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Research Article

PHARMACOGNOSTICAL EVALUATION OF STEM BARK OF *TERMINALIA ARJUNA*

UDAYSING HARI PATIL*[1] AND DATTATRAYA K. GAIKWAD[2]

***[1]Department of Botany, Bhogawati Mahavidyalaya, Kurukali. Tal-Karveer, Dist- Kolhapur [MS] India-416001, [2]Department of Botany, Shivaji University Kolhapur, Maharashtra, India-416004. Email: superoxide2311@gmail.com**

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ABSTRACT

Arjuna is potent cardiotonic in the Ayurveda. The bark was analyzed with respect to reducing sugars, total sugars, amylose, amylopectin, starch, crude fibers, and crude protein, total ash value, total polyphenols, water soluble tannins, total flavonoids, total alkaloids, nitrates, and total oxalate. The concentration of phytoconstituents except oxalate and total ash was recorded maximum in the apical stem bark than the middle and mature inner bark. The oxalate and total ash were higher in the mature inner bark than the apical stem bark and middle bark samples. The present study enrolls biochemical standardization of bark in the pharmacological point of view.

Key words: *Terminalia arjuna,* Total polyphenols, Tannins, Total flavonoids, Total alkaloids.

INTRODUCTION

Terminalia arjuna (Wight & Arn.) is an important medicinal plant, belongs to the family Combretaceae. *T. arjuna* is distributed throughout India, Burma and Sri-Lanka[1]. It mainly grows along the banks of the river and streams[2]. Bark powder boiled with water and inhaled to cure headache and to kill worms in the teeth[3]. The powdered bark is useful into the treatment of heart troubles[4], juice is used as antacid[5] and fruits are found useful as tonic[2]. Bark ash is used in the treatment of the snakebite and scorpion sting[4]. Bark is useful as expectorant, aphrodisiac, tonic and diuretic[2]. In the present study an attempt has been made to standardize this cardiotonic drug pharmacognosticaly.

MATERIALS AND METHODS

Different bark samples (apical stem bark, middle bark and mature inner bark) of *Terminalia arjuna* were collected from the hilly regions Radhanagari of Kolhapur district. In the winter season the bark was collected in the month of January and summer collection was followed in the month of May. The bark samples were cut into pieces, sun-dried then oven dried at 60^{0}C. Dried bark samples were ground into powder and stored in an air tight plastic container.

Qualitative analysis

Preliminary phytochemical analysis was carried according to the standard methods[6, 7].

Quantitative analysis

Reducing sugars

The reducing sugars were estimated according to the method introduced by Nelson[13]. The soluble sugars were extracted from 0.250mg of oven dried powdered bark tissue with 80% ethanol. The extract was filtered through Buckner's funnel using Whatman No. 1 filter paper. The filtrate obtained was condensed to 5 ml on water bath and to this 2g lead acetate and potassium oxalate (1:1) were added for decolourization. To this 40ml distilled water was added and the solution was filtered. The volume of filtrate was measured and it served as an extract for determination of reducing sugars. To the test tube containing 0.4mL plant extract, distilled water was added and volume was adjusted to 1ml. 1ml Somogyi's alkaline copper tartarate reagent was added to each test tube and all these test tubes were transferred to boiling water bath for 10 minutes. Tubes were removed and cooled to room temperature and 1ml Arsenomolybdate reagent was added to each test tube. The contents were diluted to 10mL with distilled water and after 10 minutes, the absorbance of the reaction mixture was measured at 660nm on UV-visible double beam spectrophometer (Shimdtzu UV-190). The amount of reducing sugar was calculated with the help of standard curve obtained by using different concentration of standard glucose solution (0.1mg/ml) and was expressed in g.100g^{-1} dry tissue.

