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# Phytochemical Evaluation and Radical Scavenging Activity of *Bauhinia variegata, Saraca asoka* and *Terminalia arjuna* Barks

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## ABSTRACT

Phytochemical estimation and radical scavenging activity were carried out in the barks of *Bauhinia variegata, Saraca asoka* and *Terminalia arjuna* collected from central India with the objective to determine optimum harvesting age (girth class). Bark samples were analyzed for total phenols, flavonoids and tannins. Radical scavenging activity of the barks was evaluated using DPPH assay. Significant variation was observed in total phenols and tannins in *B. variegata*; flavonoids and tannins in *T. arjuna* with respect to different girth classes. However, no significant variation was found in chemical constituents among different girth classes of *S. asoka*. Gallic acid was used as standard having $IC_{50}$ value of 1.06±0.10 μg $mL^{-1}$. Radical scavenging activity showed significant variation in the barks of different girth classes of *B. variegata, S. asoka* and *T. arjuna*. The results revealed that the optimum girth class to harvest barks of *B. variegata* was 36-55 cm, *S. asoka* 61-90 cm and *T. arjuna* 121-150 cm as these were found to contain maximum concentration of active ingredients and possess high radical scavenging activity. Among the studied species *T. arjuna* showed maximum radical scavenging activity and thus can be termed as a potent antioxidant species whose bark can be used for making various formulations containing natural antioxidants.

**Key words:** Phytoconstituents, polyphenols, antioxidant activity, harvesting age, flavonoids

## INTRODUCTION

*Bauhinia variegata, Saraca asoka* and *Terminalia arjuna* are important medicinal trees being used in several herbal formulations (Vileges *et al*., 1997; Paarakh, 2010). The therapeutic properties of herbal drugs are due to the presence of certain chemical constituents (secondary metabolites) present in plant which varies according to their age and maturity (Pandey and Kori 2009). Among different chemical constituents polyphenols (flavonoids, phenolics, condensed and hydrolysable tannins) are major bioactive compounds responsible for the prevention of chronic diseases and health care (Sati *et al*., 2010). They have been reported to exert antiinflammatory, antimicrobial, antioxidant, anticarcinogenic and body mass reducing activities (Arts and Hollman, 2005; Youdim *et al*., 2004).

*Bauhinia variegata* Linn. (Kachnar) belonging to family Fabaceae is a medium sized deciduous tree with a short bole and spreading crown. The various plant parts viz., stem, stem bark, leaves, seeds, roots, flower buds and flowers are used in various indigenous systems of medicine and

popular among the various ethnic groups in India for the cure of variety of ailments. The bark is astringent, antileprotic, antigoitrogenic, antitumour and used in fever, skin diseases and wound healing (Kurien, 2001; Daniel, 2006; Thakur *et al*., 1992; Singh and Aswal, 1992). It is also reported to be useful in obesity. The leaves are used in treatment of skin diseases and stomatitis (Balajirao *et al*., 1995). The roots of the plant are used as an antidote for snake poisoning, in dyspepsia, flatulence and as carminative (Kurien, 2001). The stem bark is reported to contain 5,7-dihydroxy and 5,7-dimethoxy flavanone-4-O-α-L rhamnopyrosyl-β-D-glycopyranosides, kaempferol-3-glucoside, lupeol and betasitosterol (Yadava and Reddy, 2001; Zhao *et al*., 2005).

*Saraca asoca* Roxb. De Wilde. (Ashoka) belonging to family Fabaceae is a medium sized evergreen tree upto 9 m in height with numerous spreading and drooping glabrous branches. Bark is bitter and used as astringent, anthelmintic, stomachic and in various other diseases. The bark has a stimulating effect on the endometrium and ovarian tissue and is useful in menorrhagia during uterine fibroids. It also has great benefits for its uterine activity (Satyavati *et al*., 1970). Leaves are useful in stomachalgia and flowers are use in vitiated condition of pitta, syphilis, hyperdipsia, inflammation, dysentery, haemorrhoids and scabies in children (Nadkarni and Nadkarni, 2005). Chemical investigation showed the presence of catechols, sterols, tannins, flavonoids, glycosides, leucopelargonidin and leucocyanidin in bark (Sadhu *et al*., 2007).

