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AG Deshmukh
AICRP on Medicinal Aromatic Plants and Betelvine, Nagarjun Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

AR Pawar
AICRP on Medicinal Aromatic Plants and Betelvine, Nagarjun Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Varsha Tapre
AICRP on Medicinal Aromatic Plants and Betelvine, Nagarjun Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

KM Deshmukh
AICRP on Medicinal Aromatic Plants and Betelvine, Nagarjun Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

NK Patke
AICRP on Medicinal Aromatic Plants and Betelvine, Nagarjun Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

JN Parmar
AICRP on Medicinal Aromatic Plants and Betelvine, Nagarjun Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

BM Muradi
AICRP on Medicinal Aromatic Plants and Betelvine, Nagarjun Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

DL Rathod
AICRP on Medicinal Aromatic Plants and Betelvine, Nagarjun Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Correspondence
AG Deshmukh
AICRP on Medicinal Aromatic Plants and Betelvine, Nagarjun Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Post-harvest storage studies in *T. arjuna* bark powder

AG Deshmukh, AR Pawar, Varsha Tapre, KM Deshmukh, NK Patke, JN Parmar, BM Muradi and DL Rathod

Abstract
Terminalia arjuna is a deciduous and evergreen tree belonging to Combretaceae family. The bark leaves and fruits of *T. arjuna* have been used in indigenous system of medicine for different ailments such as cardio tonic, antidysentric, urinary astringent, expectorant, lithontriptic, fractures, ulcers, anaemia, fatigue, bronchitis, tumor, inflammations, wound healing, cirrhosis of liver and hypertension. It is one of the most important ingredient of many Aurvedic formulations used to treat cardiovascular disorder. Vagbhata, a well-known Indian Physician of ancient times has been credited as the first to use this product for heart conditions in the seventh century A.D. Research on *T. arjuna* has been going on since the 1930s, but studies have provided mixed results. Various bioactive tannins, triterpenoids, terpenoids saponins, flavonoids, flavonoid glycosides have been isolated from the tree. Considering the possible deterioration of active components such as phenols and tannins during storage conditions, the present study was undertaken to evaluate different commonly available packaging materials. It was observed that black polybag followed by HDPE material was superior over other tested materials for storing the *T. arjuna* bark powder.

Keywords: *T. arjuna*, phenols, tannins, antioxidant potential, moisture content, storage materials

Introduction
Terminalia arjuna [Roxb. Ex DC] Wight & Arnot is a deciduous and evergreen tree belonging to Combretaceae family. The bark leaves and fruits of *T. arjuna* have been used in indigenous system of medicine for different ailments such as cardio tonic, antidysentric, urinary astringent, expectorant, lithontriptic, fractures, ulcers, anaemia, fatigue, bronchitis, tumor, inflammations, wound healing, cirrhosis of liver and hypertension [1]. It is one of the most important ingredient of many Aurvedic formulations used to treat cardiovascular disorder. Various bioactive tannins, triterpenoids, terpenoids saponins, flavonoids, flavonoid glycosides have been isolated from the tree [2]. In Ayurveda, *Terminalia arjuna* has been used to balance kapha, pitta, and vata. It has been used for scorpion stings, poisonings, asthma and bile duct disorders. The bark of Arjuna has been used in India since 3000 years, as a heart remedy [3]. Vagbhata, a well-known Indian Physician of ancient times has been credited as the first to use this product for heart conditions in the seventh century A.D. Research on *T arjuna* has been going on since the 1930s, but studies have provided mixed results. It has been evaluated for its cardiovascular hepatoprotective, cholesterol-reducing, and antioxidant properties as well as for its role in cancer therapy.

Arjun stem bark is simple, smooth and pinkish-gray in color in external view. An internal view, the bark is soft and reddish in color [4]. It is astringent, sweet, acrid, cooling, aphrodisiac, urinary astringent, and expectorant, but, chiefly used as cardio tonic as it improves blood supply to heart. Clinical studies have been conducted in coronary heart disease using *T. arjuna* bark extract at doses of 500 mg every 8 hours for up to 3 months [5]. It is also useful in ulcer treatment, fractures, and cirrhosis of liver, ischaemic heart disease, and hypertension. Arjuna has an astringent effect which acts to detoxify the blood. Its homeostatic properties help to relieve bleeding conditions and control aggravated pitta states. The general purification effect of this herb to cleanse urinary infection like UTI. In females it can be used to regulate the hormonal cycle. It is given for all kind of hormonal imbalances, fibroids, cysts, endometriosis etc. Arjuna has an effective balancing effect on the kapha and pitta doshas of the body. In this way it helps to heal wound and ulcerations. Arjuna is mostly known as a cholesterol lowering herb. It controls the blood cholesterol levels and good for people having atherosclerosis i.e cholesterol plaques block arteries at one or multiple sites. Arjuna has therapeutic effects over respiratory system. It helps to keep the airways clear and helps to fight serious lung diseases. Arjuna plays an important role as cardiac tonic [6] which improve energy levels in the body and increases the stamina so that, people can work in a better way without feeling too stressed

