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Variations in Tannin and Oxalic acid Content in *Terminalia arjuna* (Arjuna) Bark

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ABSTRACT

***Terminalia arjuna* (Arjuna),** belonging to family combretaceae, grows along the streams or rivers and often in the shallow streambeds and riverbeds in central India. It has been considered by the Ayurvedic physicians as well as by the modern practitioners as a cardiac tonic. Clinical evaluation indicated that it has been found beneficial in the treatment of coronary artery disease, heart failure, and possibly hypercholesterolemia. It has also been found to possess antibacterial, antimutagenic and antioxidant activities. Demand for *T. arjuna* bark, both in India and abroad has been increasing rapidly for over a decade. About 95 percent of the requirement is met from the wild and collected in a pattern that is not concomitant with sustainable harvesting practices. The quality of the bark is directly dependent on harvesting technique and time. There is also a clear relationship between the part of the plant harvested, harvesting method used, and the impact of these on the plant. Keeping above into consideration it has been planned to carry out systematic study on phytochemical investigation of Arjuna bark collected from various parts of the tree at different harvesting time. The bark samples were analyzed for tannin and oxalic acid. The tannin and oxalic acid content varied from 6.75 to 14.82 % and 7.66 to 20.05 % respectively in various samples of *T. arjuna* bark collected from various places of Madhya Pradesh. The middle-aged trees having GBH around 130 cm were found to contain more amount of tannin. The study gives important information to obtain better quality of *T. arjuna* bark on sustainable basis.

Keywords: *Terminalia arjuna*, sustainable harvesting, tannin, oxalic acid.

INTRODUCTION

Terminalia arjuna L. (Combretaceae) is a large deciduous tree found throughout India growing to a height of 20-25 metres. It commonly grows on banks of rivers, streams and dry watercourses in sub-Himalayan tract, Central and South India and West Bengal; also planted for shade or as ornamental tree in avenues and parks. It has been a common observation that the Arjun tree thrives better in areas with sandy soils or areas with shallow soil layer. Therefore ecologically it can be said that the natural abundance of Arjun is due to higher water table in the area. Our inquiries with the local people revealed that no one has planted Arjun specifically. Also there has not been any religious reason for abundance of Arjun trees. Nevertheless people are aware about its medicinal properties. Local people use the fallen leaves as manure and the woody fruits as a fuel. In addition Arjun serves as minor timber in making agricultural implements. In the case of Arjun trees on tank bunds and field bunds, it was the local people who planted them. Profuse seeding is another character of the Arjun tree. The seeds are wind dispersed. Farmers replant the germinated seedlings and saplings growing in their cultivation lands onto the bunds.

Bark of *T. arjuna* is flat or slightly curved, external surface pink or flesh colored with a mealy coating; inner surface reddish brown, finely striated, peeling out in thin flakes, odorless, gritty and astringent. The bark has been used in India's native Ayurvedic medicine for centuries, primarily as a cardiac tonic. Clinical evaluation of this botanical medicine indicates that it can be beneficial in the treatment of coronary artery disease, heart failure, and possibly hypercholesterolemia (1-2). It has also been found to

possess antibacterial, antioxidant and antimutagenic activities. The pharmacology of *T. arjuna* have been discussed (3) and pharmacological activities are mainly due to the tannins present in their barks.

Demand for *T. arjuna* bark, both in India and abroad has been growing rapidly for over a decade. Presently the bark of *T. arjuna* is being extracted through unscientific and destructive harvesting practices. Presently the collectors harvest the bark by making a blaze too deep and wide, damaging the cambium and ray cells responsible for the transport of nutrient and water from the roots to other parts of the tree. This is evident by many injured trees in natural habitat. About 95 percent of the requirement is met from the wild and collected in a pattern that is not concomitant with sustainable harvesting practices. Harvesting commercial quantity of bark can also affect tree population.

