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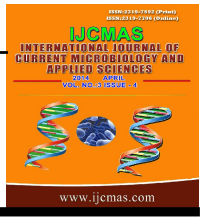

Original Research Article

Evaluation of antioxidant activities of flower extract (fresh and dried) of *Saraca indica* grown in West Bengal

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Keywords

Saraca indica; Antioxidant; Polyphenols; Flavonoids,; Tannin; Ascorbic acid; DPPH radical scavenging activity.

A B S T R A C T

Saraca indica has been greatly used as traditional medicine for women related problems, such as leucorrhoea, menorrhagia, dysfunctional uterine bleeding, bleeding haemorrhoids etc. In this study different Phytochemicals and free radical scavenging activity were measured in the ethanolic and water extract of fresh and dried flowers of *Saraca indica* collected from in and around Barrack pore area, West Bengal. The Phytochemicals studied from flower extracts are total polyphenols, flavonoids, ascorbic acid and tannins. Free Radical scavenging activities of the extracts were evaluated using DPPH assay method. The results revealed that total polyphenols, flavonoids and tannins content were relatively higher in ethanolic extract of the fresh flower and water extract of the dried flower of *Saraca indica*. Whereas both extract of dried flower contained higher amount of ascorbic acid. The free radical scavenging activity was higher in fresh flower extract (both ethanol and water) in comparison to dried flower. The results revealed that the antioxidant property of both flower extracts may improved human health status and stay away from many diseases.

Introduction

Most bioactive food constituents are derived from plants; those so derived are collectively called phytochemicals. The large majority of these phytochemicals is redox active molecules and therefore defined as antioxidants. Antioxidants can eliminate free radicals and other reactive oxygen and nitrogen species, and these reactive species contribute to most chronic diseases. It is hypothesized that antioxidants originating from foods may work as antioxidants in their own right in vivo, as well as bring about beneficial health effects through other mechanisms, including acting as inducers of mechanisms related to antioxidant defense

(Kensler et al., 2007; Jeong et al., 2006), longevity (Baur et al., 2006; Wood et al., 2004), cell maintenance and DNA repair (Astley et al., 2004).

Saraca indica is highly regarded as a universal panacea in the ayurvedic medicine. It is one of the universal plant having medicinal activities. Saraca indica has been greatly used as traditional medicine for women related problems, such as leucorrhoea, menorrhagia, dysfunctional uterine bleeding, bleeding haemorrhoids etc (ayurvedic pharmacopoeia of India. 2001). The antimicrobial activity of the stem and bark of *Saraca indica* have been evaluated against standard strain of *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium* (Shilpakala Sainath et al, 2009). The *leaves* of *Saraca indica* also evaluated for anthelmintic activity (Manjunath et al, 2006**;** Nayak et al, 2011), analgesic and antipyretic activities (Pradhan et al, 2010), CNS depressant activity (Yadav et al, 2008).

The reports of quantitative estimation of different antioxidants of the flower of *Saraca indica* are hardly available. The antioxidant property is also related to the condition of soil and environment where the plant is grown. So in this investigation we are quantitatively estimate different phytochemicals such as total polyphenols, flavonoids, ascorbic acid and tannins and free radical scavenging activities of DPPH to evaluate antioxidants properties of the flower of *Saraca indica.*

Materials and Methods

Plant material

The fresh flowers of *Saraca indica* were collected from in and around Barrackpore, West Bengal, India during the month of July to August, 2013.

Chemicals:

Folin-Ciocalteu reagents, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) reagent, gallic acid, aluminium trichloride ($AlCl_3$), butylated hydroxytoluene (BHT), catechin and 2, 6-dichloroindophenol (DCIP) were purchased from SRL India, Sulphric acid, Sodium nitrates ($NaNO_2$), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3) were purchased from Merck (India). Double distilled water was used for the complete study.

