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Qualitative and Quantitative screening of Phytochemicals in polar and non polar solvent extracts of stem bark and leaves of *Saraca indica* (L.)

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Abstract: *Saraca indica L. (Ashok) is a small evergreen tree. This plant is rich in phytochemicals viz. tannins, flavonoids, steroids, volatile oil, glycosides, various steroidal glycosides, various carbohydrates, gallic acid and egallic acid, sarcasin, sarcadin, waxy substances, proteins, carbohydrates and steroids, fatty acids like oleic, linoleic, palmitic and stearic acid. Saraca asoca has many uses mainly in the medicine to treat the women gynecological disorders, in all types of abnormal discharges from vagina, in uterine inertia, uterine pain, urinary calculus, dysurea, etc.*

Phytochemical analysis conducted on the Saraca asoca leaves and bark extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. The phytochemical screening of the bark and leaves of Saraca indica was done with chloroform, petroleum ether, ethanol, methanol, hexane and distilled water. Among the six solvent extracts studied the carbohydrate was present in aqueous extract of bark as evidenced by positive Fehling's test. All the six solvent extracts of bark showed positive Benedict's test for carbohydrate. Only the chloroform and hexane extracts of bark showed positive Iodine test for carbohydrate.The phenols and tannins were detected only in chloroform, ethanol, methanol and hexane extracts but not in petroleum ether and aqueous extracts. All the six solvent extracts of bark showed negative alkaline reagent test which indicated the absence of flavonoids. Saponin was detected in four solvent extracts of bark viz. ethanol, methanol, hexane and distilled water as evidenced by positive froth foam test. Glycosides were not detected in any of the six solvent extracts of bark. Phenolic compounds were detected in all extracts except petroleum ether. The steroids were recorded in all the six solvent extracts of bark.

In the solvent extracts of leaves of Saraca indica carbohydrates were detected in all the six solvent extracts. Phenols and tannins were detected in all except chloroform and aqueous extracts. Flavonoid was detected in all except chloroform, and saponins in all except chloroform and petroleum ether extracts. Petroleum ether extract showed negative results for the presence of glycosides. In petroleum ether and hexane extract of leaves glycosides were not detected. Phenolic compounds and steroids were detected in all the solvent extracts of leaves except hexane and distilled water extracts.

The bark and leaves of Saraca indica contain a significant amount of phytochemicals viz. alkaloid, flavonoids, phenolic, saponins and tannin. The bark of Saraca indica contained relativelely higher amounts of phytochemicals than leaves. The amount of flavonoids in bark was maximum (45.65mg/gm) followed by phenols (25.85mg/gm), alkaloids (20.75mg/gm), saponins (17.65mg/gm) and tannins (16.35mg/gm). The amount of phytochemicals in leaves also showed a similar trend i.e. the amount of flavonoids was maximum (38.75mg/gm), followed by phenol (21.67mg/gm), saponins (18.75mg/gm), alkaloids (18.35mg/gm) and tannins (14.45mg/gm).In all the six solvent extracts it was found that the bark of Saraca indica contained higher amount of phytochemicals in comparison to leaves.

Key words: *Saraca indica, bark extracts, leaf extract, polar solvents, non polar solvents, phytochemicals*

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I. Introduction

Saraca indica L. (Ashok) is a small evergreen tree of sub family Caesulpinoideae of family Leguminosae. The leaves are paripinnate, oblong and and rigidly sub- coriaceus with 6- 7 leaflets. This tree has orange coloured flowers with a beautiful aroma, 7- 8 stamens are found in flower and fruits are smooth, leathery and flat pods including 6- 8 seeds inside. Bark of this tree is rich in tannins, flavonoids, steroids, volatile oil, glycosides, and various steroidal glycosides. Leaves contain various carbohydrates, tannins, gallic acid and

egallic acid. Flowers are rich in sarcasin, sarcadin, waxy substances, proteins, carbohydrates and steroids. Seeds of this plant contain various fatty acids like oleic, linoleic, palmitic and stearic acid.

