



# Screening of the antioxidant potential of young and mature leaves of *Saraca indica* and *Ficus religiosa* a comparative study

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## Manuscript details:

Received: 01.07.2019  
Accepted: 10.12.2019  
Published: 30.12.2019

### Cite this article as:

Khetade Roshan H, Bharsagade Prachi D, Patil Pranali and Umekar Milind J (2019) Screening of the antioxidant potential of young and mature leaves of *Saraca indica* and *Ficus religiosa* a comparative study, *Int. J. of Life Sciences*, Volume 7(4): 753-759.

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Available online on  
<http://www.ijlsci.in>  
ISSN: 2320-964X (Online)  
ISSN: 2320-7817 (Print)

## ABSTRACT

*Saraca indica*, commonly known as Asoka-tree, ashok or simply Asoca is a plant belonging to the subfamily Detarioideae of the family Fabaceae. In Ayurveda, the bark is used in indigestion, pyrexia, burning sensation, colic, ulcers, menorrhagia, vaginal discharge and acnes and *Ficus religiosa* or sacred fig is a species of fig native to the Indian subcontinent and Indochina that belongs to Moraceae, the comparative study for total tannin content by folin-denis reagent for *Saraca indica* for the methanolic extract of young leaves of *Saraca indica* contains more percentage of tannic acid as compared to mature leaves and young leaves of *Ficus religiosa* contains more percentage of tannic acid as compared to mature leaves. Total flavonoid content for *Saraca indica* for the methanolic extract of young leaves of *Saraca indica* contains more percentage of flavonoids as compared to mature leaves and young leaves of *Ficus religiosa* contains more percentage of flavonoids as compared to mature leaves. The Antioxidant activity of methanolic extract of young and mature leaves of *Saraca indica* by metal chelating method using Ferrosin Ferric chloride for the concentration of 0.04 mg/ml and 0.08 exhibited potent in vitro antioxidant activity by showing 84.87% and 63.21 and *Ficus religiosa* concentration of 0.06 mg/ml and 0.08 exhibited potent in vitro antioxidant activity by showing 92.32% and 73.01inhibition of Ferrosin- ferric chloride complex respectively. So it was planned to explore the antioxidant potential of young and mature leaves of both the plants.

**Keywords:** *Saraca indica*, *Ficus religiosa*, Ferrosin Ferric chloride, flavonoid content, Antioxidant potential.

## INTRODUCTION

Plant based medicines are the oldest remedies used by the human being. The demand for herbal products increases all over the world and major pharmaceutical companies are currently conducting research on medicinal plants on large scale for their potential medicinal value.

Plant medicine has been used for the treatment of various ailments throughout the world before the advent of modern synthetic drugs (Anitha et al. 2008). *Saraca indica* (Fabaceae) is indigenous plant with a number of traditional importance. (Sharma et al. 2005). In Ayurveda, the bark is used in indigestion, pyrexia, burning sensation, colic, ulcers, menorrhagia, vaginal discharge and acnes. Several Pharmacological activities of *Saraca indica* have been reported such as larvicidal activity, antimicrobial activity, CNS depressant activity, analgesic, antipyretic, antiulcer activity, anti-inflammatory activity etc. This review contains phytochemistry and pharmacological activities of various parts of plant and the ethano botanical uses (Sharma et al. 2005). *Ficus religiosa* is a variety of fig tree that was already known as the Bodhi tree, even before Gautama Buddha sat under its branches meditating and achieved enlightenment. It is the oldest depicted tree in Indian art and literature and it can be said that this is the mythical 'World Tree' or the 'Tree of Life' of the Indian subcontinent. This plant is considered sacred by the followers of Hinduism, Jainism and Buddhism, and hence the name 'Sacred Fig' was given to it. Siddhartha Gautama is referred to have been sitting underneath a Bo Tree when he was "enlightened" (Bodhi) or "awakened" (Buddha). Thus, Bo Tree is well-known symbol for happiness, prosperity, longevity and good luck. The comparative study *Saraca indica* and *Ficus religiosa* for antioxidant potential. (Chauhan and Merh, 1999). An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants are substances that neutralize free radicals and their actions. There are natural antioxidants enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase, thioredoxine thiols and disulphides bonding which form the buffering system in every cell. Antioxidant activity can be monitored by a variety of assays with different mechanisms, including hydrogen atom transfer (HAT), single electron transfer (ET), reducing power, and metal chelation, among others. Understanding the principle mechanisms, advantages and limitations of the measurement assay is important for proper selection of method (s) for valid evaluation of antioxidant potential in desired applications. This contribution provides a general and up-to-date overview of methods available for measuring antioxidant activity and the chemistry behind them (Fereidon, 2015).

