Pak. J. Agri. Sci., Vol. 57(4), 1171-1179; 2020
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/20.8657
http://www.pakjas.com.pk

ASSESSING THE PHYSICOCHEMICAL AND ANTIOXIDANT POTENTIAL OF *Terminalia arjuna* BARK BASED FUNCTIONAL DRINK AND ITS SENSORIAL ATTRIBUTES

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Modernization and sedentary lifestyle are resulting in a drastic increase in non-communicable diseases (NCDs) such as obesity, CVDs and hypercholesterolemia. The present study was planned to assess the antioxidant potential and physicochemical properties along with sensorial attributes of (arjun tree) *Terminalia arjuna* bark based functional drink. *Terminalia arjuna* (TA) bark extracts were obtained through traditional and non-traditional extraction techniques *i.e.* conventional solvent extraction (CSE), supercritical fluid extraction (SFE) and microwave-assisted extraction (MAE). *Terminalia arjuna* bark extract based functional drinks along with control were prepared using 0.5% extract powder prepared after freeze drying of extracts. The physiochemical properties of the prepared drinks *i.e.* acidity, pH, Brix to acid ratio were affected significantly with the control and selected treatments and storage intervals while Brix was statistically unchanged. The sensorial properties *i.e.* taste, flavor, color and overall acceptability differed significantly with the treatments, however; mouthfeel and sweetness differed non-significantly. All the drinks were ranged as acceptable regarding sensory characteristics, while, drink having SFE got the highest score. Antioxidant potential of the drinks indicated significant variations for TPC, flavonoids, DPPH, ABTS and FRAP with non-significant variations with storage. Conclusively, different extraction techniques significantly affected the antioxidant potential and sensorial attributes of TA bark based functional drink

Keywords: *Terminalia arjuna*, antioxidants, supercritical fluid extraction, microwave assisted extraction, total phenolic contents, flavonoids

INTRODUCTION

One in 4 middle-aged adults in Pakistan has prevalent coronary artery disease. More than 80 percent of deaths and 85 percent of disabilities from cardiovascular diseases (CVDs) are found in low and middle income countries (Reddy, 2004). According to the NCD (Non-Communicable Disease) country profiles of 2014 released by WHO, life style related disorders are the biggest killers resulting over 38 million deaths annually. Most of these death (28 million) are in less developed countries, including India, Pakistan, Sri Lanka and African countries. South Asian countries like Pakistan, India, Sri Lanka, Bangladesh and Nepal are hosting 20 percent part of the world population and are have the highest prevalence of heart related disorders in the world (Yusuf *et al.*, 2001). CVDs are responsible for over 18 million deaths annually that make 31% of global mortalities. More than 75% of deaths due to CVDs occur in low- and middle-income countries. Among total deaths due to CVDs, almost 85% deaths are due to heart attack and stroke (W.H.O., 2018). CVDs mainly arise due to the deposition of fat in blood vessels, tobacco usage and higher levels of low-density lipoproteins (LDLs), cholesterol and blood sugar (Hansson, 2005). A decade ago, synthetic drugs were the only reliable way to reduce plasma cholesterol. Afterward, especially in European countries, the use of functional foods and nutraceuticals increased. Health professionals recommend interventions such as dietary modifications, lifestyle changes and the use of functional foods (Poli *et al.*, 2013).Functional foods have metabolic and physiological benefits and decrease the risk factors for lifestyle related issues (Gul *et al.*, 2016). Nutraceuticals are nutritionally important bioactive components that are extracted and isolated from natural sources to prevent or treat health hazards *i.e.* hypercholesterolemia, cardiovascular diseases, liver malfunctioning and diabetes (Chauhan *et al.*, 2013). The use of bark, seeds, roots, berries, leaves and flowers of different plant species for therapeutic purposes is well documented (Poli *et al.*, 2013). Different medicinal plants are believed to have beneficial effects in cardiovascular diseases due to their unique phytochemical properties in "Atharva Veda" an old treatment process, the origin of "Ayurveda" (Dwivedi, 2007). *Terminalia arjuna* (Arjuna) is extensively used in such a medicinal system and has been investigated for its hypolipidemic and cardio protective potential (Chatha *et al.*, 2014).