Ashok tree has been an integral part of Indian history. It is commonly called a tree which is important to decrease our sorrows. It has got religious significance and is also worshipped by some people in parts of India. . It has a number of medicinal properties hence used by physicians since centuries in Unani system of medicine along with Ayurveda [1] (Kokate et al. 2007). It is primarily used for the management of female reproductive problems. Married women in India are known to eat Ashoka flower buds as a ritual to invoke deities for child protection as well as gynecological problems. Women suffering from menorrhagia drink a decoction on an empty stomach in the morning, which is prepared from the bark of Ashoka in water in combination with other herbs such as *Terminalia chebula* and *Coriandrum sativum* [2] (Begum et al. 2014). In leucorrhoea, the decoction of Ashoka bark in water and milk after evaporation of water is consumed by women. In India, Srilanka, Bangladesh and Pakistan Ashoka bark is used by womenfolk in treating menorrhagia, menstrual and uterine disorders [3,4] (Mishra et al. 2013; Mollik et al. 2010).

Saraca indica is a rain- forest tree. It is native of Asia and South America. It is originally distributed in the central areas of Deccan plateau. It is also found in Western Ghats of the Indian subcontinent. It is also widely distributed in the center and the Eastern Himalayas and in the hills of Khasi, Garo and available in West Bengal. It is common to all parts of Indian and other countries. In India it is easily available in West Bengal, Kerala, Maharashtra, Andhra Pradesh and Meghalaya [1, 5] (Kokate et al. 2007; Prajapati et al.2003).

This plant has cooling properties. It is very useful for the body to bring down excessive heat in the organs due to fatigue or hormonal imbalance. It helps to regulate blood composition and stabilize blood circulation making it optimally available to all the body parts. Its pain relieving action can help relieve painful dysmenorrheal, swelling and pain at any site of the body. In females it is very commonly used to regularize hormones and menstrual cycles. It improves the strength and stamina in young females having menstrual irregularities such as dysmenorrea and leucorrhea. Many at times a combination of *Aloe vera* and Ashok is given to females to improve their reproductive health and blood condition. Anemia which is very common health problem in females is also recovered with the right combination of herbs along with Ashok derivatives. It not only works on uterine structures but also helps to cleanse the system so that any kind of microbial infestation that may be causing leucorrhea and other associated infections in the reproductive organs in females can be checked.

Ashok is also a cardiac tonic that can act as a supportive therapy for people suffering from hypertension, circulatory problems, edema, congestive heart failure etc. Its bark has natural detoxification properties which make it very useful to improve skin complexion and keep the body free from toxins inside out. Its natural cleansing properties can help the body stay toxin free. When the body has a lot of toxic load free radicals are produced. These free radicals then start damaging the body cells and all signs of ageing, disease and malfunctions are produced. For general pitta aggravated states also, Ashok bark acts as a coolant and helps to relieve thirst, excessive burning sensation, anger, emaciation, sweating etc. These are all common signs of pitta aggravation which can be relieved with the use of Ashok bark in different ways. It also has some digestive properties. Common problems of digestion like bloating, flatulence, burping, colicky pain in abdomen, ascites etc. can be relieved with the use of Ashok. It is not exactly a direct indication of the herb but it does help because all diseases have root from a malfunctioning gut and digestive system overtime.

Saraca asoca has many uses mainly in the medicine to treat the women gynecological disorders, in all types of abnormal discharges from vagina, in uterine inertia, uterine pain, urinary calculus, dysurea, etc. *Saraca asoca* (Ashoka) plant contains the presence of glycoside, flavonoids, tannins and saponins [6] Pradhan et al. (2009). It is used as spasmogenic, oxytocic, uterotonic, antibacterial, anti implantation, anti tumour, anti progestational, anti estrogenic activity against menorrhagia and anti cancer agent. The plant is useful in dyspepsia, fever, burning sensation, colic, ulcer, menorrhagia, leucorrhoea, pimples, etc Srivastav et al. (1988) [7]. *Saraca asoca* dried bark has been used for menorrhagia in India (Middelkoop and Labadie, 1986; Bhandari et al. 1995) [8, 9]. In India *Saraca asoca* dried bark as well as flower is given as a tonic to ladies to treat Uterine disorders. *Saraca asoca* stem bark also used in case of all disorder associated with the menstrual cycle (Kumar et al. (1980; Middelkoop and Labadie, 1985) [10, 11]. Ashoka is blood purifier and used in all skin diseases, ammenorhea, dysmenorrhea menopause, menorrhagia, painful menstruation blood circulation and purification, cancer, diarrhea, dysentery, edema, heart disease, hepatitis, herpes, jaundice, joint pain, kidney and gall stones, paralysis, skin problems, rheumatoid arthritis, obstructions in urinary passages (Nadkarni, 1994) [12].