Total sugars

Phenol-sulphuric acid method described by Dey[14] was used to estimate total sugars. The plant material (0.250mg of oven dried bark powder) was suspended in 20ml of 90% ethanol in 50mL test tube. The test tubes were sealed with cork and the suspension was incubated for one hour in hot water bath maintained at 60^{0}C. The extract was filtered and the filtrate was collected in 25ml capacity volumetric flask. The residue was re-extracted with another 10ml volume of 90% ethanol. Both the fractions were collected and final volume was made 25ml with 90% ethanol. For the estimation, 0.2ml plant extract was taken in a test tube and volume was made 1ml with distilled water. 1ml (5%) phenol was carefully added and mixed thoroughly. To these test tubes, 5ml concentrated sulphuric acid (analytical grade) was added rapidly but very carefully. This was mixed thoroughly by vertical agitation with a glass rod. The mixture was cooled at room temperature in air and the absorbance was recorded at 485nm against blank containing distilled water instead plant extract. The amount of soluble sugars was estimated with the help of standard curve of glucose (0.1mg/ml) and expressed in g.100g^{-1} dry tissue.

Starch content

For estimation of the starch, the insoluble residue along with the filter paper obtained after filtering the alcoholic extract of reducing sugar was transferred to a 100ml capacity conical flask containing 50 ml distilled water and 5ml concentrated HCL. The contents were hydrolyzed at 15lbs pressure for half an hour. The contents were cooled to room temperature and neutralized by addition of anhydrous sodium carbonate and filtered through Buckner's funnel. The volume of filtrate was measured and used for the further analysis of starch. Filtrate contains reducing sugars (glucose) formed as a result of hydrolysis of starch. These sugars represent the starch content in the residue. Other steps are essentially similar as described for estimation of reducing sugars. The amount of starch was estimated with the help of standard curve obtained by using different concentration of standard glucose solution (0.1mg/ml^{-1}) and was expressed in g.100g^{-1} dry tissue.

Amylose content

Amylose content was estimated according to the method described by Sadasivam and Manickam[15]. Powdered bark 250mg was taken in 100ml beaker. To this, 1ml distilled ethanol and 10mL 1N NaOH was added and digested on boiling water bath for 10 minutes. The reaction mixture was cooled and diluted to 100mL with distilled water. This extract was used for the estimation of amylose. In test tubes containing 2.5ml plant extract, 20mL of distilled water and few drops of phenolphthalein indicator (0.1%) were added. To each test tube 0.1N HCl was added until the pink colour disappeared. 1ml iodine reagent (1g of iodine and 10g of KI dissolved in water and

diluted to 500mL) was added and volume was adjusted to 50mL. The intensity of the colour measured at 590nm on double beam UV-spectrophotometer. The amylose contents were calculated with the help of standard curve obtained by using different concentrations of standard amylose solution (1mg/mL) and expressed as g.100g^{-1} of dry tissue.

Amylopectin content

Amount of Amylopectin was calculated by subtracting the amylose content from the starch content and expressed as g.100g^{-1} of dry weight.

Crude fiber content

Crude fiber contents in the bark samples were estimated according to the method described by Maynard[16]. 2g of oven dried bark powder was transferred to 500ml conical flask and 200ml 0.255N H_2SO_4 was added to it. The contents were boiled for 30 minutes with bumping chips on hot plate. The flask was cooled and the contents filtered through muslin cloth. The residue was washed several times with hot distilled water. The residue thus obtained boiled with 200ml, 0.313N NaOH (1.25g of NaOH dissolved in 100ml distilled water). The contents were filtered through muslin cloth and the residue washed with 25ml, 1.25% H_2SO_4, three portions of water 50ml each and 25ml alcohol. The residue was removed and transferred to pre-weighed ashing dish (W_1g). The residue was dried at 130 ± 2^{0}C for 2hr. Ashing dish was cooled and weighed (W_2g). The residue was ignited at 600 ± 15^{0}C. Ashing dish was cooled and weighed (W_3g). Crude fiber contents in the bark samples were calculated by using following formula and expressed g.100g^{-1} of dry weight.

$$\frac{\text{Loss in weight on ignition (W2 − W1) − (W3 − W1)}}{\text{Weight of the sample}} \text{X } 100$$