and fatigued. Aqueous extract of *T. arjuna* contains 23% calcium salts and 16% tannins [4]. It is reported that water extract of *T. arjuna* barks shows maximum amount of antimicrobial activities against *Proteus Vulgaris, Klebsiella aerogenes, Eschrichia coli* and *Pseudomonas aerogenis* [7]. Organic extracts of *T. arjuna* bark were also prepared using the sequential methods with a number of organic solvents such as hexane, benzene, chloroform, acetone, dichloromethane, ethyl acetate, butanol, ethanol, methanol and ether, etc., to extract various phytochemical constituents. The chemical structures of available compounds were confirmed by various advanced techniques like HPLC, UPLC, and LC-ESI-MS/MS analysis. Polyphenols, flavonoids, tannins, triterpenoids, saponins, sterols and minerals are the major constituents of *T. arjuna*. Such amino acids like tryptophan, tyrosine, histidine and cysteine are also the main ingredients in *T. arjuna* [4].

The aim of the present investigation is to evaluate quality parameters such as total phenols, tannins and antioxidant potential of bark of *T. arjuna* powder periodically at an interval of three months under storage conditions different

Material and Methods

The bark samples were collected from the plantation block of *T arjuna* available at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The packaging material were procured locally. 250 g powder was stored in each packaging material at ambient temperature. The statistical analysis was done using CRD design with three replications. The moisture content, total phenols, tannins and antioxidant activity was estimated after every three months of interval using routine biochemical methods.

Treatment Details: Various packaging material were used to store Arjun bark powder. These include: Brown paper bag (T1), Plastic bottle (T2), Polypropylene transparent bag (T3), Black poly bag (T4), LDPE transparent bag (T5) and HDPE transparent bag (T6)

Methodology

1. **Moisture content:** Moisture content from the given sample was evaluated by accurately weighing one gram of powder and heating it to 105 ^{0}C for three hours, cooling down and again weighing. The procedure was repeated twice and the moisture content was calculated based on the difference between the initial and final weights.
2. **Estimation of Phenols and Tannins:** The phenols and tannins were estimated by a method described by Makkar *et al* 1993 [8]

A. Extraction of Phenolics (Simple Phenolics and Tannins):1 g of dried and finely ground bark sample was extracted with 10 mL of aqueous acetone (70%) in an ultrasonic water bath for 60 min at room temperature. The content of the beaker was then transferred to centrifuge tubes and centrifuged for 10 min at approximately 3000 *g* at 4 °C. The supernatant was kept on ice.

B. Preparation of Calibration Curve: 0.0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µL of standard tannic acid solution(0.1 mg/mL)was placed in 5-mL glass test tubes separately and then added an adequate quantity of distilled water for respective test tubes to bring the volume to 500 µL. To this solution, 250 µL of the Folin-Ciocalteu reagent (1 N) and 1.25 mL of the sodium carbonate solution (final volume 2.0 mL; tannic acid concentration 0.0 to 10 µg) were added and the contents were vertexes for 5 minutes and kept at room temperature under dark condition. The absorbance at 725 nm against the reagent blank was measured after 40 min.

C. Analysis of Total Phenols: Suitable aliquots of the above sample extract were taken in test tubes and made the volume to 500 µL with distilled water. 250 µL of the Folin-Ciocalteu reagent (1 N) and then 1.25 mL of the sodium carbonate solution were added to it. The tubes were vertexes and absorbance was record at 725 nm after incubation for 40 min under dark conditions. The total phenols were calculated as tannic acid equivalent from the above calibration curve and expressed as total phenolics as grams per100 g on a dry matter basis.

D. Determination of Total Tannins (Non-Tannin Phenolic): 100 mg PVPP was added in a test tube. To it 1.0 mL distilled water and then 1.0 mL of the sample extract were added, vertexes and kept at 4°C for 15 min, vortexed it again, and centrifuged at 3000 *g* for 10 min. The phenolic content of the supernatant were measured as mentioned above and expressed the content of non-tannin phenolics on a dry matter basis. From the above results, the tannin content of the sample was calculated as:

Total phenolics (%) – Non tannin phenolics (%) = Tannin (%)

3. Antioxidant Activity

Antioxidant activity was measured by DPPH assay according to Gulcin, 2005 [9].