Phytochemicals are primary and secondary compounds that are occurring naturally in various medicinal plants, leaves, vegetables and they are found to exert defence mechanism to protect plants against various diseases [13] (D. Krishnaiah et al. 2007). Scientific evaluation of medicinal plants are important not only to the discovery of novel drugs but also it put forth to assess toxicity risks associated with the use of herbal preparations. Plant derived extracts contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties [14] (M. Kumaraswamy et al. 2011). Plant-derived medicines have been

part of traditional healthcare in most parts of the world for thousands of years and there is increasing interest in plants as sources of agents to fight against microbial diseases [15] (V. Ashok Gomashe et al. 2014).

Saraca asoca is reported to contain glycoside, flavonoids, tannins and saponins [16] (P. Pradhan et al. 2010). The *asoca* tree has many health benefits and has long been used in traditional Indian medicine as a key ingredient in various therapies and cures. It is used as protective drug for spasmogenic, oxytocic, uterotonic, anti-bacterial, antiimplantation, anti-tumour, antiprogestational, antiestrogenic activity against menorrhagia and anti-cancer. One of the uses of the *asoca* herb is in the treatment of menstrual disorders associated with excessive bleeding, congestion, pain, dysmenorrhoea, abdominal pain, uterine spasms and miscarriage [16, 17, 18] (P. Pradhan et al. 2010; M. A. H. Mollik et al. 2010; S. N. Begum et al. 2014). It also has a nourishing effect on the circulatory system, thereby making it an effective remedy in arrhythmia and cardiac weakness [19] (A. H. M. V. Swamy et al. 2013). The *asoca* herb also helps in encouraging urine flow and thus helps in treating conditions that cause painful urination. It also has specific analgesic properties and it is said to improve the complexion of skin [20] (A. Mishra et al. 2013). The various phytoconstituents have been reported in leaves and bark of the plant. All parts of plant viz. bark, leaves, flowers are regarded as medicinally important and used as therapeutic agent in treatment of diabetes, cancer and hemorrhagic dysentery, bleeding piles, uterine infections and bacillary dysentery. An antioxidant molecule, the gallic acid has been reported in *Saraca asoca* flower [21] (S. Singh et al. 2015). Dried flower buds are reported to have antibacterial activity [22] (P. Pradhan et al. 2009). Aqueous suspension of *Saraca asoca* flower has antiulcer activity in albino rats [23] (V. Maruthappan et al. 2010). *Saraca asoca* bark and flowers exhibit antitumour activity against DLA, S-180 and Ehrlich ascites carcinoma tumour cell lines [24] (T. R. Cibin and D. G. Devi, 2012). Larvicidal activity has also been recorded [25] (N. Mathew et al. 2009). Chemopreventive activity of flavonoid fraction of *Saraca asoca* is reported in skin carcinogenesis [26] (Cabin et al. 2010). Flower extract is bitter in taste and bark has a stimulatory effect on endometrium and ovarian tissue and used in uterine fibrosis, menorrhagia, bleeding hemorrhoids and also as astringent.

The aim of the present study is to analyse the important phytochemicals in the six different solvent extracts of bark and leaves of *Saraca indica* qualitatively as well as quantitatively.

II. Materials and Methods

Fresh bark and leaves of *Saraca indica* were collected in the month of February 2017 from the campus of College of Commerce (Patna). The collected plant materials were brought to the laboratory on the same day. Plant samples were washed with water and air–dried at room temperature for 7 days, oven – dried at 40 °C to remove the residual moisture. The dried leaves and bark were powdered using a mixer grinder and stored in air-tight container for future use. Six different solvents such as Chloroform, Petroleum ether, Ethanol, Methanol, Hexane and Distilled water were used for extraction. About 1 gm of the plant samples were added respectively into the test tubes containing 5 ml solvents, and were extracted at room temperature. The extracts in all the six solvents of bark and leaves were tested for the presence of biological compounds following standard methods.