Preparation of extracts:

The flowers were collected and healthy flowers were shade dried and then powdered using electric blender to get a coarse powder. The Fresh whole of flowers was also used in these studies. All the plant materials were stored at 4^oC for further use.

Ethanol and water extracts from Dried and Fresh Flowers

The ethanol and water extracts of dried and fresh flowers were prepared by mortar-pestle using respective solvents (water and ethanol) separately and grounded paste of flowers with solvent were then shake for 24 h in a shaker. The extracts were filtered through glass wool. The extraction process was repeated twice. The collected filtrates were dried at room temperature and weighted by electronics balance and diluted by distilled water to a desired concentration (10□g/ml) (Maneemegalai and Naveen, 2010). Theses extracts were stored in refrigerators (4^oC) for further used.

Antioxidant measurement assay methods: All the experiments were performed in triplicate using the following procedure:

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activities of extracts of fresh flower and dried flower powder were assessed by method reported by Sasidharan et al. (Sasidharan et al, 2007) with some modification. 0.002% DPPH was prepared in ethanol. 250 µl of DPPH solution was mixed with 5 µl of flower extracts and final volume of 1000µl was made up by adding ethanol. The mixtures were kept in dark for 20 min and optical density was measured at 517 nm using Spectrophotometer (Systronics make, Model no 2202) ethanol (750µl) with DPPH solution (250 µl) was used as control. The percentage of inhibition of DPPH activity was calculated (Chorage et al, 2013) using the formula given below:

$$\text{Percent of inhibition of DPPH activity} = \frac{(\text{absorbance of control} - \text{absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Total phenolic content assay

The total phenolic content was measured using Folin-Ciocalteus reagent based on procedures described by Singleton et al. (Singleton et al., 1999), with some modifications. Briefly, 0.5 ml of sample was mixed with 1.5 ml (1:10 v/v diluted with distilled water) Folin-Ciocalteau's reagent and allowed to stand for 22°C for 5 min. Then 2 ml of sodium carbonate (Na_2CO_3, 7%, w/v) was added and the mixture were allowed stand for another 90 min and kept in the dark with intermittent shaking. Then the absorbance of the blue colour that developed was measured at 725 nm using spectrophotometer (Systronics make, Model no 2202). Gallic acid was used for constructing the standard curve (20 to 100 µg/ml;) and the total phenolic compounds concentration in the flower extract was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) of extract.

Total Flavonoid content assay

Total flavonoid content of the flower extract was determined according to colorimetric method described by Zhishen et al. (Zhishen et al., 1999), with some modification. Briefly 0.5 ml sample was mixed with 2 ml of distilled water and 0.15 ml of sodium nitrite ($NaNO_2$, 5% w/v), allowed to stand for 6 min, 0.15 ml aluminium trichloride ($AlCl_3$, 10% w/v) was added and allowed to stand again for 6 min, followed by addition of 2 ml of sodium hydroxide (NaOH, 4% w/v). The final volume was make up to 5 ml by distilled water. The reaction mixture was mixed thoroughly and allowed to stand for another 15 min. The absorbance of pink colour that developed was measured at 510 nm using spectrophotometer (Systronics make, Model: 2202). Distilled water was used as blank. All the experiment was carried out in triplicate. The total flavonoid content was expressed in mg of catechine per gram of flower extract.

Tannin assay

Content of Tannins in the flower extract was determined by Folin Denis method (Polshettiwar and Ganjiwale, 2007). Briefly 1 ml of sample or standard solution of Tannic acid (5µg/ml - 40µg/ml) was mixed with 0.25 ml Folin Denis reagent and 0.50ml saturated Na_2CO_3 solution ware added to it. The volume was made up to 5 ml with distilled water and absorbance

was measured at 700 nm after 30 min of incubation. The total tannic acid content was expressed as mg of tannic acid equivalent per gram of dry weight of the sample (Kalpana et al, 2013)

Vitamin C assay

Vitamin C of the flower extract was measured titrematrically by 2, 6-dichloroindophenol (DCIP) solution. The 2, 6-dichloroindophenol (DCIP) solution was prepared by dissolving 52 mg of 2, 6-dichloroindophenol in about 500 mL of water. Sodium bicarbonate (42 mg) is then added and dissolved. The resulting solution is finally diluted to 1 L with distilled water.

