqueous >Methanol >Ethanol >Acetone)
Total flavonoids content (mg CE/g)	Bahera >Triphala >Amla >Harad (Methanol >Acetone >Aqueous >Ethanol)

Trends in extract yield (g/100g) of promising extracts of Amla, Bahera, and Harad along with Triphala

Parameter	Order
Extract yield (g/100g)	Harad > Triphala > Amla > Bahera (Aqueous > Methanol > Ethanol > Acetone)

Table 9: IC_{50} values (μg/ml) of different fruits extracts (aqueous, methanol, ethanol, and acetone) of Triphala and its fruit constituents: Amla, Bahera, and Harad using total antioxidant capacity (%)

Samples ↓ Solvents →	IC_{50} values from total antioxidant capacity data				
	Aqueous	Methanol	Ethanol	Acetone	Mean
Amla	1199	1236	1306	1333	1269
Bahera	1322	1314	1342	1408	1347
Harad	1407	1357	1391	1605	1440
Triphala	1225	1284	1309	1370	1297
Mean	1288	1298	1337	1429	

Trends in total antioxidant potential using various available methods

Parameters	Order
% DPPH free radical scavenging activity	Bahera >Amla >Harad >Triphala
%Total antioxidant capacity	Amla >Triphala >Bahera >Harad
IC_{50} (µg/ml) from %DPPH	Bahera <Amla <Harad <Triphala (Methanol<Ethanol<Aqueous<Acetone)
IC_{50} (µg/ml) from %TAC	Amla <Triphala <Bahera <Harad (Aqueous <Methanol <Ethanol <Acetone)

Conclusion

Plants and herbs have been used an important contributor to the quality of human life for thousands of years. The traditional system of herbal medicinal practice out-competes the use of synthetic drugs due to the fact of natural origination, cost-effectiveness, and least side effects. As compared, synthetic drugs evince toxic and mutagenic effects than natural drugs. These attributes bring extensive use of these herbal medicines for thousands of years in developing and developed countries. Further, synthetic antioxidants such as butylated hydroxyanisole and BHT have restricted use in food because of their carcinogenic properties. As a result of herbal medicinal practice, several fresh and non-drug substances were innovated. Hence, it is of prime importance to formulate novel medicines by exterminating restraints in the herbal medicinal practices.

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