Terminalia arjuna bark has been widely used as a cardiotonic and for acute and chronic renal disease in ayurvedic system

of medicine (Kumar *et al.,* 2012). It has been reported to exhibit antioxidative, hypocholesterolaemic (Chatha *et al.,* 2014), antimutagenic, hypolipidemic (Subramaniam *et al.,* 2011) and antibacterial effects (Morshed *et al.,* 2011).

Terminalia arjuna bark is rich in polyphenols including flavonoids, phenylpropanoids as well as tannins (20–24%) and is used for the therapy of fractures, asthma, ulcers and blood disorders (Silva and Serrano, 2015). It also contains bioactive components that are useful in hepatic, congenital and viral diseases (Saxena *et al.,* 2007; Manna *et al.,* 2009). Major bioactive components responsible for therapeutic potential are terpenoids, arjunolic and arjunic acids, arjunin, punicalin, casuarinin, galloylglucose, catechins, gallocatechins and epigallocatechins (EGC) are also found in arjun tree. Phenolic acids like gallate, ellagic acid and their derivatives are also present in *T. arjuna* bark. Other bioactive components of arjun tree are arjungenin, glycosides, proanthocyanidins, flavonoids (arjunone, arjunolone, luteolin) and phytosterols (Dhingra *et al.,* 2013).

In this context, there is a dire need to develop formulations that can reduce the risk of CVDs with little or no side effects and *T. arjuna* is a good candidate for this effect. Purposely, arjuna bark based functional drink was formulated through optimized extraction techniques (50:50 water to methanol ratio; 6 minutes microwave exposure and 5500 psi pressure in supercritical fluid extraction) and assessed for its physicochemical and antioxidant potential as well as sensory evaluation along with storage study.

MATERIALS AND METHODS

The proposed study was carried out at Grain Science Laboratory, National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad, Pakistan. The raw materials used, and methods followed in this study are described below.

Raw material procurement:

Arjun (*Terminalia arjuna*) tree bark was obtained from the trees at the University of Agriculture, Faisalabad. The trees from same locality were selected to get homogenous raw material for extraction. The bark was cleaned to remove any foreign matter and dust. Grinding of bark was carried out through mortar and pestle to increase its extraction efficiency. The ground bark was stored at room temperature in airtight polyethylene bags for further usage.

Preparation of arjun tree bark extracts: The solvent extract was prepared with methanol and water (50:50 v/v) to determine the extraction efficiency as described by Tian *et al.,* (2008). The super critical extract was prepared by using SFE at 40ºC at 5500 psi pressure using SFT-150 (supercritical fluid extractor incorporation, USA) (Pourmortazavi and Hajimirsadeghi, 2007). Microwave-assisted extraction (MAE) was performed at 600Wfor 6 minutes by the method of Tewari *et al.* (2015). Filtration of extracts was done through filtration assembly and concentrated at 40ºC temperature using a rotary evaporator (N-N series, EYELA, Japan) by the method of Rusak *et al.,* (2008). These concentrated extracts were stored at refrigeration temperature for further study.

Development of functional drink: Liquid arjuna bark extracts were collected in a flask and filtered. Solvents from extracts were evaporated by keeping them in freeze dryer by following the protocol of Chang *et al.* (2006). Equal quantity of dried arjuna bark powder (0.5%) was added to formulate functional drink. Control treatment (D_0) with no extract was also formulated for comparison with other treatments. Ingredients used for drink development were included table sugar, citric acid, aspartame, sodium benzoate. While carboxy methylcellulose (CMC) was added as a stabilizer. No added color and flavor were used during drink development to identify the true sensory parameters. Drinks were made by mixing all the ingredients including respective arjuna extract in water and heated for 1 min at 90°C. All the prepared drinks were cooled up to 15°C and packed in transparent plastic bottles and stored at refrigeration temperature.