Qualitative estimation of Phytochemicals: Qualitative analysis of phytochemicals was done for carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, cardiac glycosides and alkaloids

Test for Carbohydrates

Fehling's test

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's test

Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Iodine test

Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

Test for Phenols and Tannins

Crude extracts were mixed with 2ml of 2% solution of $FeCl_3$. A blue–green or black coloration indicated the presence of phenols and tannins.

Test for Flavonoid

Alkaline reagent test

Crude extracts were mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for Saponins (Frothing test)
Crude extracts were mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponin.

Test for Glycosides
Liebermann's test

Crude extracts were mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Salkowski's test
Crude extracts were mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown color indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

Keller-kilani test (Cardiac Glycosides)

Crude extracts were mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the inter phase indicated the presence of cardiac glycoside.

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Test for Alkaloids:
The crude extract of all the six solvents was boiled in 10 ml methanol and filtered separately. 1% HCl was added followed by 6 drops of Dragendroff reagent, and the brownish-red precipitate was taken as evidence for the presence of alkaloids.

Phlobatannins:
The deposition of a red precipitate denoted the presence of phlobatannins when crude extract of all the six solvent of plant material was dissolved in 10 ml of aqueous extract and few drops of 1% HCl were added in the boiling tube.

Anthraquinones: All the six solvent extracts of leaves were boiled in 10% HCl for 5 mins separately and the filtrate was allowed to cool. An equal volume of $CHCl_3$ with few drops of 10% NH_3 was added to the 2ml filtrate. The formation of rose-pink colour implies the presence of anthraquinones.

Quantitative estimation of phytochemicals: Phytochemicals were determined quantitalively for alkaloids, flavonoids, tannins, saponins and total phenols

Determination of Alkaloids

Alkaloids content was measured by method suggested by Harborne (Harborne, 1973) [27]. A suspension was prepared by dispersing 5 gm of the dried leaves in 10% acetic acid solution in ethanol and kept at 28^0C for 4hrs which was further filtered through Whatman No. 42. Thereafter alkaloid was precipitated by concentrating the filtrate to one-quarter of its original volume and drops of conc. aqueous NH_4OH were added. Finally, the precipitate was washed with 1% ammonia solution and dried at 80^0C in the oven. The content of alkaloid was calculated and expressed as mg/g of sample.

Determination of Flavonoids

The flavonoids content was also determined by Harborne27 (Harborne, 1973) method. 5 gm of leaves were boiled in 2M HCl for 30 min under reflux condition and filtered after cooling. An equal volume of ethyl acetate was then added drop wise to the filtrate. The weight of precipitated flavonoid was determined and recorded as mg/g.

Determination of Tannins

The finely powdered leaves and barks of *Saraca indica* were kept separately in a beaker containing 20 ml of 50% methanol covered with parafilm and then heated at 80oC in a water bath for 1 hr with continuous stirring. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and rinsed with 50% methanol. 1 ml of sample extract was treated with 20 ml distilled water, 2.5 ml Folin-Denis reagent

and 10 ml of 17% Na_2CO_3 for the development of a bluish-green colour and was allowed to stand for 20 mins. The absorbance was measured at 760 nm and the amount of tannin was calculated by comparing it with a standard curve prepared in the range of 0-10 ppm.

Determination of Saponins

100 ml Isobutyl alcohol was added to 1 gm of the finely powdered sample and stirred for 5 hrs. 20 ml of 40% saturated solution of Magnesium carbonate was added to the mixture and filtered. 2 ml of 5% FeCl3 solution and 50ml volume of distilled water was added to 1ml of colourless solution and kept for 30 mins for colour (blood red) development The absorbance of the samples as along with the standard were read at 380 nm and calculated in mg/g. Standard saponin solution was prepared in the reference range of 0-10 ppm.