Total polyphenols

Total polyphenol contents were estimated according to the method given by Folin and Denis[17]. Dry powdered bark (500mg) was homogenized in 15ml acetone (80%) and filtered through Buckner's funnel. The residue was washed several times with 80% acetone and final volume was adjusted to 50ml with 80% acetone. The reaction mixture in Nessler's tubes contained 1ml plant extract, 10ml 20% Na_2CO_3 and 2ml Folin-Denis reagent (prepared by mixing 100g of sodium tungstate with 20g phosphomolybdic acid in about 800ml distilled water and 200ml, 25% Phosphoric acid and the mixture was refluxed for 2-3 hours, cooled to room temperature and final volume was adjusted to 1000ml with distilled water). Final volume of the reaction mixture was adjusted to 50ml with distilled water and allowed to develop blue colour. After 20 minutes absorbance of blue colour developed was measured at 660nm on double beam UV-visible spectrophotometer. Total polyphenols were calculated with the help of standard curve of tannic acid (0.1mg/ml) and expressed as g.100g^{-1} dry weight.

Water soluble tannins

Method of Schanderl[18] was employed for determination of water soluble tannins. 500mg of powdered bark sample along with 75ml distilled water were transferred to 250ml capacity conical flask. The flask was gently heated on hot plate and material boiled for 30 minute. The contents was cooled to room temperature and centrifuged at 2000rpm for 20 minutes. The residue was discarded and volume of supernatant was adjusted to 100ml with distilled water. This extract was used for the estimation of the tannins in the bark samples. 1ml of the tannin source was transferred to 100ml capacity volumetric flask containing 75ml distilled water. To this, 5ml Folin-Denis reagent and 10ml sodium carbonate solution were and diluted to 100ml with distilled water. Contents in the flasks were thoroughly mixed and absorbance was measured after 30 minutes at 700nm on double beam UV-visible spectrophotometer (Shimadzu-190). A blank was prepared with water instead of the sample. Amount of water soluble tannins was calculated with the help of standard curve of tannic acid and expressed as g.100g^{-1} of dry weight.

Total flavonoids

Total flavonoids were estimated according to method given by Luximon-Ramma *et al.*[19]. Powdered bark (500mg) was extracted in 10ml acetone (80%) using mortar and pestle. The homogenate was filtered through Buckner's funnel using Whatman No. 1 filter paper. The volume of filtrate adjusted to 50ml with 80 % acetone. The reaction mixture contained 1.5ml the plant extract and 1.5ml, 2% methanolic Aluminum Chloride (2g Aluminium chloride dissolved in 100ml pure methanol). Blank was prepared with distilled water in place of sample. The absorbance of reaction mixture was measured at 367.5nm on a UV-visible double beam spectrophotometer (Shimadzu-190). Total flavonoid contents were calculated with help of standard curve of rutin (0.3mg/ml) and expressed as g.100g^{-1} of dry weight.

Total alkaloids

A method described by Singh *et al.*[20] was followed to determine the total alkaloid contents in the bark samples. 100mg bark powder was extracted in 10ml 80% ethanol. This was filtered through muslin cloth and centrifuged at 5000rpm for 10 min. Supernatant obtained was used for the further estimation total alkaloids. The reaction mixture contained 1ml plant extract, 1ml of 0.025M $FeCl_3$ in 0.5M HCl and 1ml of 0.05M of 1,10- phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70 ± 2^{0}C. The absorbance of red colour was measured at 510nm against reagent blank. Alkaloid contents were estimated and calculated with the help of standard curve of colchicines (0.1mg/ml) and expressed as g.100g^{-1}of dry weight.

Crude protein content

Crude protein contents were calculated by multiplying the total nitrogen content by factor 6.25.

Nitrate content

The nitrate contents in bark powder were determined using method given by Cataldo *et al.*[21]. In test tubes containing 100mg of dry bark powder, 10ml of de-ionized water was added. The suspension was incubated at 45^{0}C for one hour. After incubation, sample was centrifuged at 5000rpm for 15 minutes. The residue was discarded and the supernatant was taken for nitrate estimation. In 50ml test tubes, 0.2ml extract was mixed thoroughly with 0.8ml, 5% (w/v) salicylic acid (prepared in concentrated H_2SO_4). After 20 minutes at room temperature, 19ml of 2N NaOH was added slowly to raise the pH above 12. Samples were cooled to room temperature and absorbance was measured on a double beam spectrophotometer at 410nm. The amount of nitrate (µg of NO_3.g^{-1} dry tissue) was calculated with the help of a standard curve obtained by taking different concentrations of KNO_3.