Physicochemical analysis of the drinks: *Terminalia arjuna* bark extract-based functional drink was analyzed for pH, color, acidity, brix and acid/brix ratio.

***pH*:** The pH of the drinks was measured at 20ºC using a calibrated pH meter (Ino Lab 720, Germany) by the AOAC (2006) method. Calibration of pH meter was done before measurement using 0.1M sodium acetate buffers of different pH (4, 7 and 10).

***Acidity*:** The acidic value of the drinks was measured by the procedure described by AOAC, (2006) with acidity meter (QA supplies LLC, USA). In this method, 300 µL of the functional drink was mixed with 30 mL of distilled water. Thorough mixing was done, and the mixture was poured to the detector of acidity meter. Acidity value was recorded based on % acidity of citric acid.

***Brix*:** The brix of arjun tree bark extract-based drinks was determined by using Digital Refractometer by following AOAC (2006) Method.

***Acid/Brix ratio*:** The acid to brix ratio of the arjun tree bark drinks was evaluated as per AOAC (2006) Method.

***Phytochemical screening assays*:** The phytochemical assay of extracts was carried out through TPC and flavonoids assays explained below.

***Total Phenolic Contents (TPC)*:** TPC was carried out by Folin-Ciocalteu reagent assay using gallic acid equivalent, by the method of Sengul *et al.* (2009). Purposely, 125 µL of bark extract was mixed with an equal quantity of Folin-Ciocalteu mixture. Subsequently, 500 µL distilled water was added and the mixture was left to stay for 5 minutes at 22ºC. Afterward, 4.5mL $NaHCO_3$ solution (7%) was added to the mixture and absorbance was measured at 765 nm after 90 minutes against reagent blank using a UV/Vis spectrophotometer (CE7200 Cecil Instruments Limited, UK). TPC was expressed in gallic

Table 1. Treatments for arjun tree bark based functional drink

Product	Optimized condition	Treatment
Control		D_0
Solvent extracted drink	50:50 water: methanol ratio	D_1
Supercritical extracted drink	5500 psi pressure	D_2
Microwave-assisted extracted drink	6 minutes exposure	D_3

D_0= Control drink, D_1= Solvent extract-based drink, D_2= Supercritical extract-based drink, D_3= Microwave assisted extract-based drink

acid equivalent (GAE)/g against 100 µg/mL standard gallic acid.

***Flavonoids*:** Flavonoid content was measured by the procedure of Ghasemzadeh and Jaafar (2013). Precisely measured, 1 mL ofbark extract was 0.3 mLNaNO2 (5%). After 5 minutes, 0.3 mL $AlCl_3$ (10% solution) was added followed by 2mL NaOH (1M) solution was mixed with distilled water and total volume was raised upto 10mL.Absorbance was measured at 510 nm wavelength, using UV/Vis spectrophotometer (CECIL CE7200). Results were measured as catechin equivalent (CE)/g by comparing it against 300 mg/L standard catechin.

Antioxidant activity assays:

***Free radical scavenging ability*:** The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of extracts was measured according to the procedure described by Cheel *et al.* (2007). For this purpose, 1 ml methanol solution of DPPH was prepared and thoroughly mixed with extract. The mixture was incubated for 30 minutes at 25°C. At 520 nm, absorbance was measured through spectrophotometer (CECIL CE7200). DPPH assay was calculated through the expression given below

$$\text{Absorbance reduction (\%)} = \frac{\text{AB} - \text{AA}}{\text{AB}} \times 100$$

In which, AB = Blank sample absorbance at 0 minute, AA = Tested extract absorbance at 30 minutes

***ABTS assay*:** The ABTS assay (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) of extracts was carried out according to the method of Hossain *et al.*, (2010). For each assay, 20 µL of diluted extract was poured to 1 mL chromogen and incubated at 37 °C. Afterward, the initial absorbance was measured. Subsequently, 200 µL H_2O_2 was mixed in the mixture and incubated in the dark room at 37°C temperature. The final absorbance was measured after 3 minutes. To get the resultant absorbance, initial absorbance was deducted from the final absorbance. Antioxidant capacity was calculated through obtained value as compared to synthetic antioxidant Trolox (0.1 mM-0.4 mM).