### **SARACA INDICA**

**Scientific Name-** *Saraca indica*

**Common Name-** Ashoka tree, Sita Ashoka

#### **Scientific classification**

Kingdom: Plantae  
Order: Fabales  
Family: Fabaceae  
Subfamily: detarioideae  
Species: *Ficus religiosa*

**Other scientific name-** *Jonesia roxb*

### **FICUS RELIGIOSA**

**Scientific Name-** *Ficus religiosa*

**Common Name-** Peepal tree

#### **Scientific classification**

Kingdom: Plantae  
Clade: Angiosperms, Eudicots  
Order: Rosids  
Family: Moraceae  
Genus: *Ficus*  
Species: *Ficus religiosa*

**Other scientific name-** *Ficus caudata* Stokes, *Ficus peepul* Griff, *Ficus superstiosa* Link, *Urostigma affine* Miq, *Urostigma religiosum* (L.) Gasp.

### **MATERIAL AND METHODS**

#### **Collection of *Saraca Indica* Material Leaves:**

The young and mature leaves of plant *Saraca indica* were collected from the garden of Smt. Kishoritai Bhojar Collage of Pharmacy, New Kamptee, Nagpur District, and Maharashtra, India during March 2017. The plant was identified and authenticated by Dr. V. Tiwari. Dr. Vijay J Tiwari, Associate professor, Department of botany, J.M. Patel College, Bhandara.

#### **Collection of *Ficus religiosa* Leaves:**

The young and mature leaves of plant *Ficus religiosa* were collected from the village of Gangner - Khandala, Nagpur District, Maharashtra, India, during March 2017. The plant was identified and authenticated by Dr. Vijay J Tiwari, Associate professor, Department of botany, J.M. Patel College, Bhandara.

#### **Methanolic Extract**

About 25g of each dried powdered of young and mature leaves of *Saraca indica* and *Ficus religiosa* were macerated in 100 ml of methanol for 7 days at room temperature. The resulting extract was filtered. The residue was further extracted using the same procedure. The filtrate obtained were combined and

then evaporated to dryness. After drying the extracts were subjected for determination of total tannin content, total flavonoid content and antioxidant activity evaluation.

#### **Total Tannin Content:**

Tannins are the polyphenolic compounds which can be classified as hydrolysable and non-hydrolysable. Citrous fruits, red wine and tea leaves are the important source of natural tannins. They have property to bind and precipitate proteins.

#### **Preparation of Folin-Denis Reagent:**

Sodium tungstate (10gm) and phosphomolybdic acid (2gm) were dissolved in distilled water (75ml) and later 5ml phosphoric acid was added into solution. Mixture was refluxed for 2 hor and volume was made to 100ml with distilled water.

#### **Preparation of carbonate solution:**

Sodium carbonate (17.5gm) was dissolved in distilled water and volume was made upto 50ml. mixture was allowed to stand overnight and filtered.

#### **Preparation of standard curve of tannic acid:**

Stock solution of tannic acid of concentration (1mg/ml) was prepared by using distilled water. 5ml of stock solution was diluted to 100ml with distilled water in another volumetric flask to get concentration of 50µg/ml tannic acid. From this working stock solution, 1ml to 5ml were taken in clear separated test tubes and 0.5ml of Folin-Denis Reagent and 1ml of sodium carbonate solution were added to each test tube and volume was made up to 10ml with distilled water. All the reagents in each tube were mixed well and kept undisturbed for about 30 minutes and absorbance was read at 700nm against reagent blank using UV spectrophotometer.

#### **Preparation of test solution:**

Accurately weighed (0.1gm) of the powdered material were dissolved in distilled water and volume was made to 100ml in volumetric flask. Later 5ml stock solution was diluted to 100ml with distilled water in another volumetric flask. From the test solution 1-5ml working standard solution were taken in clear separated test tube. Further procedure is similar as standard curve of tannic acid. (Khadabadi and Deore, 2011).

#### **Total Flavonoid Content**

##### **Aluminium Chloride Colorimetric Method for Preparation of calibration curve:**

In this method, quercetin was used as standard. Stock solution of quercetin of concentration (0.1mg/ml) was prepared by using Methanol. From this stock solution, 1 to 6 ml of solutions was taken separately in clear test tubes. Then volume was made up to 10ml with methanol (10-100µg/ml). From each of the above test tubes 0.5ml standard solution was taken separately in different test tubes and to each, 1.5ml of 95% ethanol, 0.1ml of 10% aqueous aluminium chloride, 0.1ml of 1M potassium acetate and 2.8 ml distilled water were added and mixed well. A calibration curve was made by measuring the absorbance of each mixture at 415 nm with a Shimadzu UV- 1800 spectrophotometer. Sample blank was prepared in similar way by replacing aluminium chloride with distilled water.