Oxalic acid content

The oxalic acid contents were estimated according to the method given by Abaza *et al.*[22]. For estimation of oxalic acid, 1g powdered bark, 10ml 3N HCl and 65ml double distilled water were taken in 100ml capacity volumetric flask. The flask was kept in boiling water bath for 1hr to digest the plant material. After digestion flask was removed, cooled and diluted up to the mark of 100ml and filtered through Whatman No. 1 filter paper. Two aliquots of 50ml extract were placed in 150ml beakers and 20ml 6N HCl were added in each beaker to increase acidity and to avoid pectin retention. The mixture was evaporated to half of its original volume and filtered through Whatman No. 1 filter paper. The precipitate on the filter paper was washed several times with warm double distilled water. To this filtrate, 3-4 drops of methyl red indicator (0.01% in alcohol) were added and then concentrated ammonia solution was added until the solution turned faint yellow.The solution was heated carefully on water bath maintained at 90-100^{0}C, cooled and filtered to remove interfering ferrous ions containing precipitate. The filtrate thus obtained was heated to 90-100^{0}C on water bath and to this 10ml 5% $CaCl_2$ was immediately added along with 20-25 drops of ammonia solution to restore yellow colour. The solution was kept overnight to settle the precipitate. On next day, the solution was filtered through

ashless filter paper (Whatman Filter Paper No. 44). The precipitate on the filter paper was washed several times with double distilled water to make free from Ca (to check whether the ppt is free from Ca^{++} or not, few drops of 5% sodium oxalate were added to 3ml of washing filtrate in test tube. Formation of turbidity indicated presence of Ca^{+2} and demanded further washing of ppt). Residue along with filter paper was dissolved in hot 1:5 H_2SO_4 and diluted to 125ml with double distilled water and transferred to 250ml conical flask. Contents of the conical flask were heated to 90 – 100^{0}C and titrated against 0.05N $KMnO_4$. The percentage of oxalate was calculated by using following formula,

$$\frac{\text{'ml' of KMnO4 used x 0.05 x 45.02 x 100}}{\text{1000 x dry weight x 50/100}}$$

Total ash content

Total ash content was determined by the method described in Indian Pharmacopeias[23].1g of dry powdered bark was accurately eighed and transferred to the previously ignited and weighed silica crucible. The bark powder was spread at the bottom of the crucible and the crucible incinerated at a high temperature not exceeding 450^{0}C until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight and the percent of total ash was calculated with reference to the air dried powder.

RESULTS AND DISCUSSION

The physico-chemical parameters are mainly used in judging the purity and quality of the drug. Hence, the present investigation deals with the biochemical evaluation of bark of potent cardiotonic *Terminalia arjuna.* Qualitative analysis of methanolic extract of different bark samples of *Terminalia arjuna* is shown in the Table No. 1. Table shows that the concentration of the secondary metabolites was higher in the apical stem bark than middle and mature inner bark. Quantitative estimation of different phytochemicals during summer and winter seasons is shown in the Table No. 2. The concentration of reducing sugars, total sugars.

Table 1: Qualitative analysis of stem bark samples of *Terminalia arjuna*.