***FRAP assay*:** The reducing power of the extracts was measured through ferric reducing ability of plasma (FRAP) through the procedure of Baek *et al.* (2008). Purposely, 1 mL of sample extract was added in 1mL potassium ferricyanide (1%) and 1 mL sodium phosphate buffer (pH 6.6, 200 mM) and the mixture was incubated for 20 minutes at 50°C. Afterward, 1mL trichloroacetic acid (10%) was added and centrifugation was done for 5 minutes at 13400×g.The supernatant was collected and 0.1 mL ferric chloride (0.1%) was added along with 1mL distilled water. Absorbance was recorded at 700 nm. An aqueous solution of $FeSO_4.7H_2O$ (100-1000 µM) was used for preparation of standard curve. Values were given in micromoles Fe (II) per g.

***Sensorial attributes*:** The sensorial attributes of the drinks were evaluated using hedonic scale (9-point) by a group of trained panelists (Meilgaard *et al.*, 2007).In separate booths, panel of 5 trained judges (from 35-45 years of age, staff members of Faculty of Food, Nutrition and Home Sciences) was provided with fluorescent lights and drinks to be evaluated in transparent glass labeled with random code numbers. Distilled water and unsalted bland crackers were given to the judges to neutralize their mouth receptors for better judgment during the hedonic response.

Statistical analysis?

RESULTS AND DISCUSSION

***Physicochemical analysis of arjuna bark extract based functional drink*:** Physicochemical parameters like pH, acidity, brix and brix to acid ratio of the drinks were analyzed through their respective procedures. Results and their discussion regarding the aforementioned parameters are given below.

***pH Value*:** Mean squares revealed significant differences of treatments on pH value of arjuna bark extract based functional drinks. However, storage intervals and their interaction with treatments varied non-significantly. Among the treatments, highest mean value (4.66±0.19) for pH was observed for D_0 followed by D_2 (4.60±0.15), D_1 (4.55±0.15) and D_3 (4.48±0.20) (Table 2). It is revealed from mean values of different treatments that incorporation of arjuna bark extract slightly lowered their pH, while storage had no effect on the pH value of the drinks.

Our findings for pH value are comparable to the results reported by Verma *et al.* (2013), who analyzed physicochemical parameters of *Terminalia arjuna* bark extracts obtained through different solvents. The reported pH value of aqueous solution was 4.56. Likewise, Rasheed and co-workers (2013) compared the physicochemical properties of an in house sample of *Terminalia arjuna* bark with a market sample. The pH recorded of an in-house sample was 5.46 for an aqueous solution while a little higher of (5.51) market sample. Later, Garg and Ahuja (2015) developed lemon, amla and ashwagandha based functional drink as a

Table 2. Means for pH of arjuna bark-based drink

Storage Interval (Days)	Treatments				
	D_0	D_1	D_2	D_3	Mean
0	4.76±0.15a	4.64±0.10a	4.69±0.06a	4.55±0.19a	4.66±0.12a
30	4.68±0.16a	4.57±0.15a	4.61±0.21a	4.49±0.13a	4.58±0.16ab
60	4.54±0.23a	4.46±0.15a	4.51±0.16a	4.40±0.21a	4.47±0.18b
Mean	4.66±0.18a	4.55±0.13ab	4.60±0.14ab	4.48±0.17b	

Means having same alphabets are statistically similar

Table 3. Means for acidity of arjuna bark-based drink

Storage Interval (Days)	Treatments				
	D_0	D_1	D_2	D_3	Mean
0	0.12±0.00c	0.13±0.00bc	0.13±0.00bc	0.14±0.01ab	0.13±0.00b
30	0.13±0.00bc	0.14±0.01ab	0.14±0.01ab	0.15±0.00a	0.14±0.00a
60	0.13±0.01bc	0.15±0.01a	0.14±0.01ab	0.15±0.01a	0.14±0.01a
Mean	0.13±0.00c	0.14±0.00b	0.14±0.00b	0.15±0.00a	