*Terminalia arjuna* Roxb. Wight and Arn. (Arjuna) belonging to family Combretaceae is a large deciduous and evergreen tree, standing 20-30 m above ground level with fissured bark and numerous dropping branches. It has been considered by the Ayurvedic physicians as well as by the modern practitioners as a cardiac tonic (Dwivedi, 1996). Clinical evaluation of this botanical medicine indicates that it is beneficial in the treatment of coronary artery disease, heart failure and possibly hypercholesterolemia (Alpana *et al*., 1997). It has also been found to possess antibacterial, antioxidant, antimutagenic (Kaur *et al*., 2002) and hepatoprotective activities (Subasini *et al*., 2007). The pharmacology of *T. arjuna* have been discussed by Patnaik *et al*. (2007) and pharmacological activities are mainly due to the tannins present in its bark. Tannins also contribute to the hypotensive action of *T. arjuna* bark (Takahashi *et al*., 1997). It is also speculated that tannins are responsible for its astringent, wound healing and anti microbial activity (Chaudhari and Mengi, 2006; Bele *et al*., 2010).

Phenolic compounds are a unique category of phytochemicals especially in terms of their vast potential health-benefiting properties. They have multiple biological effects and also act as antioxidants by preventing the oxidation of Low-Density Lipoproteins (LDL), platelet aggregation and damage of red blood cells (Cheynier, 2005). These chemical constituents (secondary metabolites) present in plant vary according to their age and maturity. Root causes of numerous chronic diseases involve oxidative damage to the cellular components. The use of antioxidants to minimize the oxidative damage is one of the important approaches to the primary prevention of these health problems. Several studies have indicated that the antioxidant activities of some plants were highly correlated with their total phenolic contents (Palav and Dmello, 2006; Oboh, 2008; Gupta *et al*., 2010). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Osawa, 1994). Further, phenolic compounds are effective hydrogen donors, which make them antioxidant (Rice-Evans *et al*., 1995). Flavonoids have also been reported to have multiple biological effects, including an antioxidant activity (Gil *et al*., 1999). Many important medicinal tree species possess antioxidant properties due to the presence of phenolic compounds.

Keeping the above into consideration a study was carried out to evaluate phytochemical constituents and radical scavenging (antioxidant) activity in the barks of *B. variegata*, *S. asoka* and *T. arjuna* collected from central India for optimizing the harvesting age (girth class).

## MATERIALS AND METHODS

The bark samples of *B. variegata* and *T. arjuna* were collected from Jabalpur (Madhya Pradesh) and *S. asoka* were collected from Bolangir and Khurda (Orissa) during 2008-2010. The trees were grouped according to girth class representing different age groups. In *B. variegata* and *S. asoka* the girth class ranged from 15-90 cm as trees of higher girth classes were not found. However, in *T. arjuna* it ranged from 30-180 cm. The barks were collected following sustainable harvesting practices.

The harvested bark samples were brought to laboratory and dried under shade. Fresh and dry weights of the barks were recorded to determine the moisture content. The dried bark samples were ground into coarse powder and used for chemical analysis. Total phenols, flavonoids and tannins were analyzed. Total phenols in the barks were estimated by Folin-Ciocalteau method (McDonald *et al.*, 2001), tannin by Folin-Denis method (Sadasivam and Manikam, 1996) and for flavonoids, aluminium chloride colorimetric technique was used (Chang *et al.*, 2002). DPPH assay was used for estimation of radical scavenging activity (Nickavar *et al.*, 2006).

**Estimation of radical scavenging activity:** The DPPH assay has been largely used as a quick, reliable and reproducible parameter to evaluate the *in vitro* radical scavenging activity of plant extracts (Koleva *et al.*, 2002; Goncalves *et al.*, 2005). The evaluation of the free radical scavenging activity of each of the extract was carried using the DPPH (2, 2-diphenyl-1- picrylhydrazyl) assay as described by Nickavar *et al.* (2006) with slight modification.

**Sample preparation:** Five gram of dried and powdered plant material was taken in a conical flask containing 100 mL of 2 N hydrochloric acid (HCl). The content was kept in a boiling water bath for 30 min, cooled and filtered. The filtrate was transferred to a separating funnel and extracted with 150 mL (50×3) of diethyl ether. The combined ether layer was then washed with distill water and dried over anhydrous sodium sulphate. The residue thus obtained was dissolved in 10 mL methanol and various concentrations of sample extracts were prepared in methanol. One mililiter of 0.3 mM DPPH in methanol was added to 2.5 mL solution of the extract or standard and allowed to stand at room temperature in dark for 30 min. The change in colour from deep violet to light yellow was then measured at 518 nm using spectrophotometer. Blank consisted of 1 mL methanol and 2.5 mL of each sample solution, negative control contained 1 mL DPPH solution and 2.5 mL methanol. Gallic acid was used as standard.