Sr. no.	Parameter	Samples		
		Apical bark	Middle bark	Mature Inner Bark
1	Polyphenol	+++	++	+
2	Flavonoid	+++	++	+
3	Tannin	+++	++	+
4	Alkaloid	+++	++	+
5	Flavones	+++	++	+
6	Terpenoids	+++	++	+
7	Saponins	+++	++	+
8	Cardiac glycosides	+++	++	+
9	Sterols	+++	++	+

'+++'- High concentration; +'+'- Moderate concentration; '+'- low concentration and '-'absent

Table 2: Quantitative evaluation of phytochemicals

Sr. no.	Parameter	Samples					
		Apical Bark		Middle Bark		Mature inner Bark	
		Summer	Winter	Summer	Winter	Summer	Winter
1	Reducing Sugars	6.54	4.33	5.50	3.83	5.10	3.24
2	Total Sugars	11.69	10.87	11.23	10.35	10.51	9.59
3	Amylopectin	14.66	18.70	12.32	17.20	12.08	16.79
4	Amylose	0.65	0.75	0.58	0.73	0.54	0.71
5	Starch	15.31	19.44	12.90	17.93	12.62	17.49
6	Crude fiber	16.2	18.9	12.55	15.85	12.3	14.7
7	Crude Protein	11.94	4.34	7.60	3.33	5.80	1.08
8	Total ash	21.30	18.5	25.1	21.1	31.3	29.7
9	Total Polyphenols	20.42	14.92	19.14	14.27	15.24	13.89
10	Tannin	6.80	6.25	6.27	5.73	5.31	4.50
11	Flavonoid	1.58	0.87	1.46	0.72	1.38	0.54
12	Total Alkaloid	2.12	1.44	1.99	1.34	1.88	1.13
13	Total Oxalate	6.75	5.94	8.04	6.26	8.97	6.59
14	Nitrate (µg of $NO_3.g^{-1}$ dry tissue)	5492	5464	5082	5055	4945	4508

crude fibers, crude protein and inorganic residual ash was higher during summer while that of amylose, amylopectin and starch was marked higher during winter. Higher level of reducing sugars were estimated for apical stem bark (6.54%) during summer and mature inner bark (2.89%) during winter accumulated less amount of reducing sugars. The reducing sugar contents reported in the present study are much higher than Black locust bark (0.9% to 3.2%) [19]. Similar pattern of total sugar accumulation was noticed during summer and winter where, sugar contents fluctuated from maximum value of 11.66% in apical stem bark to its minimum 9.29% in mature inner bark during summer and winter seasons respectively. Total sugars in the middle bark varied in between apical and mature inner bark i.e. from 10.35-11.23%. Sugars estimated in our study falls in the range of *Saraca declinata* (11-12%) and *Saraca asoca* (15%) reported by Khatoon *et al.*[20] and *Ficus racemosa* (5.70 ± 0.06%) on fresh weight basis and 15.0 ± 0.15% on dry weight basis[21]. Cold winter accumulated maximum level of amylopectin in apical bark (18.70%) while, lowest quantity was determined in mature inner bark (12.08%) during summer. Amylose also showed its maximum level during winter in apical stem bark (0.75%) and low level in mature inner bark (0.54%) during hot summer. Accumulation of photosynthetic product starch was noticed higher during winter in apical stem bark (19.44%) and decreased during summer to its minimum 12.62% in mature inner bark. Amount of starch estimated for middle bark was in between apical and mature inner bark i.e. 12.32-17.20%. It is clear from the results that no significant changes have been noticed in the starch, amylose and amylopectin content during summer and winter cold. The starch content in the bark of *Terminalia arjuna* varied from 12.62-19.44% which is much higher than Ash tree (6.9%), Alder

(2.8-2.9%), Oak (2.5-2.7%), Maple (0.5- 0.6%) and Birch (0.3-0.4%)[22] and lower than the starch in the bark tissue of *Polyalthea longifolia,* (65-70%) and *Saraca declinata* (51-52%) reported by Khatoon *et al*.[20]. Quantity of crude fiber was maximum in apical stem bark (18.9%) during winter and lowest amount was recorded in mature inner bark (12.3%) during summer and that of middle bark was moderate (12.55-15.85%). Crude fiber content estimated in our study are in the range of *Ficus racemosa* (20.5%) [21], Shri Lankan Cinnamon (21.27%) [23] and *Desmodium oojeinense* (14.49%)[24] whereas, fiber content estimated from the barks of *Careya arborea* (51%)*, Gmelina arborea* (50%) and *Shorea robusta* (40%) by Santra *et al*.[25] are higher than reported in present investigation. Crude proteins estimated were found to be increased during summer and the amount was significantly higher in summer than winter. Higher contents of crude proteins were noticed in apical stem bark (11.94%) and least value was obtained for mature inner bark (1.08%). Protein value determined for Arjuna bark in the present study correlates with *Gmelina arborea* (11.37%) *Careya arborea* (5.37%) as reported by Santra *et al*.[25] and Shri Lankan Cinnamon (4.99 ± 0.10 %%) [23].