Determination of total phenols

Five gms of the powdered leaves were boiled with 50 ml of ether for 15 mins and distributed in the ratio 1:2 (extract: distilled water). 2ml of ammonium hydroxide followed with 5ml of pentanol was added to it and incubated at the room temperature for 30mins. The absorbance was read at 505 nm wavelength.

For measuring alkaloids a suspension was prepared by dispersing 5 gm of the dried bark and leaves separately in 10% acetic acid solution in ethanol and kept at 28^0C for 4hrs which was further filtered through Whatman No. 42. Thereafter alkaloid was precipitated by concentrating the filtrate to one-quarter of its original volume and drops of conc. aqueous NH_4OH were added. Finally, the precipitate was washed with 1% ammonia solution and dried at 80^0C in the oven. The content of alkaloid was calculated and expressed as mg/g of sample.

For determining flavonoids 5 gm of bark and leaves were boiled separately in 2M HCl for 30 min under reflux and filtered after cooling. An equal volume of ethyl acetate was then added drop wise to the filtrate. The weight of precipitated flavonoid was determined and recorded as mg/g.

For measuring tannin the finely powdered bark and leaves of *Saraca indica* were kept separately in a beaker containing 20 ml of 50% methanol covered with parafilm and then heated at 80^0C in a water bath for 1 hr with continuous stirring. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and rinsed with 50% methanol. 1 ml of sample extract was treated with 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na_2CO_3 for the development of a bluish-green colour and was allowed to stand for 20 mins. The absorbance was measured at 760 nm and the amount of tannin was calculated by comparing it with a standard curve prepared in the range of 0-10 ppm.

For determining saponin content 100 ml Isobutyl alcohol was added to 1 gm of the finely powdered bark and leaf samples separately and stirred for 5 hrs. 20 ml of 40% saturated solution of Magnesium carbonate was added to the mixture and filtered. 2 ml of 5% $FeCl_3$ solution and 50ml volume of distilled water was added to 1ml of colourless solution and kept for 30 mins for colour (blood red) development The absorbance of the samples as along with the standard were read at 380 nm and calculated in mg/g. Standard saponin solution was prepared in the reference range of 0-10 ppm.

For determining total phenolic content five gms of the powdered bark and leaves of *Saraca indica* were boiled with 50 ml of ether for 15 mins and distributed in the ratio 1:2 (extract: distilled water). 2ml of ammonium hydroxide followed with 5ml of pentanol was added to it and incubated at the room temperature for 30mins. The absorbance was read at 505 nm wavelength.

Quantitative analysis of phytochemical constituents in six different solvent extracts

Six solvent extract of bark and leaves of *Saraca indica* viz. chloroform, petroleum ether, ethanol, methanol, hexane and distilled water were prepared by soaking 10gm of the powdered sample in 200 ml of each of the solvent separately for 12 hrs. The extracts were then filtered using filter paper. The extracts were then concentrated to ¼ of the original extracts i.e. 50 ml.

The amount of total phenolics in extracts was determined by the Folin–Ciocalteu method. Gallic acid was used as a standard by using different concentrations of (20-200µg) from which the total phenol content in the extract was expressed in terms of gallic acid equivalent (mg GAE /gm) extract. Different aliquots of 0.1 to 1.0 ml of plant extract were also prepared in methanol and 0.5 ml of each sample were introduced into test tubes and mixed with 2.5 ml of a 10-fold dilute Folin- Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 30 mins at room temperature. Phenols react with the phosphomolybdic acid in Folin- Ciocalteau reagent in alkaline medium and produce blue coloured complex (Molybdenum blue). The absorbance of the resulting solutions was measured at 760 nm against reagent blank. A standard calibration curve was prepared by plotting absorbance against concentration and it was found to be linear over this concentration range. The concentration of total phenol in the test sample was determined from the calibration graph. The assay was carried out in triplicate and the mean values with ± SD are presented.

The aluminium chloride colorimetric method was used for flavonoids determination. Each solvent extract (0.5 ml of 1:10 gm ml^{-1}) was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was kept at room temperature for 30

min; the absorbance of the reaction mixture was measured at 418 nm. The percentage of total flavonoids were calculated from the calibration curve of Quercetine equivalent (QE) (200-1000µg) plotted by using the same procedure and total flavonoids was expressed as Qeurcetin Equivalent (QE) equivalents in mg per gm sample. The results obtained have been presented in Table- 1, 2, 3and 4 and Fig- 1 and 2.