Means having same alphabets are statistically similar

Table 4. Means for TSS/brix of arjuna bark-based drink

Storage Interval (Days)	Treatments				
	D_0	D_1	D_2	D_3	Mean
0	12.72±0.39a	12.93±0.27a	12.88±0.17a	13.04±0.53a	12.89±0.40a
30	12.96±0.44a	13.11±0.44a	13.02±0.59a	13.26±0.38a	13.08±0.27a
60	13.07±0.67a	13.18±0.45a	13.09±0.47a	13.33±0.64a	13.16±0.17a
Mean	12.91±0.53a	13.07±0.44a	12.99±0.43a	13.21±0.59a	

Means having same alphabets are statistically similar

substitute for soft drinks during summer. The pH value of freshly prepared drink was 4.5 while a slight decrease was witnessed during storage. Similarly, Harsha and Aarti (2015) carried out a physicochemical analysis of traditional herbal juice during 20 days of storage. They reported decline in pH of the drink during storage which is contradictory to our findings. Breakdown of acidic compounds during storage resulted in decrease in pH of functional drink. The nature and chemical composition of functional ingredients is a major factor that defines the pH of a drink (Jan and Masih, 2012).

***Acidity*:** The F value regarding acidity of the drinks depicted significant variations with treatments and storage while interaction between them effected non-significantly. Means demonstrated little increment in acidity with the addition of arjuna bark extracts. The highest value for acidity (0.15±0.01) was measured in a functional drink with microwave extract (D_3) and lowest (0.13±0.01) for control drink (D_0). Meanwhile, storage unveiled slight increment in acidity when drinks were stored for longer period (Table 3).

Ahmed *et al.* (2008) prepared a polyphenolic functional drink with mandarin (*Citrus reticulata*) and reported an acidity value of 0.14 at the beginning of the storage study which increased to 0.21. Similarly, Ayub and co-workers (2010) formulated a functional drink with strawberry and delineated that acidity of drink slightly increased during 3-month storage. This increase in acidity may be attributed to the production of ionized acids and their salts as well as oxidation of reducing sugars.

***Total soluble solids/ brix*:** Statistical analysis pertaining to treatments and days of storage depicted non-significant effect on brix and their interaction also revealed non-significant variations.

Means for brix of arjuna bark drink ranged from 12.91±0.53 to 13.21±0.59 for treatments. Results from storage study explicated that total soluble solids/brix increased non-momentously as number of storage days increased. At 60th day, highest brix value (13.16±0.17) was recorded as compared to the 30th day (13.08±0.27) and first day (12.89±0.40) (Table 4).

Murtaza and others (2004) observed similar brix value for strawberry drink. They found that brix value increased non-significantly during storage period from 13.33 to 13.63. Hussain *et al.* (2003) and Majumdar *et al.* (2010) also observed non-significant increase in TSS of drinks with storage period. However, results by Harsha and Aarti (2015) for brix/total soluble solids demonstrated slightly increasing trend with an increase in storage duration. Brix of medicinal juice recorded at first day as 12.1 that increased to 13.8 on 20th day. An increase in TSS can possibly be attributed to a loss in moisture during storage.

***Brix to Acid Ratio*:** Mean squares for brix to acid ratio in functional drink exhibited significant variations as an effect of treatments and storage, while non-significant variation was

Table 5. Means for brixto acid ratio of arjuna bark-based drink

Storage Interval (Days)	Treatments				
	D_0	D_1	D_2	D_3	Mean
0	106.00±3.29a	99.46±2.09ab	99.08±1.29ab	93.14±3.82bc	99.42±3.08a
30	99.69±3.39ab	93.64±3.13bc	93.00±4.19bc	88.40±2.56c	93.68±1.97b
60	100.53±5.13ab	87.87±2.99c	93.50±3.37bc	88.87±4.27c	92.69±1.20b
Mean	102.07±4.18a	93.66±3.18bc	95.19±4.28b	90.14±2.61c	