The decrease in absorbance by the DPPH radical with increase in concentration of the extract which manifested in the rapid discolouration of the purple DPPH, suggest that samples have antioxidant activity due to their proton donating ability (Adesegun *et al.*, 2007). The decrease in absorbance was then converted to percentage antioxidant activity using the following formula:

$$\text{Antioxidant activity (\%)} = [\text{Abs}_{\text{control}}-(\text{Abs}_{\text{sample}}-\text{Abs}_{\text{blank}})/\text{Abs}_{\text{control}}]\times 100$$

**Statistical analysis:** Results are expressed as Mean±SD of triplicates. Using statistical package for social sciences (SPSS) version 14 did the analysis.

## RESULTS AND DISCUSSION

Phytochemical analysis of *B. variegata* revealed that total phenols content was maximum (55.38±1.01%) in girth class 36-55 cm and minimum (22.48±7.57%) in girth class 15-35 cm. Tannins were found maximum (24.12±1.77%) in girth class 56-75 cm and minimum (15.71±0.88%) in girth class 15-35 cm. Inhibitory concentration ($IC_{50}$), i.e., the amount of sample required to inhibit 50% DPPH, was found maximum (7.68±0.04 mg $mL^{-1}$) in girth class 15-35 cm and minimum (5.63±0.04 mg $mL^{-1}$) in girth class 36-55 cm. Total phenols, tannins and antioxidant activity ($IC_{50}$) showed significant variation with respect to girth classes. However, significant variation was not observed in the moisture content and flavonoids concentration with increase in girth class. The results are depicted in Table 1.

Phytochemical analysis of *S. asoka* bark samples showed that the maximum concentration of total phenols (7.25±0.94%), flavonoids (0.23±0.04%) and tannins (40.15±3.55%) was found in girth class 61-90 cm and minimum concentration of total phenols (6.54±0.71%), flavonoids (0.17±0.01) and tannins (29.92±6.98%) was found in girth class 15-30 cm. $IC_{50}$ was found maximum (4.82±0.04 mg $mL^{-1}$) in girth class 15-30 cm and minimum (2.29±0.03 mg $mL^{-1}$) in girth class 61-90 cm. The data revealed that there was not any significant variation in the moisture content and concentration of all active ingredients with respect to girth class. However, $IC_{50}$ value showed a significant variation among different girth classes. The results are showed in Table 2.

Concentration of total phenols in *T. arjuna* bark was found maximum (17.95±2.39%) in girth class 121-150 cm and minimum (12.48±2.70%) in girth class 30-60 cm. Flavonoids content was found maximum (0.59±0.01%) in girth class 151 cm and above and minimum (0.32±0.06%) in girth class 30-60 cm. Tannins were found maximum (12.5±0.30%) in girth class 121-150 cm and minimum (10.2±0.46%) in girth class 30-60 cm. $IC_{50}$ was found maximum (3.01±0.05 mg $mL^{-1}$) in girth class 30-60 cm and minimum (1.21±0.03 mg $mL^{-1}$) in girth class 121-150 cm. Flavonoids, tannins and $IC_{50}$ showed significant variation, however, moisture content and total phenols did not show any significant variation with respect to girth classes. The results are presented in Table 3.

Radical scavenging activity of the sample extracts increased with the increase in concentration. Figure 1 represents the antioxidant activity of gallic acid having $IC_{50}$ value of 1.06±0.10 μg $mL^{-1}$. Figure 2 shows the comparison between the antioxidant activities of studied species depicting that *T. arjuna* bark have highest DPPH percent inhibition at all the concentrations, whereas the other two species possess lower activity at all the levels tested.