Table- 1: Qualitative phytochemical analysis of bark of *Saraca indica*

Tests		Extracts used					
		Chloroform	Petroleum ether	Ethanol	Methanol	Hexane	Distilled water
Carbohydrates	Fehling's test	-	-	-	-	-	+
	Benedict's test	+	+	+	+	+	+
	Iodine test	+	-	-	-	+	-
Phenols and Tannins	Ferric chloride test	+	-	+	+	+	-
Falavonoids test	Alkaline reagent test	-	-	-	-	-	-
Saponin test	Froth Foam test	-	-	+	+	+	+
Glycosides	Libermann's test	-	-	-	-	-	-
	Salkowski test	+	+	+	+	+	+
	Killer- Killani test	+	+	+	+	+	+
Phenolic compounds		+	-	+	+	+	+
Steroids		+	+	+	+	+	+
Tests for some other phytochemicals	Phlobatannin	+	+	+	+	+	+
	Terpinoid	+	+	+	+	+	+
	Diterpinoid	+	+	+	+	+	+
	Emodins	+	-	+	+	+	+
	Anthraquinones	+	+	+	+	+	+
	Carotenoids	+	+	+	+	+	+
	Anthocyanin	+	+	+	+	+	+
	Coumarin	+	+	+	+	+	+
	Phytosterol	+	+	-	-	+	+
	Fatty acids	+	+	+	+	+	+
	Proteins	+	+	+	+	+	+
	Amino acids	+	+	+	+	+	+

Table- 2: Qualitative phytochemical analysis of leaves of *Saraca indica*

Tests		Extracts used					
		Chloroform	Petroleum ether	Ethanol	Methanol	Hexane	Distilled water
Carbohydrates	Fehling's test	+	+	+	+	-	-
	Benedict's test	+	-	+	+	+	+
	Iodine test	-	+	+	+	+	+
Phenols and Tannins	Ferric chloride test	-	+	+	+	+	-
Falavonoids test	Alkaline reagent test	-	+	+	+	+	+
Saponin test	Froth Foam test	-	-	+	+	+	+
Glycosides	Libermann's test	+	-	+	+	-	+
	Salkowski test	-	-	+	+	+	+
	Killer- Killani test	+	-	+	+	-	-
Phenolic compounds		+	+	+	+	-	-
Steroids		+	+	+	+	-	-
Tests for some other phytochemicals	Phlobatannin	+	+	+	+	+	+
	Terpinoid	+	+	+	+	+	+
	Diterpinoid	+	+	+	+	+	+
	Emodins	+	-	+	+	+	+
	Anthraquinones	+	+	+	+	+	+
	Carotenoids	+	+	+	+	+	+
	Anthocyanin	+	+	+	+	+	+
	Coumarin	+	+	+	+	+	+
	Phytosterol	+	+	-	-	+	+
	Fatty acids	+	+	+	+	+	+

	Proteins	+	+	+	+	+	+
	Amino acids	+	+	+	+	+	+

Table- 3: Quantitative estimation of phytochemicals in bark and leaves of *Saraca indica* (amount in mg/gm)

Phytochemicals	Bark	Leaves
Alkaloids	**20.75±0.26**	**18.35±0.45**
Flavonoids	**45.65±0.47**	**38.75±0.35**
Phenols	**25.85±0.35**	**21.67±0.21**
Saponins	**17.65±0.32**	**18.75±0.37**
Tannins	**16.35±0.24**	**14.45±0.31**

Mean ± SD of five measurements

Phenols are expressed as Gallic acid equivalent (GAE) and Flavonoids are expressed as Quercetin equivalents (QE) in mg/100 gm