Briefly 5.00 mL of the sample or standard ascorbic acid solution was taken into a 250-mL Erlenmeyer flask, 2 mL of the 3% Metaphosphoric acid mixture and about 25 mL of distilled water to the flask was added. This mixture was then titrated with the DCIP solution until a permanent (lasting more than 30 sec) light red or pink colour appears. The volume of DCIP needed to oxidize the sample and standard ascorbic acid was correlated to find out ascorbic acid content in the sample. The result was expressed in mg of ascorbic acid per gram of extract.

Statistical analysis

All the analysis were carried out in triplicate and expressed as mean ± SD. Analyses of variance were performed using the one-way analysis of variance (ANOVA). Significant differences between means were determined by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant

Results and Discussion

Phytochemicals and antioxidant activity analysis of water and ethanol extract of *Saraca indica* fresh flowers and dried flowers were given in Table.1 and Table.2 respectively.

Total polyphenol contents (mg/g of flowers) of ethanolic extract of fresh and dried flowers of *Saraca indica* were 4.509 mg/g and 3.146 mg/g respectively (Figure.1). Where as in the water extract of fresh and dried flowers, total polyphenol contents were 1.068 mg/g and 2.190 mg/g respectively. From these observations, we can say that total polyphenol content in ethanolic extract is relatively 46.31% higher in fresh flowers then dried flowers. But the water extract of dried flowers contain 105.05% more of total polyphenol than fresh flowers.

Total ascorbic acid contents (mg/g of flowers) of *Saraca indica* flowers are also presented in the Figure 2. Ethanol extract of fresh flowers contained ascorbic acid 0.124mg/g and dried flowers contained 0.426 mg/g. Whereas water extract of fresh flowers and dried flowers contained ascorbic acid 0.113 mg/g and 0.415 mg/g respectively. Therefore from the above results it can be seen that ascorbic acid content of ethanolic and water extract of dried flowers are 235.48% and 267.25% respectively higher than fresh flowers.

Total tannin content (mg/g of flowers) of *Saraca indica* flowers is presented in the Figure 3. Ethanol extract of fresh flowers contained tannin 0.720mg/g and dried flowers contain 0.486mg/g, whereas water extract of fresh and dried flowers

Table.1 Phytochemical and antioxidant activity analysis of water and ethanol extract of fresh flowers of *Saraca indica*

Antioxidant property	**Total polyphenol** (mg/g of whole flowers ± SD)	**Ascorbic acid** (mg /g of whole flowers ± SD)	**Tannin Content** (mg /g of whole flowers± SD**)**	**Flavonoid content** (mg /g of whole flowers ± SD)	**DPPH Activity** (% of inhibition/g of whole flowers ± SD)
Water extract	1.068 ± 0.02	0.113 ± 0.02	0.155 ± 0.05	0.032 ± 0.07	79.88 ± 0.01
Ethanol extract	4.509 ± 0.03	0.124 ± 0.02	0.720 ± 0.02	0.466 ± 0.01	81.34 ± 0.01

Table.2 Phytochemical and antioxidant activity analysis of water and ethanol extract of dried flowers powder of *Saraca indica*

Antioxidant property	**Total polyphenol** (mg/g of whole flowers ± SD)	**Ascorbic acid** (mg /g of whole flowers ± SD)	**Tannin Content** (mg /g of whole flowers± SD**)**	**Flavonoid content** (mg /g of whole flowers ± SD)	**DPPH Activity** (% of inhibition/g of whole flowers ± SD)
Water extract	2.190 ± 0.01	0.415 ± 0.02	0.778 ± 0.03	0.136 ± 0.03	23.82 ± 0.1
Ethanol extract	3.146 ± 0.03	0.426 ± 0.03	0.486 ± 0.02	0.303 ± 0.01	58.20 ± 0.01