Although it is commonly believed that most tree species completely regenerate their bark after it has been damaged. *T. arjuna* trees are thick-barked and withstand fire damage, but are vulnerable to fungal or borer attack once their bark is removed. The ability to withstand bark damage offered the potential for sustainable harvesting of *T. arjuna* bark.

A fair amount of chemical work has been done on this plant. The major active constituents include tannins, triterpenoid saponins (arjunic acid, arjunolic acid, arjungenin, arjunglycosides), flavonoids (arjunone, arjunolone, luteolin), oxalic acid, gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs), phytosterols, calcium, magnesium, zinc, and copper (4-5). It contained unusually, large quantities of calcium salts with small amounts of aluminum and magnesium salts; about 12% of tannins, consisting mainly pyrocatechol tanning; an organic acid with a high melting point and a phytosterol; some coloring matters, and sugars, etc. *T. arjuna* bark has been found to be a potent source of oxalic acid (6-7). Many factors such as soil composition, water stress, temperature and humidity can affect levels of phenolics present in plants (8). Tannin content altered during the development of the plant and also as a response to the environmental changes (9-11). These variations influence directly the quality of the plant for medicinal use.

The quality of the bark is directly dependent on harvesting technique and time. There is also a clear relationship between the part of the plant harvested, harvesting method used, and the impact of these on the plant. Keeping above into consideration this study was carried out to find out the variation in tannin and oxalic acid content in *T. arjuna* bark.

MATERIALS AND METHODS

The surveys were conducted to different areas of Madhya Pradesh to select Arjun growing areas. Arjun trees of different age group and girth size were selected for extraction of bark. The experiments were laid out in the forest areas of Balaghat and Jabalpur as well as in the farmer's field on randomized block design for the extraction of bark. The girth of selected trees at breast height (GBH) ranged between 77-228 cm. Trees of less than 60 cm GBH were rejected. Care was taken not to include trees with pollarded crown, broken branches or those infected with fungi and insects.

Bark Harvesting

The bark of Arjuna was harvested by putting blazes of different sizes e.g.,19x30, 24x30, 30x30, 22.5x45, 25x45, 30x45, 32x45, 28x60, 30x60, 37.5x60, 41x60, 45x60, 38x90, 42x90, 57x90, 31x120, 45x120, 46x120 cm etc. The sizes of blaze were according to GBH and age of tree. The breadth of the blaze was ¼ or $1_{/3}$ of the girth of the trunk at breast height. However, the length of the blazes was 30,45,60,90 and 120 cm. The harvested bark was brought to laboratory for chemical analysis. The Fresh and dry weights of the bark were recorded. Data on regrowth (regeneration of bark) was recorded on quarterly basis. The physical appearance of bark regrowth was also recorded. The bark's regenerative properties were determined by the time taken to regenerate the bark.

Chemical analysis

Tannin content in the bark was estimated by Folin-Denis Method (12) and oxalic acid content by using methods of Bhatia(6).

Estimation of tannins: By Folin-Denis method

0.5 g of the powdered bark was taken in a 250 ml conical flask. 75-ml distilled water was added in it. The flask was gently heated and boiled for 30 minutes. Centrifuged at 2000 rpm for 20 minutes and filtered. The supernatant liquid was collected in 100 ml volumetric flask and the volume was made up 100 ml. 1ml of the sample extract was transferred to a 100ml volumetric flask containing 75-ml distilled water. Added 5 ml of Folin-Deins reagent, 10 ml of 35% sodium carbonate solution and diluted to 100 ml with distilled water. The solution was shaken well and the absorbance was read at 700 nm after 30 minutes. Blank was prepared with water instead of the sample. Standard graph was also prepared by using 0 - 100µg tannic acid. The tannin content of the samples as

tannic acid equivalents from the standard graph was calculated.