#### **Preparation of test solution:**

10mg of extract was accurately weighed and transferred to 100ml volumetric flask and made up the volume with methanol. Stock solution of plant extract of concentration (0.1mg/ml) was prepared by using Methanol. From this stock solution, 1 to 6 ml of solutions was taken separately in clear test tubes. Then volume was made up to 10ml with methanol (10-100µg/ml). Further procedure is similar as calibration curve of quercetin (Khadabadi and Deore, 2011).

#### **Antioxidant Activity for Metal chelating method**

**Preparation of standard quercetin:** The calibration standards (0.01—0.1mg/ml) were prepared from the stock solution by the serial dilution of methanol. From each calibration standard 0.25ml of solution was pipette out to test tubes containing 1.75 ml of methanol, 0.25 ml of ferrous chloride and 0.25 ml of ferrozine. The reaction mixture was shaken and kept for 10 minutes at room temperature in dark place and then absorbance was measured at 562nm using UV Spectrophotometer. Then the % of metal chelating activity at different concentrations was determined (Singh and Singh, 2008), (Pullab Kalita and Barman Tapan, 2013) (Tambe and Bhambar, 2014).

#### **Formula for calculating metal chelating activity is:**

$$\% \text{ activity} = \frac{\text{Absorbance of the control} \times \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

**RESULT & DISCUSSION**

**Total Tannin Content by Folin-Denis Reagent for *Saraca Indica***

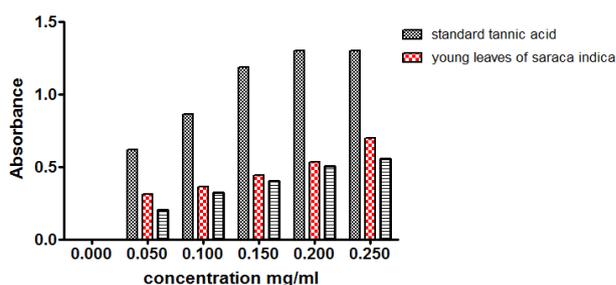
Table 1 and Figure 1. Indicates that methanolic extract of young leaves of *Saraca indica* contains more percentage of tannic acid as compared to mature leaves. At concentration of 0.25 mg/ml, the methanolic extract of young and mature leaves showed 48.07 %

and 38.1% of tannins respectively as compared with standard tannic acid.

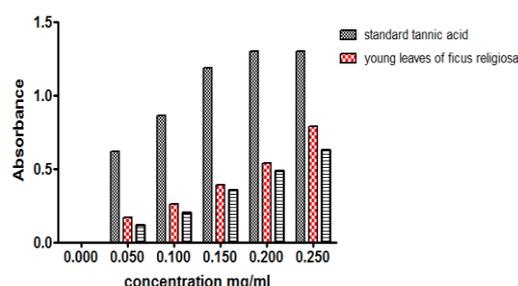
Table 2 and Figure 2. Indicates that methanolic extract of young leaves of *Ficus religiosa* contains more percentage of tannic acid as compared to mature leaves. At concentration of 0.25 mg/ml, the methanolic extract of young and mature leaves showed 54.3% % and 43.4% of tannins respectively as compared with standard tannic acid.

**Table 1: Total tannin content of methanolic extract of young and mature leaves of *S. indica*.**

Sr. no	Std sol tannic acid (in ml)	Conc mg/ml	Abs of std solution	Abs mature leaves	Abs of young leaves
1	1	0.05	0.620	0.200	0.311
2	2	0.1	0.864	0.361	0.364
3	3	0.15	1.186	0.400	0.442
4	4	0.20	1.302	0.501	0.532
5	5	0.25	1.452	0.554	0.698



**Figure 1**



**Figure 2**

**Table 2: Total tannin content of methanolic extract of young and mature leaves of *F. religiosa*.**

Sr.no	Std sol of tannic acid (in ml)	Concentration mg/ml	Absorbance of std sol	Abs of mature leaves	Abs of young leaves
1	1	0.05	0.620	0.116	0.170
2	2	0.1	0.864	0.205	0.289
3	3	0.15	1.186	0.357	0.389
4	4	0.20	1.302	0.489	0.536
5	5	0.25	1.452	0.631	0.789