Figure.1 Total Polyphenol content (mg/g of flowers) of fresh and dried flowers of *Saraca indica*

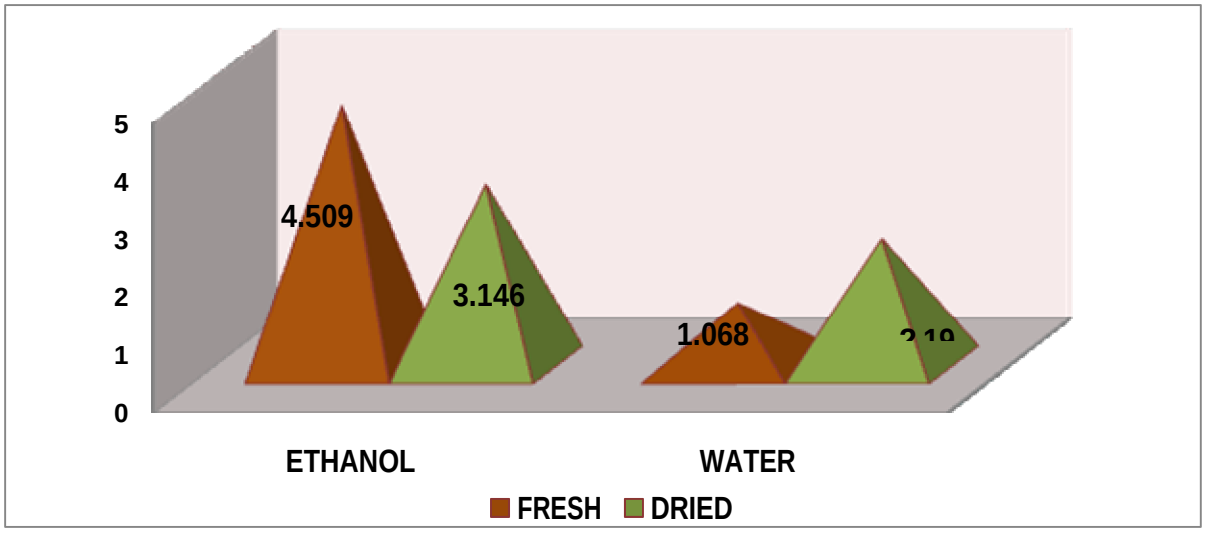


Figure.2 Total Ascorbic Acid content (mg/g of flowers) of fresh and dried flowers of *Saraca indica*

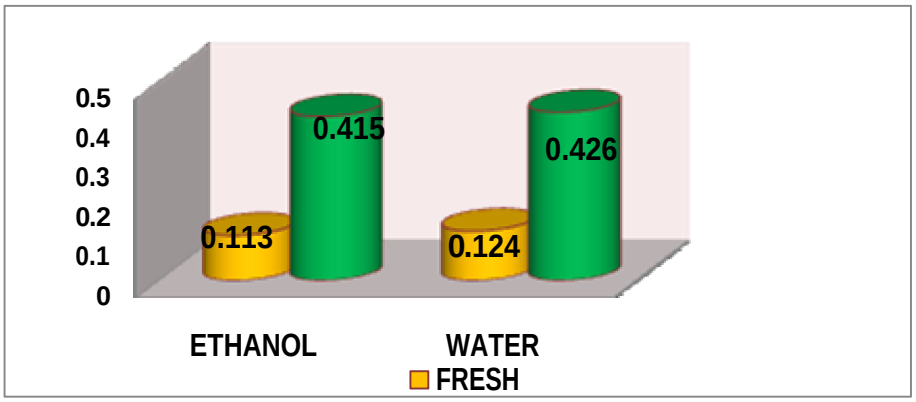


Figure.3 Total Tannin content (mg/g of flowers) of fresh and dried flowers of *Saraca indica*