Table 1: Phytochemical analysis and antioxidant activity of *Bauhinia variegata* bark

| Girth class (cm) | Moisture (%) | Total phenols (%) | Flavonoid (%) | Tannins (%) | $IC_{50}$ (mg $mL^{-1}$) |
|---|---|---|---|---|---|
| 15-35 | 69.33±2.9$^a$ | 22.48±7.57$^a$ | 0.01±0.00$^a$ | 15.71±0.88$^a$ | 6.94±0.11$^a$ |
| 36-55 | 67.56±0.77$^a$ | 55.38±1.01$^b$ | 0.02±0.00$^a$ | 16.00±0.36$^a$ | 5.63±0.04$^b$ |
| 56-75 | 70.41±0.42$^a$ | 33.99±3.87$^c$ | 0.02±0.01$^a$ | 24.12±1.77$^b$ | 7.68±0.04$^c$ |
| ±SE | 1.43 | 4.03 | 0.005 | 0.95 | 0.059 |

Values are presented as Mean±SD (n = 3). Mean value within each column followed by different letter differ significantly at $p<0.05$

Table 2: Phytochemical analysis and antioxidant activity of *Saraca asoka* bark

| Girth class (cm) | Moisture (%) | Total phenols (%) | Flavonoids (%) | Tannins (%) | $IC_{50}$ (mg $mL^{-1}$) |
|---|---|---|---|---|---|
| 15-30 | 63.83±3.75$^a$ | 6.54±0.71$^a$ | 0.17±0.01$^a$ | 29.92±6.98$^a$ | 4.82±0.04$^a$ |
| 31-60 | 64.39±2.11$^a$ | 6.67±0.41$^a$ | 0.19±0.02$^a$ | 36.40±2.91$^a$ | 3.88±0.05$^b$ |
| 61-90 | 55.56±8.67$^a$ | 7.25±0.94$^a$ | 0.23±0.04$^a$ | 40.15±3.55$^a$ | 2.29±0.03$^c$ |
| ±SE | 4.56 | 0.59 | 0.021 | 4.03 | 0.032 |

Values are presented as Mean±SD (n = 3). Mean value within each column followed by different letter differ significantly at $p<0.05$

Table 3: Phytochemical analysis and antioxidant activity of *Terminalia arjuna* bark

| Girth class | Moisture (%) | Total phenols (%) | Flavonoids (%) | Tannins (%) | $IC_{50}$ (mg $mL^{-1}$) |
|---|---|---|---|---|---|
| 30-60 | 52.88±2.81[a] | 12.48±2.70[a] | 0.32±0.06[a] | 10.20±0.46[a] | 3.01±0.05[a] |
| 61-90 | 58.31±4.01[a] | 15.27±0.40[a] | 0.38±0.01[a] | 10.47±0.58[a] | 2.08±0.04[b] |
| 91-120 | 64.20±8.98[a] | 16.20±4.04[a] | 0.36±0.06[a] | 11.67±0.80[b] | 1.90±0.05[c] |
| 121-150 | 63.45±5.29[a] | 17.95±2.39[a] | 0.55±0.02[b] | 12.50±0.30[b] | 1.21±0.03[d] |
| 151 and above | 61.56±3.89[a] | 14.25±1.58 | 0.59±0.01[b] | 11.97±0.45[b] | 2.37±0.02[e] |
| ±SE | 4.44 | 2.07 | 0.03 | 0.47 | 0.029 |

Values are presented as Mean±SD (n = 3). Mean value within each column followed by different letter differ significantly at $p<0.05$

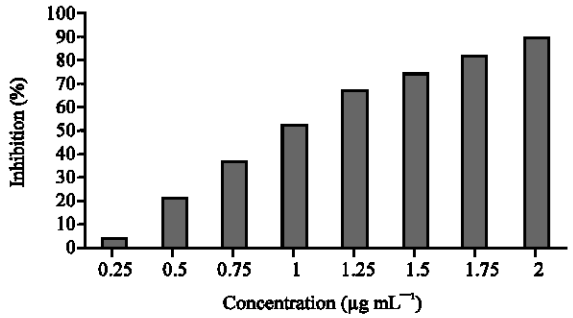


Fig. 1: Antioxidant activity of Gallic acid

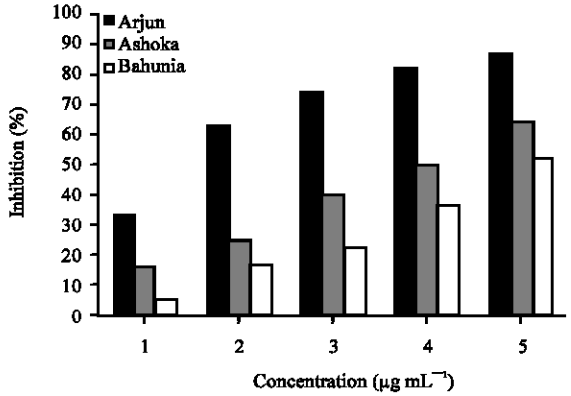


Fig. 2: Percentage inhibition of DPPH in *Bauhinia variegata, Saraca asoka* and *Terminalia arjuna*