Means having same alphabets are statistically similar

Table 6. Means for sensory characteristics of arjuna bark-based drink

Treatments	D_0	D_1	D_2	D_3	F value
Color	6.27±0.35b	7.33±0.29a	7.74±0.39a	7.95±0.32a	14.50**
Flavor	6.63±0.20b	7.42±0.30a	7.89±0.16a	7.53±0.38a	11.30**
Mouth feel	6.92±0.21a	7.23±0.29a	7.51±0.15a	7.62±0.38a	3.99^{NS}
Sweetness	7.83±0.23a	7.69±0.31a	7.55±0.15a	7.62±0.38a	0.54^{NS}
Taste	6.70±0.20b	7.21±0.29ab	7.84±0.16a	7.36±0.37ab	9.23**
Overall Acceptability	6.50±0.20b	7.18±0.29ab	7.71±0.15a	7.47±0.37a	11.60**

Means having same alphabets are statistically similar

found for the interaction between them. Mean values regarding brix to acid ratio illustrated a decreasing trend with an increase in storage duration. Highest brix to acid ratio was observed in D_0 (106±3.29) followed by D_1 (99.46±2.09), D_2 (99.08±1.29) and D_3 (93.14±3.82) (Table 5). Control drink represented least acidity value due to the absence of arjuna extract, so it resulted in a higher brix to acid ratio. At the initiation of storage, highest brix to acid value was recorded among all the treatments. It indicates the relative sweetness or tartness that influences the sensorial characteristics of the product (Ahmed, *et al.*, 2008). Decrease in brix to acid ratio was influenced by the changes in acidity during storage period.

***Sensory Evaluation*:** Color is one of the basic parameters for a food product to be selected by the customer. Statically, F value exhibited significant variations as an effect of treatments. Maximum scores were given to D_3 (7.95±0.32) followed by D_2 (7.74±0.39) and D_1 (7.33±0.29) while D_0 received the lowest score (6.27±0.35) (Table 6). Control drink was formulated without added color while drinks with arjuna extracts had bright red color tonality with varying extent and shades (Figure 1).

Figure 1: Arjuna bark-based functional drink

Perception of food flavor is a multifaceted procedure that involves the sense of smell, taste and chemesthesis (chemical sense; also referred to as pungency or irritation). The addition of arjuna bark extract changed the flavor of final product as compared to the control drink having no arjuna extract. The flavor of D_2 was assigned highest score (7.89±0.16) by the judges followed by D_3 (7.53±0.38), D_1 (7.42±0.30) and D_0 (6.63±0.20) (Table 6). Scores for mouthfeel assigned by judges ranged from 6.92±0.21 to 7.62±0.38 (Table 6). The sweetness of control drink was 7.83±0.23 while it ranged from 7.69±0.31 to7.55±0.15 for arjuna bark-based drinks (Table 6). The addition of arjuna extract resulted in a slightly bitter taste in drinks owing to bioactive components present. Drink prepared with supercritical fluid extract (D_2) received high liking for taste (7.84±0.16) followed by drink based on microwave extract (D_3) and conventional solvent extract (D_1) with scores of 7.36±0.37 and 7.21±0.29, accordingly (Table 6). D_0 was liked the least (6.70±0.20) by the panel due to bland sweet taste with no arjuna extract. Addition of arjuna extract in drinks resulted in peculiar taste that got high scores. Overall acceptability is the basis of consumer decision towards a food product. Statistically, significant difference was observed among drinks for overall acceptability. Control drink (D_0) got the lowest scores (6.50±0.20) of overall acceptability while drink with SFE extract (D_2) got highest score (7.71±0.15). CSE drink (D_1) and MAE drink (D_3) were ranked with7.18±0.29 and 7.47±0.37 scores, accordingly (Table 6).