Stock solution of samples were prepared by extracting 100 mg of dried powder in 100 ml of ethanol to give conc of 1mg/ml. DPPH solution was prepared freshly by dissolving 4.3 mg DPPH in 3.3 ml ethanol.150µl DPPH solution was added to 3ml ethanol and absorbance was taken immediately at 516 nm for control reading. Different volume level of test sample (10, 20, 30, 40, 50µl) were screened and made 100µl of each dose level by dilution with ethanol. Diluted with ethanol with upto 3 ml.150µl of DPPH was added to each tube and absorbance was taken at 516 nm after 15 min using ethanol as a blank. The median inhibitory concentration IC_{50} was calculated based on 50 % inhibition of free radical formation using a calibration curve in the linear range by plotting the extract concentration vs the corresponding scavenging effect.

Results and Discussion

The most important component of medicinal plant is its active principal which being a biomolecule may be susceptible to degradation under various conditions of processing and storing. The factors that can cause degradation include biotic factors such as infection, infestation and abiotic factors such as temperature, and humidity. *T arjuna* bark powder is getting popular due to its cardio protective properties.

Oxidation is a process where highly reactive and harmful chain reactions of oxygen species are generated which cause damage to the living organism. These highly reactive and free radical systems present in biological systems from a wide variety of sources and may oxidize nucleic acids, proteins, lipids and can initiate degenerative diseases like cancer, diabetes, neurodegenerative disorders, atherosclerosis and other cardiological disorders [10]. An antioxidant is a molecule which can terminate the chain reaction by removing the free radical intermediates. There is a worldwide increase in

concern towards the use of natural phytochemical based antioxidants [11]. Therefore it is important to study the effect of different packaging conditions to preserve its activity. Since phenols and tannins and hence antioxidant potential (12) are major active components, the present investigation was directed to evaluate the effect of different packaging material on total phenol, tannin and antioxidant potential of *T arjuna* bark powder estimated at an interval of three months.

The effect of storage conditions on moisture content is shown in Table No 1. After 18 months of storage the mean moisture content decreased over time from 10.43 % to 6.93 % which was almost 33.56 % decrease over control. Lowest moisture content was observed in T6 i.e HDPE bag (6.02±0.64 %) while highest moisture content was observed in T1 i.e brown paper bag (8.38±0.21%).

The trend in total phenol content was also observed to decrease with time (Table No 2). The mean phenol content decreased from 14.24 ± 0.33 % at 0 months after storage i.e initially to 8.70±0.27 % with 38.93 % decrease over control after 18 months of storage. Highest phenol content was observed in T4 i.e black polybag (9.56±0.36%) followed by T6 i.e HDPE bags (9.51±0.24). Lowest content was observed with T1 i.e storing in brown paper bag (6.68±0.25%) with 53.07 % decrease over the control. Same trend was observed with tannins as tannin (Table No 3). After 18 months of storage the mean tannin content was reduced from 13.28±0.37 % in control i.e 0 months of storage to 7.83±0.27 % which is 40.87% decrease over control. Highest tannin was observed in T4 i.e black polybag (8.71±0.36%) followed by T6 i.e HDPE bags (8.64±0.24). Lowest content was observed with T1 i.e storing in brown paper bag (5.83±0.25%) with 56.09 % decrease over the control.

Since phenol and tannins are responsible for antioxidant activity, it also corresponds to the same trend as total phenols and tannins. Antioxidant activity was estimated by DPPH method (13). The DPPH• test is based on the ability of the stable 2, 2-diphenyl-1-picrylhydrazyl free radical to react with hydrogen donors. The DPPH• radical displays an intense UV-VIS absorption spectrum. In this test, a solution of radical is decolorized after reduction with an antioxidant (AH) or a radical (R•) in accordance with the following scheme: DPPH• + AH → DPPH•-H + A•, DPPH• + R• → DPPH•-R [14]. This method is very simple and also quick for manual analysis. Determination of the antioxidant activity is one of the ways of nutritional and biological quality evaluation of plant. The mean value of antioxidant activity was found to be decreased after 18 months of storage (Table No 4). Highest antioxidant activity was observed with T4 i.e black poly bag (38.06±1.38 IC_{50}µg/ml) while least activity was observed in T3 i.e polypropylene transparent bag (44.04±1.00 IC_{50}µg/ml).It has been proved that antioxidant activity depends on the type of phenolics present in the fruit [15]. The ability of plant polyphenols to scavenge reactive oxygen radicals contributes in the plant defense. However the total amount of antioxidants in various plant need not to represent the total antioxidant capacity [16], almost all phenolic compounds in plants demonstrate some antioxidant activity [17].