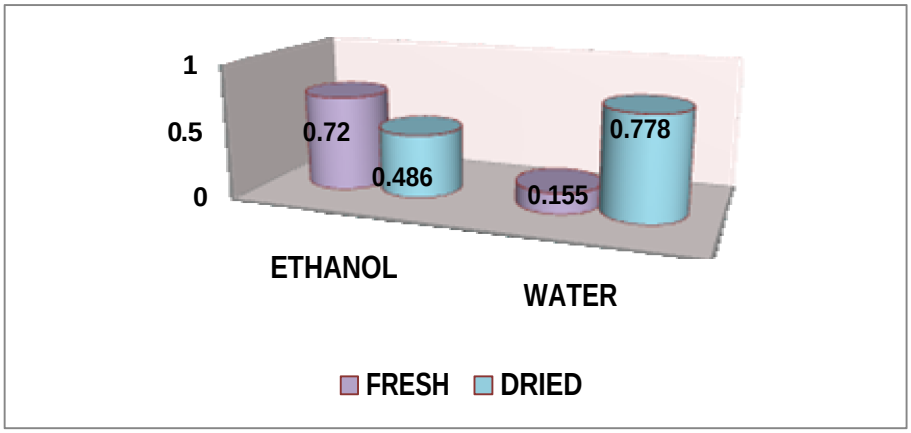


Figure.4 Total Flavonoid content (mg/g of flowers) of fresh and dried flowers of *Saraca indica*

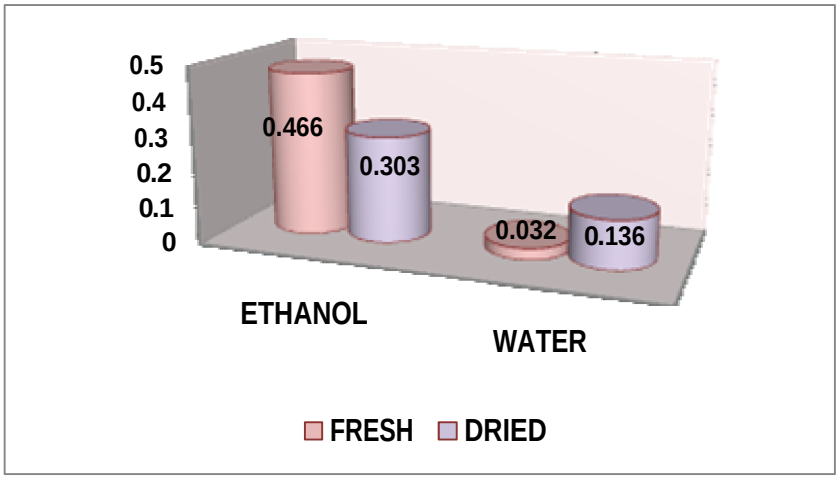
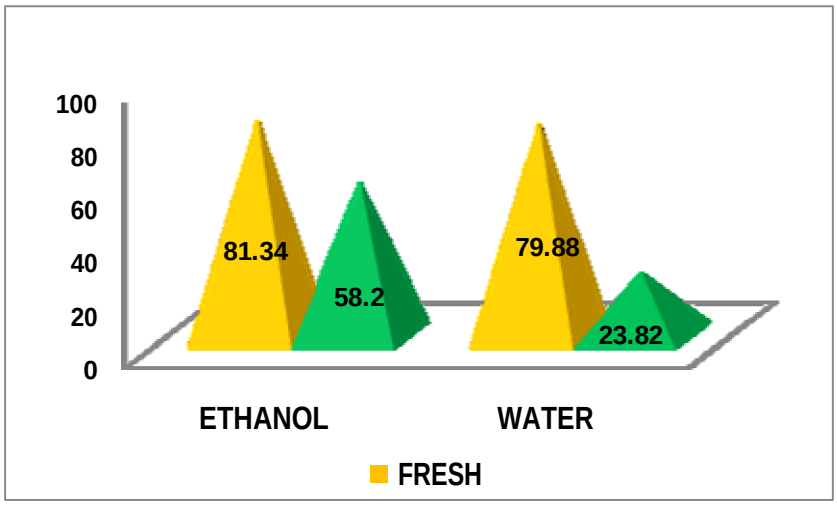