Our finding for sensorial properties is supported by the results of Rathnayaka (2012) who evaluated readily to serve medicinal drink prepared with *Terminalia catappa* for its sensory attributes by 7-point hedonic scale. Color, taste and overall acceptability were categorized as “liked” and neither liked nor disliked” by the judges. Likewise, Harsha and Aarti (2015) also developed a traditional Indian medicinal drink

having cholesterol lowering and anti-diabetic properties. Sensory evaluation of the developed drink resulted in acceptable scores by the judges for color (7.4) and overall acceptability (7.8) while for taste and flavor, slightly higher scores were assigned. Sohail *et al.* (2018) developed a functional drink using licorice extracts obtained through different extraction techniques and observed a similar range of scores for overall acceptability (7.38-7.75).

***Phytochemical and antioxidant potential of functional drinks*:** Mean squares of TPC revealed statistically significant variations for the treatments, however, storage period and treatment interaction revealed non-significant differences. Drink with supercritical fluid extract had highest TPC (9.79±0.35 mg GAE/g) while the lowest TPC was observed in drink prepared with solvent extract (4.85±0.28 mg GAE/g) (Table 7). Non-significant impact of storage revealed stability of phenolics over 2 months of refrigeration.

Analysis of variance regarding total flavonoids depicted significant variations for treatments and storage, though, their interaction resulted in non-significant differences. The highest mean value recorded was 4.35±0.18mg GAE/g in D_2followed by 3.41±0.17mg GAE/g in D_3 and 2.17±0.15mg GAE/g in D_1. During storage, slight decrease in flavonoids of the drink was noticed as the values decreased from 2.29±0.11 mg GAE/g to 2.08±0.10 mg GAE/g in D_1, from 4.45±0.27 mg GAE/g to 4.25±0.12 mg GAE/g in D_2 and from 3.51± mg GAE/g to 3.33± mg GAE/g in D_3 during 60 days of storage (Table 8).

Mean squares regarding DPPH elucidated significant variation for treatments and non-significant with respect to storage and its interaction with treatments. Amongst treatments, highest radical scavenging ability was observed in D_2 (44.95±1.97%) and lowest in D_1 (36.17±1.29%) while D_3 exhibited 40.70±2.46% free radical scavenging ability (Table 9).

The F value depicted significant effects of treatments and storage on ABTS values of arjuna bark extract based functional drink, while interaction between them resulted in non-significant variations. The highest radical scavenging activity was found in D_2 (1.57±0.08mMol TE/g) trailed by D_3 (1.33±0.08 mMol TE/g) and D_1 (1.20±0.02mMol TE/g). Slight reduction in radical scavenging activity was noticed over 60 days of storage as D_1 reduced from 1.26±0.04mMol TE/g to 1.15±0.03mMol TE/g, D_2 from 1.61±0.09mMol TE/g to 1.54±0.06mMol TE/g and D_3 from 1.37±0.08mMol TE/g to 1.29±0.10mMol TE/g (Table 10).

Statistical analysis of FRAP presented significant differences among treatments, while storage and its interaction with treatments exhibited non-significant differences. Amongst the treatments, D_2 exhibited highest FRAP value (69.82±3.84µM Fe^{2+}/g) followed by D_3 (60.13±2.94µM Fe^{2+}/g) and D_1 (53.54±2.95 µM Fe^{2+}/g). Over 60 days of storage, little

Table 7. Means for TPC (mg GAE/g) of arjuna bark-based drink

Storage Interval (Days)	Treatments			
	D_1	**D_2**	**D_3**	**Mean**
0	4.93±0.21c	9.92±0.58a	7.23±0.32b	7.36±0.37a
30	4.86±0.23c	9.81±0.22a	7.05±0.39b	7.24±0.28a
60	4.76±0.39c	9.64±0.26a	6.92±0.37b	7.10±0.34a
Mean	4.85±0.28c	9.79±0.35a	7.06±0.36b	