Determination of Oxalic acid

One part of the air-dried, water-extracted *T. arjuna* bark was treated with three parts of 20% H_2SO_4 acid at boiling temperature for one hour. After acid treatment the material was filtered while hot and the bark chips washed with hot water till free of acid (usually 3-4 washings). The filtrate was left overnight at room temperature when most of the calcium sulphate with some quantity of oxalic acid separated and was isolated by filtration. The filtrate was concentrated over a water-bath to a density of 1.15 or when the colour of the liquor just changed from red to dark red. At the stage oxalic acid crystals started appearing. The liquid was allowed to cool and oxalic acid crystals appeared in bulk. These crystals were separated by filtration and the filtrate was further concentrated to obtain a second crop of the oxalic acid crystals. A subsequent crop was also likewise obtained. The material obtained in the beginning with calcium sulphate and in 1st, 2nd and 3rd crops was dissolved in water; a pinch of activated carbon was added to the solution and the solution was warmed over water-bath. The solution was filtered and concentrated to obtain crystals of oxalic acid. Total oxalic acid was recrystallized using distilled water.

RESULTS AND DISCUSSION

The bark of *T. arjuna* was analysed for their tannin and oxalic acid content. The data pertaining to tree, blaze size, tannin and oxalic acid content is depicted in Table 1. Minimum and maximum girth of the trees selected for study was 77 cm and 228 cm respectively. Bark thickness at breast height ranged from 8.12 to 20.96 mm. Mean bark thickness at breast height in Arjuna trees was 15 mm. Mean thickness of bark varied from trees to trees. It is irrespective from the age/girth of tree. Mean bark yield per square centimeter ranged between 0.22 gm to 1.14 gm and found varying from tree to tree. The tannin content ranged from 6.75 to 14.82 gm per 100 gms. The amount of oxalic acid in the bark ranged between 7.66 gm to 20.05 gms per 100 gms. The variation was observed in tannin and oxalic acid content with season and age of the tree. The variation in protein, phenol, tannin, nitrate, oxalate in addition to vitamin C, anthocyanin and chlorophyll in the leaves was reported by Srivastava *et al*. 1997 (13). Seasonal variations in leaf tannins have already been reported for deciduous trees such as: *Quercus robur* (14) and *Betula pubescens* (11). These variations occurred during leaf growth and development, from spring to fall, and were related with herbivore resistance of plants. In the present study only bark tannins were evaluated and they also showed seasonal variations. Bark (inner bark is the potent part, and contains more tannin in comparison to dry inactive outer portion to the bark. Variation in tannin and oxalic acid content may be due to variation in climatic conditions (rainfall, humidity and mean temperature). Barks collected in the March contained higher amount of tannins (14.82%) followed by the bark collected in November.

Tannin and oxalic acid content in terms of girth/age of the tree is depicted in Table 2. The data revealed that there is direct relationship between tannin and oxalic acid content in terms of the girth of the tree. The trees of girth size 40 cm contained minimum amount of tannin (7.56%) and oxalic acid (11.54%) while trees of girth size 116 cm and 100 cm contained higher tannin (14.25 %) and oxalic acid (20.05 %).

Barks of different plant parts e.g. trunk, branch and twigs were also analysed for tannin and oxalic acid content. The data presented in Table 3 revealed that the trunk bark contains maximum amount of tannin (13.03 %) and oxalic acid (18.46%), whereas lowest tannin (6.32 %) and oxalic acid (10.08 %) content was determined from the bark of the twigs.