Figure.5 Free Radical Scavenging Activity of fresh and dried flowers of *Saraca indica* (% of inhibition/g of flowers)



contain 0.155mg and 0.778mg/g of flowers respectively. From the above result, it has been seen that ethanol extract of fresh flowers has 48.75% more tannin level then dried flowers. But water extract has shown the opposite result. The water extract of dried flower contained 401.93% higher level of tannin than water extract of fresh flower.

Total flavonoids content (mg/g of flowers) of *Saraca indica* flowers is presented in the Figure 4. Ethanol extract of fresh and dried flowers contain 0.466mg/g and 0.303mg/g of flower flavonoids respectively, whereas water extract of fresh flowers contained flavonoids 0.032 mg/g and dried flowers contained 0.136 mg/g of flowers. Therefore we can say that ethanol extract of fresh flower contain 54.15% more flavonoids then dried flower where as water extract of fresh flower has 325% less amount of flavonoids then dried flowers.

Total percentage of scavenging activity of *Saraca indica* flowers is presented in the Figure 5. Ethanol extract of fresh flowers contained 81.34% and dried flowers contained 58.20% of scavenging activity per gram of flowers. Whereas water extract of fresh flowers have shown 79.88% and dried flowers shown 23.82% of scavenging activity per gram of flowers. From the result it has been seen that both the extract (ethanol and water) of fresh flower show more percentage of scavenging activity then the extracts of dried flowers.

Different phytochemical properties of different parts of *Saraca indica was* extensively reviewed by Pradhan et al (Pradhan et al., 2009). The leaves of Saraca indica was investigated as antidepression activity upon central nervous system (Verma et al, 2010) anthelminthic activity (Nayak and Sahoo, 2011) and also possess a pronounced effect upon the uterine activity (Satyavati et al, 1970). *S. asoca* leaves also possesses antihyperglycemic and antioxidant properties as well improves body weight, liver profile, renal profile and total lipid levels. It can justify folklore uses of the plant in diabetes (Kumar et al, 2012).

Preliminary phytochemical analysis of *S. asoca* leaves showed the presence of flavonoids, tannins, saponins, sterols and triterpenoids which are known bioactive principles [Dhawan et al, 1977; Rao et al, 2003].

The phytochemical screening of flowers and flowers buds are not been reported earlier although flowers and flower buds of *Saraca indica* extract was reported to have antimicrobial activity against *enterobacteria* (Pal et al, 1985). The flowers also act against the gastric ulcer (Bhadauria et al, 2012) and possess anti-diabetic activity (Rangari, 2007). In this respect it is quite significant to know the phytochemical constituent of flowers and flower buds of *Saraca indica.* The flowers of *Saraca indica* is bloom during rainy season. In urban area the flowers are dried and used throughout the year. Hence we are evaluated the antioxidant properties of dried flower as well as fresh one. The results of different antioxidant constituents revelled that the most of the antioxidant constituents are present in ethanolic extract of the flowers than water extract in both fresh and dried flowers. The presence of different antioxidant constituents in the flowers of *Saraca indica* may also correlated with its antimicrobial (Pal et al, 1985), anti-diabetic (Rangari, 2007) activity and function against gastric ulcer (Bhadauria et al, 2012).

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