Means having same alphabets are statistically similar

Table 8. Means for flavonoids (mg CE/g) of arjuna bark-based drink

Storage Interval (Days)	Treatments			
	D_1	**D_2**	**D_3**	**Mean**
0	2.29±0.11c	4.45±0.27a	3.51±0.17b	3.42±0.18a
30	2.15±0.23c	4.36±0.14a	3.39±0.18b	3.30±0.18ab
60	2.08±0.10c	4.25±0.12a	3.33±0.15b	3.22±0.12b
Mean	2.17±0.15c	4.35±0.18a	3.41±0.17b	

Means having same alphabets are statistically similar

Table 9. Means for DPPH (%) of arjuna bark-based drink

Storage Interval (Days)	Treatments			
	D_1	**D_2**	**D_3**	**Mean**
0	36.28±1.34b	45.11±2.84a	40.83±2.29ab	40.74±2.16a
30	36.18±1.77b	44.93±1.69a	40.69±2.20ab	40.60±1.89a
60	36.05±0.76b	44.80±1.39a	40.59±2.88ab	40.48±1.68a
Mean	36.17±1.29c	44.95±1.97a	40.70±2.46b	

Means having same alphabets are statistically similar

Table 10. Means for ABTS of arjuna bark-based drink

Storage Interval (Days)	Treatments			
	D_1	D_2	D_3	Mean
0	1.26±0.04cd	1.61±0.09a	1.37±0.08bc	1.41±0.07a
30	1.19±0.01cd	1.56±0.09ab	1.34±0.05cd	1.36±0.05ab
60	1.15±0.03d	1.54±0.06ab	1.29±0.10cd	1.33±0.06b
Mean	1.20±0.02c	1.57±0.08a	1.33±0.08b	

Means having same alphabets are statistically similar

Table 11. Means for FRAP (µM Fe^{2+}/g) of arjuna bark-based drink

Storage Interval (days)	Treatments			
	D_1	D_2	D_3	Mean
0	53.89±4.63b	70.24±2.18a	60.54±3.75ab	61.56±3.52a
30	53.48±0.48b	69.78±4.68a	60.11±2.95ab	61.12±2.70a
60	53.25±3.73b	69.45±4.65a	59.76±5.44ab	60.82±4.61a
Mean	53.54±2.95c	69.82±3.84a	60.13±2.94b	

Means having same alphabets are statistically similar

decrement in FRAP values were seen, indicating its stability for 2 months (Table 11).

Storage stability of bioactive in functional drinks is the major consideration of scientists and food processing industry. For example, a polyherbal medicinal drink having arjuna bark extract was evaluated for its sensorial and phytochemical attributes (Garg & Ahuja (2015). The study reported a non-significant decrement in DPPH activity of the drink as an effect of storage. Contrarily, Harsha & Aarti (2015) developed an ayurvedic drink having medicinal properties. The authors concluded that DPPH activity in the drink decreased from 59.7% to 50.5% during 20 days of storage. Several factors including exposure to sunlight, change in pH, oxidation and enzymatic reactions are responsible for reducing the activity of phenolic and flavonoids in plant extracts or products (Choi *et al.,* 2002). Likewise, Holliday (2010) affirmed that SFE is better in extracting phenolics and antioxidants from oat bran. Conclusively, phenolics measurement differs with different extraction techniques and differences in reported values can be attributed to botanical, seasonal and genetic differences (Arya *et al.,* 2012).

***Conclusion*:** The current study revealed the effect of different extraction techniques on the physicochemical parameters of the arjuna bark based functional drink. However, extraction techniques (CSE, SFE and MAE) showed similar behavior during 60 days of storage regarding physicochemical characteristics. For the evaluation of sensorial attributes, drink prepared with SFE extract was liked mostly by the judges as compared to other drinks. Although, remaining drinks were also ranked as acceptable. In the phytochemical and antioxidant potential of prepared drink, SFE drink resulted in a higher amount of TPC, flavonoids, DPPH, ABTS and FRAP assay than MAE and CSE drinks.

***Acknowledgements*:** The authors are thankful to the Higher Education Commission (HEC), Pakistan for financial support for the project

Conflict of interest: There is no conflict of interest declared by the authors

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[Received 29 oct 2019; Accepted 18 June 2020; Published (online) 17 July 2020]