Table 1: Moisture content in *T arjuna* bark powder under different storage conditions

SN	% Moisture Content (MAS)						
	Feb-17 0 Month	**May-17 3 Months**	**Aug-17 6 Months**	**Nov-17 9 Months**	**Feb-18 12 months**	**May-18 15 Months**	**Aug-18 18 Months**
T1	10.43±0.92	12.55±0.39	13.21±0.38	10.97±0.61	8.58±0.55 (17.77)	7.16±0.24 (31.32)	8.38±0.21 (19.69)
T2	10.43±0.92	11.85±0.12	12.03±0.10	9.59± 0.59	7.81±0.50 (25.09)	5.78±0.55 (44.61)	6.15±0.23 (41.07)
T3	10.43±0.92	12.08±0.12	12.28±0.22	10.18±0.34	7.87±0.29 (24.54)	6.68±0.32 (35.92)	7.43±0.25 (28.80)
T4	10.43±0.92	12.11±0.27	12.36±0.30	10.06±0.41	8.04±0.13 (22.95)	6.37±0.48 (38.93)	6.92±0.15 (33.62)
T5	10.43±0.92	12.25±0.08	12.49±0.41	9.97±0.37	7.67±0.32 (26.46)	6.09±0.24 (41.58)	6.69±0.23 (35.86)
T6	10.43±0.92	11.81±0.51	12.14±0.10	9.81±0.31	7.61±0.26 (27.01)	5.55±0.21 (46.79)	6.02±0.64 (42.31)
Mean	10.43	12.11±0.21	12.42±0.25	10.10±0.44	7.93±0.34 (23.97)	6.27±0.34 (39.86)	6.93±0.29 (33.56)
Range		11.81-12.55	12.03-13.21	9.59-10.97	7.61-8.58	5.55-7.16	6.02-8.38
SE (m) ±		0.12	0.16	0.23	0.17	0.21	0.18
CD		0.38	0.49	0.71	0.52	0.62	0.55
F Test		Sig	Sig	Sig	Sig	Sig	Sig

Values are mean of triplicates ± SD; Values in parenthesis = % Decrease over control

Table 2: Total Phenol content in *T arjuna* bark powder under different storage conditions

SN	% Total Phenol Content (MAS)						
	Feb-17 0 Month	**May-17 3 Months**	**Aug-17 6 Months**	**Nov-17 9 Months**	**Feb-18 12 months**	**May-18 15 Months**	**Aug-18 18 Months**
T1	14.24±0.33	13.14±0.31 (7.75)	12.76±0.38 (10.39)	10.71±0.44 (24.81)	9.48±0.21 (33.40)	7.56±0.31 (46.89)	6.68±0.25 (53.07)
T2	14.24±0.33	14.12±0.27 (0.84)	13.61±0.10 (4.38)	12.02±0.71 (15.59)	11.08±0.27 (22.17)	10.27±0.42 (27.90)	9.31±0.21 (34.60)
T3	14.24±0.33	13.43±0.42 (5.69)	12.63±0.22 (11.28)	10.95±0.23 (23.10)	9.69±0.22 (31.93)	8.13±0.22 (42.93)	7.74±0.34 (45.67)
T4	14.24±0.33	13.68±0.35 (3.96)	13.2±0.30 (6.79)	11.89±0.50 (16.53)	10.92±0.42 (23.31)	10.61±0.25 (25.49)	9.56±0.36 (32.87)
T5	14.24±0.33	13.81±0.23 (3.04)	13.58±0.41 (4.63)	11.17±0.36 (21.56)	10.13±0.46 (28.86)	9.78±0.59 (31.32)	9.37±0.26 (34.20)
T6	14.24±0.33	13.95±0.23 (2.01)	13.64±0.10 (4.21)	11.85±0.60 (16.81)	10.79±0.57 (24.25)	10.18±0.43 (28.51)	9.51±0.24 (33.19)
Mean	14.24±0.33	13.69±0.30 (3.88)	13.25±0.50 (6.95)	11.45±0.40 (19.58)	10.35±0.25 (27.40)	9.42±0.37 (33.84)	8.70±0.27 (38.93)
Range		13.14-14.12	12.63-13.64	10.71-12.02	9.48-11.08	7.56-10.61	6.68-9.56
SE (m) ±		0.14	0.13	0.21	0.14	0.22	0.15
CD		0.42	0.38	0.64	0.44	0.68	0.45
F Test		Sig	Sig	Sig	Sig	Sig	Sig