No marked differences were recorded in total polyphenols during winter among three bark samples but, their concentration was increased during summer. These were more concentrated in the apical stem bark (20.42%) and comparatively lesser quantity of polyphenolic compounds was observed in mature inner bark (13.89%) during winter. Polyphenols estimated by Zahin et al[26] in *Holarrhena antidysenterica* (5.95%) are lower than our results. The values determined in our study are lower than the bark of *Khaya senegalensis* (47.19%) and Pterocarpus *erinaceus* (40.80%) as estimated by Karou *et al.*[27]. Amount of tannins was elevated during summer conditions whereas; level of tannins was decreased during winter in the order of- apical bark > middle bark > mature inner bark. There are no large differences observed in the tannin content among the different bark samples during both seasons. Maximum tannin contents were reported in apical stem bark (6.80%) and lower value was determined in mature inner bark (4.50%). These figures significantly correlates with *T. arjuna* bark (6.75%) [28]. There were no considerable differences observed in the flavonoid content among the three bark samples in the summer and winter but, the flavonoid level was elicited twice during summer as compared to winter. Higher concentration was reported in apical bark (1.58%) during summer and the amount of flavonoids figured minimum in mature inner bark (0.54%). Flavonoid estimated in the bark of *Terminalia arjuna* in the present investigation are in range of *Terminalia arjuna* (1.59%) and *Eugenia jambolana* (1.68%) as reported by Sultana *et al*.[26]. Alkaloid fractions in the Arjuna bark were in the range between 1-2.25%. Maximum concentration was noticed during summer in the apical bark (2.12%) while lower value 1.13% was recorded for mature inner bark during winter. Alkaloid levels estimated for *Terminalia arjuna* are in the range of *Delphinium occidentale* (1.1%) [29] and *Rauvolfia serpentina* (1.62 % to 1.83%) [30].

Ash value of a drug gives an idea of the earthy matter or inorganic composition or other impurities present along with the drug. Total ash content determined in the bark samples ranged between 31.5-19% and increased during summer. High ash value was noticed in the mature inner bark (31.3%) during summer and low values were reported in middle (21.1%) and apical bark (18.5%) during winter. These ash content are lower than *Vatica simalurensis* (26.15%) [31] and higher than Cinnamon bark (2.4%)[32] and *Acacia catechu* (1.16-2.0%)[33].

No marked increase in the oxalate content among the three bark samples was noticed during winter but, total oxalate levels increased during summer than winter. Mature inner bark accounted maximum oxalate value (8.97%) and minimum value was observed in the apical stem bark (5.94%). Pandey and Kori[28] reported 7.66 to 20.05% oxalate in *T. arjuna* bark. Oxalate contents determined in the present study correlates with the reports by Pandey and Kori[28]. Other antinutritional factor nitrate was found to be concentrated during summer in the apical stem bark (5492µg/g) and mature inner bark (4508µg/g) showed lowest value of nitrate accumulation. These values are lower than the values recorded for white radish (20421µg/g) Celery (10919µg/g) and Red radish (7266µg/g) by Taras[34]. Similar pattern bimolecular accumulation was studied in the barks of *Anogeissus latifolia*[35] and *Pterocarpus marsupium* [36].

CONCLUSION

The bark of *Terminalia arjuna* contains appreciable amount of secondary metabolite. These phytoconstituents may acts as resource of pharmacologically active agents and natural antioxidants. The present evaluation of various biochemical parameters will be helpful while standardizing the drug for its various pharmacological potentials and to check the adulteration in natural valuable drug at the time of consumption for desire pharmacological effect.

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