**Table 3: Total flavonoid content of methanolic extract of young & mature leaves of *S. indica*.**

Sr.no	Concentration µg/ml	Abs of std querecetin	Absorbance of mature leaves	Absorbance of young leaves
1	10	0.171	0.093	0.099
2	20	0.236	0.123	0.156
3	30	0.263	0.129	0.198
4	40	0.336	0.158	0.567
5	50	0.351	0.273	0.321
6	60	0.395	0.301	0.341

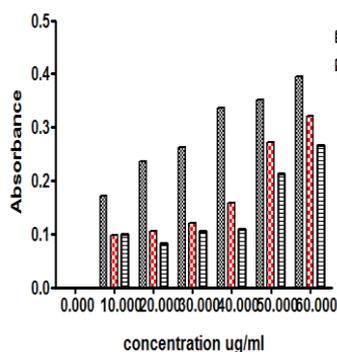


Figure 3

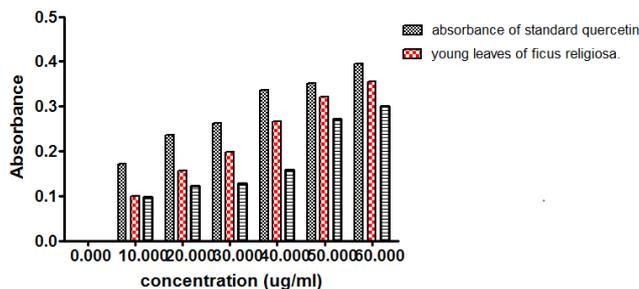


Figure: 4

Table 4: Total flavonoid content of methanolic extract of young & mature leaves of F religiosa.

Sr.no	Conc µg/ml	Abs of standard querecetin	Absorbance of mature leaves	Absorbance of young leaves
1	10	0.171	0.083	0.097
2	20	0.236	0.099	0.105
3	30	0.263	0.106	0.121
4	40	0.336	0.109	0.159
5	50	0.351	0.214	0.273
6	60	0.395	0.267	0.321

Absorbance 1) Blank = 0.0 2) Control = 1.063

Table: 5 Antioxidant activity of methanolic extract of young and mature leaves of *Saraca indica* by metal chelating method using Ferrosin and Ferric chloride

Sr.no	Conc of test sol in mg/ml	% activity young leaves	% activity of mature leaves
1	0.01	74.9	57.76
2	0.02	76.38	64.25
3	0.03	67.45	59.73
4	0.04	84.87	58.04
5	0.05	72.81	61.52
6	0.06	69.70	58.60
7	0.07	77.70	56.82
8	0.08	81.81	63.21
9	0.09	52.86	57.57
10	0.1	66.98	57.85

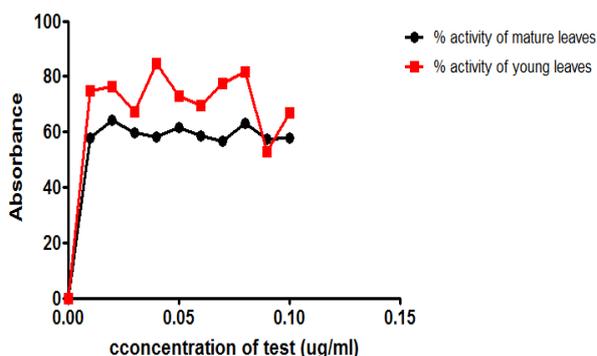


Figure 5

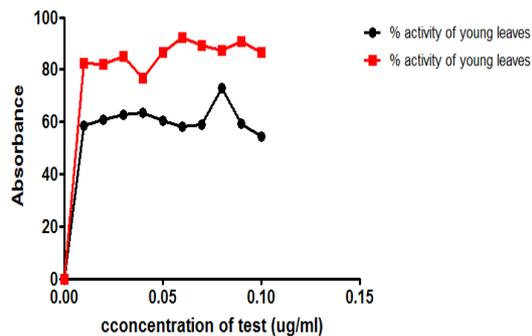


Figure 6

**Table 6: Antioxidant activity of methanolic extract of young and mature leaves of *Ficus religiosa* by metal chelating method using Ferrosin and Ferric chloride**

Sr.no	Conc of test sol in mg/ml	% activity young leaves	% activity of mature leaves
1	0.01	82.48	58.43
2	0.02	82.09	60.86
3	0.03	85.16	62.65
4	0.04	76.59	63.42
5	0.05	86.70	60.61
6	0.06	92.32	58.31
7	0.07	89.38	58.95
8	0.08	87.34	73.01
9	0.09	90.79	59.49
10	0.1	86.44	54.47

Absorbance 1) Blank = 0.0 2) Control = 0.782

#### Total Flavonoid Content for *Saraca Indica*

Table 3 and Figure 3. Indicates that methanolic extract of young leaves of *Saraca indica* contains more percentage of flavonoids as compared to mature leaves. At concentration of 60 µg/ml, the methanolic extract of young and mature leaves showed 60 % and 47.2% of flavonoids respectively as compared with standard quercetin.