Values are mean of triplicates ± SD; Values in parenthesis = % Decrease over control

Table 3: Total Tannin content in *T arjuna* bark powder under different storage conditions

SN	% Total Tannin Content (MAS)						
	Feb-17 0 Month	**May-17 3 Months**	**Aug-17 6 Months**	**Nov-17 9 Months**	**Feb-18 12 months**	**May-18 15 Months**	**Aug-18 18 Months**
T1	13.28±0.37	12.2±0.29(7.69)	11.68±0.28(12.03)	9.68±0.28(27.07)	8.61±0.22(35.20)	6.71±0.61(49.46)	5.83±0.25(56.09)
T2	13.28±0.37	13.24±0.27(0.28)	12.56±0.21(5.44)	11.46±0.50(13.73)	10.41±0.40(21.64)	9.46±0.70(28.73)	8.46±0.21(36.29)
T3	13.28±0.37	12.55±0.39(5.48)	11.49±0.32(13.45)	9.99±0.07(24.80)	8.82±0.24(33.61)	7.29±0.34(45.07)	6.88±0.34(48.16)
T4	13.28±0.37	12.80±0.32(3.62)	12.26±0.13(7.68)	11.28±0.41(15.09)	10.38±0.49(21.87)	9.74±0.29(26.67)	8.71±0.36(34.43)
T5	13.28±0.37	12.93±0.25(2.64)	12.42±0.25(5.08)	11.02±0.34(17.04)	9.98±0.46(24.87)	8.99±0.34(32.32)	8.47±0.26(35.31)
T6	13.28±0.37	13.08±0.22(1.54)	12.57±0.24(5.33)	11.36±0.20(14.46)	10.28±0.11(22.57)	9.37±0.44(29.44)	8.64±0.24(34.97)
Mean	13.28±0.37	12.81±0.29(3.54)	12.20±0.24 (8.17)	10.80±0.30(18.70)	9.74±0.32 (26.63)	8.59±0.36(35.28)	7.83±0.27(40.87)
Range		12.20-13.24	11.49-12.57	9.68-11.46	8.61-10.41	6.71-9.74	5.83-8.71
SE (m) ±		0.14	0.10	0.15	0.12	0.22	0.15
CD		0.42	0.31	0.47	0.37	0.68	0.46
F Test		Sig	Sig	Sig	Sig	Sig	Sig

Values are mean of triplicates ± SD; Values in parenthesis = % Decrease over control

Table 4: Antioxidant Capacity in *T arjuna* bark powder under different storage conditions

SN	% Antioxidant Potential DPPH Assay IC 50 (µg/ml) (MAS)						
	Feb-17 0 Month	**May-17 3 Months**	**Aug-17 6 Months**	**Nov-17 9 Months**	**Feb-18 12 months**	**May-18 15 Months**	**Aug-18 18 Months**
T1	24.19±0.51	24.02±0.66	27.13±0.17	33.38±0.67	35.09± 0.62	38.30±0.74	43.03±0.60
T2	24.19±0.51	21.92±0.59	25.12±0.35	28.95±1.29	31.96± 0.17	35.50±0.67	39.88±1.22
T3	24.19±0.51	23.39±0.88	26.47±0.54	34.60±0.76	36.15± 1.03	39.47±1.16	44.04±1.00
T4	24.19±0.51	22.87±0.73	25.99±0.44	27.45±0.58	30.14± 0.56	33.69±0.78	38.06±1.38
T5	24.19±0.51	22.59±0.49	26.10±0.34	32.39±1.03	33.89± 0.30	36.98±0.40	41.79±1.18
T6	24.19±0.51	22.28±0.48	25.52±0.32	31.47±1.33	32.64± 0.62	36.08±0.86	40.55±1.27
Mean	24.19±0.51	22.85±0.64	26.06±0.36	31.37±0.95	33.31± 0.55	36.67±0.77	41.22±1.11
Range		21.92-24.02	25.12 -27.13	27.45-34.60	30.14-36.15	33.69-39.47	38.06-44.04
SE(m) ±		0.29	0.21	0.56	0.35	0.41	0.37
CD		0.89	0.65	1.69	1.07	1.24	1.13
F Test		Sig	Sig	Sig	Sig	Sig	Sig

Values are mean of triplicates ± SD

Conclusion

Among the evaluated packaging material for *T arjuna* bark powder, black poly bag was found to be superior for preserving total phenolic content, tannins and antioxidant potential.

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