Sustainable harvest:

Remove only ¼ or 1/3 of the mature bark on total girth of the tree. Remove only outer and middle bark leaving the inner bark for regeneration. The bark should be dried in sun before storage. Regular field observations were taken on the recovery of bark. The stage of bark recovery (regrowth) varied from tree to tree. After one year, the stripped trees exhibited an average of 42% recovery based on surface area covered with fresh bark. Findings of studies conducted elsewhere indicated that some other factors like temperature, relative humidity and time of stripping influences wound healing in woody species. The trees in which the bark was harvested on 25th December 2003 by putting blaze size (30X30cm) showed almost complete bark recovery nearly after two years. However, few trees showed partial recovery of bark. The study shows that the technique of making of blaze on the tree also plays an important role in the recovery of bark. If the blaze is sharp the recovery is faster whereas if the blaze (cut) is not sharp the recovery is slow. The study shows that bark regeneration in Arjuna depends on the extent of damage on the cambium layer. With damaged cambium when the wound extends beyond the cambium and into the wood, the

Table 1. Tannin and oxalic acid content in Terminalia arjuna bark

Date of collection	Location	GBH cms	Blaze size cm	Thickness of bark (mm)	Bark wt. per cm2	Tannin %	Oxalic acid %
6.11.2004	Chillod, Balaghat	97	24x30	09.80	0.40	7.68	7.66
6.11.2004	Chillod, Balaghat	77	19x30	12.53	0.57	7.56	17.55
6.11.2004	Chillod, Balaghat	97	24x30	12.12	0.38	10.73	12.30
6.11.2004	Chillod, Balaghat	97	24x30	10.12	0.69	12.08	11.40
6.11.2004	Chillod, Balaghat	130	32x45	11.60	0.33	14.17	18.66
6.11.2004	Chillod, Balaghat	120	30x45	11.64	0.35	11.32	10.99
6.11.2004	Chillod, Balaghat	120	30x45	20.96	1.03	10.99	13.61
6.11.2004	Chillod, Balaghat	120	30x45	08.70	0.38	12.33	12.87
7.11.2004	Chikhlabaddi, Balabhat	152	38x90	15.68	0.78	11.70	13.47
7.11.2004	Chikhlabaddi, Balabhat	228	57x90	13.11	0.51	11.34	12.77
7.11.2004	Chikhlabaddi, Balabhat	172	42x90	20.94	1.14	10.55	14.51
7.11.2004	Chikhlabaddi, Balabhat	185	46x120	18.75	0.96	12.98	12.58
7.11.2004	Chikhlabaddi, Balabhat	125	31x120	10.80	0.40	13.65	13.69
7.11.2004	Chikhlabaddi, Balabhat	180	45x120	13.66	0.40	12.54	11.72
7.11.2004	Chikhlabaddi, Balabhat	144	30x60	16.18	0.58	12.43	13.40
7.11.2004	Chikhlabaddi, Balabhat	165	41x60	18.60	0.87	11.58	12.39