Total phenols have a strong negative correlation with the $IC_{50}$ values in *B. variegata* (r = -0.706) and *T. arjuna* (r = -0.631); tannins have a strong negative correlation in *S. asoka* (r = -0.722) and *T. arjuna* (r = -0.671), whereas negative correlation of $IC_{50}$ with flavonoids was found only in *S. asoka*. Higher negative suggest that the antioxidant activity is dependent upon the constituents (polyphenols), more the concentration of constituents lesser will be the $IC_{50}$ value, hence higher will be the antioxidant activity. Present result for *S. asoka* corroborates with the study of Afolayan *et al.* (2008) in which they observed radical scavenging activity due to the presence of flavonoids in *Malva parviflora* and reported a significant positive relation between total phenols and flavonoids. Palav and Dmello (2006) and Vinson *et al.* (1995) also reported correlation of polyphenols with antioxidant activity. Correlation coefficients between different constituents evaluated and $IC_{50}$ values in the studied species are given in Table 4.

Table 4: Correlation between phytochemical constituents and $IC_{50}$ values in studied species

| | $IC_{50}$ | | |
|---|---|---|---|
| Phytochemical constituents | *Bauhinia variegata* | *Saraca asoka* | *Terminalia arjuna* |
| Total Phenols | -0.706* | -0.444 | -0.631* |
| Flavonoids | -0.268 | -0.788* | -0.449 |
| Tannins | 0.732* | -0.722* | -0.671** |

*Correlation is significant at 0.05 level, **Correlation is significant at 0.01 level

The study revealed that the concentration of phenols, flavonoids and tannins in *B. variegata* and *T. arjuna* was lower in younger trees, increased upto middle age and then decreased. However, in *S. asoka*, their concentration increased with age. Berrocal *et al.* (2004) also showed that the chemical composition of *Pinus radiata* trees varied significantly with the age of tree. Nadeem *et al.* (2002) also reported significant variations in the taxol content in bark of *Taxus baccata* trees of different age. Pandey and Kori (2009) also reported direct relationship of tannin and oxalic acid content with the age of *T. arjuna* trees. Phytochemical analysis of *B. variegata* bark revealed the presence of 0.02% flavonoids whereas Parekh and Chanda (2007) did not reported flavonoids in the bark collected from the semi-arid region of Rajkot Gujarat, India. In *T. arjuna* bark tannin content ranged between 9.6 to 12.8%. Present findings are corroborated by the findings of Pandey and Kori (2009) who reported 6.75-14.82% tannins in *T. arjuna* bark. Total phenols, tannins and flavonoids were found responsible for the radical scavenging activity of *B. variegata*, *T. arjuna* and *S. asoka* respectively. Free radical scavenging activity of different flavonoids was also demonstrated by Khlebnikov *et al.* (2007) and Tsimogiannis and Oreopoulou (2004).

The results of phytochemical evaluation and antioxidant activities showed that the middle aged populations of *B. variegata* (36-55 cm) and *T. arjuna* (121-150 cm) have maximum concentration of phenols and showed highest radical scavenging activity. However, in *S. asoka*, maximum concentration of phenols and highest radical scavenging activity was found in highest girth class (61-90 cm).

## CONCLUSION

In present study significant variation was found in phytochemical constituents in *B. variegata* and *T. arjuna* barks. However, no significant variation was seen in the bark of *S. asoka*. The amounts of polyphenols present were directly proportional to the radical scavenging activity of the species. Among the studied species *T. arjuna* showed maximum radical scavenging activity and thus can be termed as a potent antioxidant species whose bark can be used for making various formulations containing natural antioxidants. The results revealed that the optimum girth class for harvesting of barks of *B. variegata* is 36-55 cm, *S. asoka* 61-90 cm and *T. arjuna* 121-150 cm.

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