Table 4 and Figure 4. Indicates that methanolic extract of young leaves of *Ficus religiosa* contains more percentage of flavonoids as compared to mature leaves. At concentration of 60 µg/ml, the methanolic extract of young and mature leaves showed 60 % and 95.4% of flavonoids respectively as compared with standard quercetin.

#### Antioxidant Activity for *Saraca Indica*

Table 5 and Figure 5 Indicates that methanolic extract of young and mature leaves of *Saraca Indica* at concentration of 0.04 mg/ml and 0.08 exhibited potent in vitro antioxidant activity by showing 84.87% and 63.21% inhibition of Ferrosin- ferric chloride complex respectively. The young leaves showed more potent antioxidant activity as compared to mature leaves.

#### **FICUS RELIGIOSA**

Antioxidant activity of young and mature leaves of *Ficus religiosa* by metal chelating method.

Table 6 and Figure 6 Indicates that methanolic extract of young and mature leaves of *Ficus religiosa* at concentration of 0.06 mg/ml and 0.08 exhibited potent in vitro antioxidant activity by showing 92.32% and

73.01% inhibition of Ferrosin- ferric chloride complex respectively. The young leaves showed more potent antioxidant activity as compared to mature leaves.

#### SUMMARY AND CONCLUSION

The young leaves of Peepal (*Ficus religiosa*) and Ashoka (*Saraca indica*) are transiently red due to presence of polyphenolic compounds. Polyphenolic compounds are one of the most important categories of natural antioxidants. So young and mature leaves of both the plants were evaluated for their antioxidant potential and total tannin and flavonoid content. The Total tannin content of young and mature leaves of *Saraca indica* and *Ficus religiosa* was estimated by Folin-Denis method. The Methanolic extract of young leaves of *Saraca indica* contains more percentage of tannic acid as compared to mature leaves. At concentration of 0.25 mg/ml, the methanolic extract of young and mature leaves showed 48.07 % and 38.1% of tannins respectively as compared with standard tannic acid. (Table 1 and Figure 1). The Methanolic extract of young leaves of *Ficus religiosa* contains more percentage of tannic acid as compared to mature leaves. At concentration of 0.25 mg/ml, the methanolic extract of young and mature leaves showed 54.3% % and 43.4% of tannins respectively as compared with standard tannic acid (Table 2 and Figure 2).

The total Flavonoid content of young and mature leaves of *Saraca indica* and *Ficus religiosa* was estimated by Aluminium chloride method. The methanolic extract of young leaves of *Saraca indica* contains more percentage of flavonoids as compared

to mature leaves. At concentration 60 µg/ml, the methanolic extract of young and mature leaves showed 60 % and 47.2% of flavonoids respectively as compared with standard quercetin. (Table 3 and Figure 3). The methanolic extract of young leaves of *Ficus religiosa* contains more percentage of flavonoids as compared to mature leaves. At concentration 60 µg/ml, the methanolic extract of young and mature leaves showed 60 % and 95.4% of flavonoids respectively as compared with standard quercetin. (Table 4 and Figure 4). The in vitro antioxidant activity was carried out for methanolic extract of young and mature leaves of *Saraca indica* at concentration of 0.04 mg/ml and 0.08 exhibited potent in vitro antioxidant activity by showing 84.87% and 63.21% inhibition of Ferrosin- ferric chloride complex respectively. The young leaves showed more potent antioxidant activity as compared to mature leaves. (Table 5 and Figure 5). And *Ficus religiosa* at concentration of 0.06 mg/ml and 0.08 exhibited potent in vitro antioxidant activity by showing 92.32% and 73.01% inhibition of Ferrosin- ferric chloride complex respectively. The young leaves showed more potent antioxidant activity as compared to mature leaves. (Table 6 and Figure 6). From the present study it was concluded that the methanolic extract of young leaves of *Saraca indica* and *Ficus religiosa* contains more percentage of tannins and flavonoids and exhibited more potent in vitro antioxidant activity as compared to mature leaves. This may be attributed to the presence of high percentage of polyphenolic compounds.

#### CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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