7.11.2004	Chikhlabaddi, Balabhat	112	28x60	13.12	0.27	7.31	13.52
28.7.2005	Dokarbandi, Karanjia	100	25x45	11.20	0.56	11.80	12.40
28.7.2005	Dokarbandi, Karanjia	95	30x45	9.32	0.44	11.24	13.68
28.7.2005	Dokarbandi, Karanjia	105	35x65	11.20	0.51	10.11	14.13
28.7.2005	Dokarbandi, Karanjia	115	37.5x60	10.65	0.42	12.12	13.57
28.7.2005	Karajiya, Balaghat	100	25x45	11.03	0.555	11.8	13.01
28.7.2005	Karajiya, Balaghat	90	30x45	9.40	0.444	11.24	14.3
28.7.2005	Karajiya, Balaghat	105	35x60	11.21	0.511	12.91	10.98
28.7.2005	Karajiya, Balaghat	115	37.5x60	10.40	0.422	12.12	16.66
28.7.2005	Chillod, Balaghat	120	30x30	10.00	0.611	11.56	7.66
28.7.2005	Chillod, Balaghat	90	22.5X45	11.00	0.790	12.31	17.55
28.7.2005	Chillod, Balaghat	130	45X60	13.00	0.814	8.11	12.30
28.7.2005	Chillod, Balaghat	92	30X45	13.02	0.370	10.28	11.40
28.7.2005	Chillod, Balaghat	144	36X60	12.13	0.687	8.82	18.66
28.7.2005	Chillod, Balaghat	130	30X60	10.00	0.527	6.75	10.99
01.9.2005	Kanjai, Balaghat	134	30x60	10.00	0.517	12.44	16.02
01.9.2005	Kanjai, Balaghat	130	30.5x60	12.00	0.589	14.24	13.19
01.9.2005	Kanjai, Balaghat	124	31x60	11.25	0.645	12.34	11.76
01.9.2005	Kanjai, Balaghat	90	22x45	12.00	0.595	11.76	14.52
01.9.2005	Kanjai, Balaghat	110	27.5x45	12.50	1.030	7.74	16.00
01.9.2005	Chillod, Balaghat	120	30x30	10.23	0.61	11.56	13.56
01.9.2005	Chillod, Balaghat	90	22.5x45	11.31	0.80	12.31	14.57
01.9.2005	Chillod, Balaghat	135	45x60	13.25	0.81	8.16	14.26
01.9.2005	Chillod, Balaghat	92	30x45	13.12	0.37	10.28	12.87
01.9.2005	Chillod, Balaghat	144	36x60	12.09	0.79	8.82	13.40
01.9.2005	Chillod, Balaghat	130	30x60	8.12	0.53	6.76	12.56
02.9.2005	Bhandaruri, Balaghat	134	33x60	10.12	0.58	12.46	16.02
02.9.2005	Bhandaruri, Balaghat	130	32.5x60	12.30	0.59	14.29	13.01
02.9.2005	Bhandaruri, Balaghat	124	31x60	11.13	0.65	12.34	13.19
02.9.2005	Bhandaruri, Balaghat	90	22.5x45	12.14	0.66	13.36	11.76
02.9.2005	Bhandaruri, Balaghat	110	27.5x45	12.15	0.53	7.74	14.52
02.9.2005	Bhandaruri, Balaghat	137	32x45	15.23	0.74	12.13	16.00
02.9.2005	Bhandaruri, Balaghat	172	43x45	14.33	0.62	11.84	10.55
02.9.2005	Bhandaruri, Balaghat	210	70x45	14.20	0.49	12.54	18.46
02.9.2005	Bhandaruri, Balaghat	170	60x45	13.18	0.58	12.44	13.40
13.1.2006	Barha, Jabalpur	280	70x70	8.12	0.549	11.81	16.62
13.1.2006	Barha, Jabalpur	150	40x45	11.00	0.638	13.37	18.08
13.1.2006	Barha, Jabalpur	80	20x45	7.15	0.222	13.66	12.88
13.1.2006	Barha, Jabalpur	140	36x45	10.21	0.570	12.15	14.66
13.1.2006	Barha, Jabalpur	131	36x60	10.18	0.497	9.98	16.00

03.3.2006	Dokarbandi, Balaghat	97	25x75	10.00	0.613	12.08	12.40
03.3.2006	Dokarbandi, Balaghat	98	30x80	9.50	0.500	11.69	13.68
03.3.2006	Dokarbandi, Balaghat	80	25x50	11.00	0.460	14.82	14.13
03.3.2006	Dokarbandi, Balaghat	150	50x100	12.20	0.325	13.79	13.51
03.3.2006	Dokarbandi, Balaghat	110	40x90	8.33	0.319	12.86	13.26

Table 2. Tannin and oxalic acid content in Terminalia arjuna bark

Girth (cm)	Blaze size (cm)	Tannin content %	Oxalic acid % (gm/100 gm)
40	10X25	7.56	11.54
50	12.5X25	8.25	15.23
60	15X25	8.56	13.52
100	25X30	13.59	20.05
116	29X40	14.25	18.55
117	29X40	13.85	16.55

Table 3. Tannin and oxalic acid content in different parts of Terminalia arjuna bark

Date of collection	Location	Plant parts	Blaze size cm	Bark wt. per cm2	Tannin content %	Oxalic acid %
13.01.06	Barha, Jabalpur	Trunk	35x60	0.523	13.03	18.46
13.01.06	--do--	Branch	30x12	0.347	8.52	15.52
13.01.06	--do--	Twig	30x12	0.152	6.32	10.08

cut may not heal or if it does, very slowly, exposing it to fungal and insect attack. Some insect and fungal infestations were observed on the blazes. Exposed part of the trunk was attached by the insects, but the damage was not severe. In some trees gums oozed out from the blazes. We did not observe any adverse trend on the overall development of tree. No tree was found to die after harvesting of bark. Although Arjuna shows remarkable bark regrowth in moist sites but this would be very early to predict at this stage of study.

Research Needs

Research needs to be undertaken on the selection of fast growing, high active-ingredient yielding *T . arjuna* cultivars.

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REFERENCES

1. R. Alpana, P. Laurai, R. Gupta, P. Kumar and V.N. Sharma. Hypochesreloamic effects of *Terminala arjuna* tree bark. *Journal of Ethnopharmacology,* **55**:65-67(1997).
2. R. Chander, K. Singh, A.K. Khanna, S.M. Kaul, A. Puri, R. Saxena, G. Bhatia, F. Rizvi and A.K. Rastogi. Antidyslipidemic and antioxidant activities of different fractions of *Terminalia arjuna* stem bark. *Indian Journal of Clinical Biochemistry*. **19(2):** 41-148 (2004).
3. D.S. Kumar and Y.S. Prabhakar. On the ethnomedical significance of the Arjun tree, *Terminalia arjuna* (Roxb.) Wight & Arnot. *J Ethnopharmacol.* **20(2):**173-90 (1987).
4. S. Dwivedi and N. Udupa. *Terminalia arjuna*: Pharmacognosy, Phytochemistry, Pharmacology and clinical use. A review. *Fitoterapia*, **60:**413-420 (1989).
5. A. Ali, S.T. Abdullah, H. Hamid M. Ali, and M.S. Alam. Two new pentacyclic triterpenoid glycosides from the bark of *Terminalia arjuna*. *Indian Journal of Chemistry*, **42B**(11):2905-2908 (2003).
6. K. Bhatia, Jia Lal and Mohd. Swalesh. Utilization of barks of *Terminalia* species from Uttar Pradesh. *Indian Forester*. **103**:273-280 (1977).
7. K. Bhatia and K.S. Ayyar. Barks of Terminalia species-A new source of oxalic acid. *Indian Forester*. **106(5)**:363-367 (1980).
8. M. Kouki and Y. Manetas. Resource availability affects differentially the levels of gallotannins and condensed tannins in *Ceratonia siliqua*. *Biochem Syst. Ecol*. **30:**631-639 (2002).
9. T. Hatano, R. Kira, M. Yoshizaki and T. Okuda. Seasonal changes in the tannins of *Liquidanbar formosana* reflecting their biogenesis. *Phytochemistry*. **25:**2787-2789 (1986).

10. S.C. Santos, W.F. Costa, J.P. Ribeiro, D.O. Guimarães, P.H. Ferri, H.D. Ferreira, and J.C. Seraphin, Tannin composition of barbatimao species. *Fitoterapia.* **73:** 292-299 (2002).
11. J.P. Salminen, V. Ossipov, E. Haukioja and K. Pihlaja. Seasonal variation in the content of hydrolysable tannins in the leaves of *Betula pubescens*. *Phytochemistry*, **57(1):**15-22 (2001).
12. S.H. Schanderi. Methods in Food Analysis, Academic Press. New York, p. 709 (1970).
13. N. Srivastava, D. Prakash and H.M. Behl. Biochemical contents, their variation and changes in free amino acids during seed germination in *Terminalia arjuna*. *Int J Food Sci Nutr*. **48(3):**215-9 (1997).
14. P. Feeny. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillar. *Ecology.* **51:** 565-581 (1970).

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