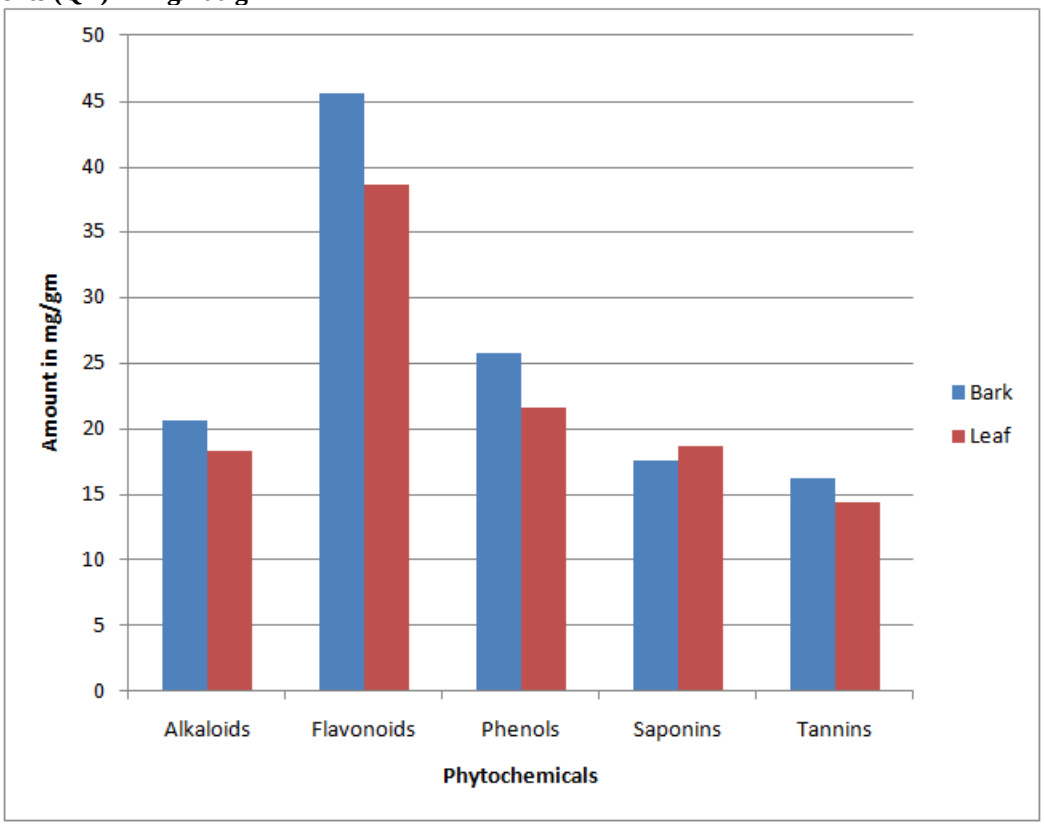

Fig- 1: Phytochemicals in bark and leaves of *Saraca indica*

Table- 4: Comparative analysis of total alkaloids, flavonoids, phenols, saponins and tannins in six different solvent extracts of bark and leaves of *Saraca indica* (amount in mg/gm)

Solvents	Extract type	Total alkaloids	Total flavonoids	Total phenols	Total saponins	Total tannins
Chloroform	**Bark**	**14.35±0.26**	**3.38±0.21**	**42.35±0.61**	**10.75±0.31**	**15.55±0.18**
	Leaf	**12.65±0.35**	**5.75±0.27**	**37.65±0.52**	**9.47±0.21**	**13.65±0.21**
Petroleum ether	**Bark**	**12.75±0.71**	**4.12±0.07**	**43.35±0.63**	**10.45±0.32**	**12.75±0.08**
	Leaf	**10.25±0.21**	**3.75±0.08**	**36.35±0.70**	**9.42±0.24**	**11.38±0.11**
Ethanol	**Bark**	**36.35±0.15**	**5.35±0.31**	**118.75±0.72**	**70.25±0.09**	**62.75±0.13**
	Leaf	**32.65±0.18**	**6.58±0.32**	**148.35±0.61**	**61.37±0.07**	**53.67±0.07**
Methalon	**Bark**	**38.45±0.17**	**4.75±0.76**	**115.98±0.75**	**71.45±0.15**	**54.35±0.16**
	Leaf	**33.35±0.25**	**5.86±0.64**	**146.37±0.64**	**63.35±0.13**	**55.65±0.30**
Hexane	**Bark**	**28.65±0.38**	**4.15±0.07**	**40.28±0.64**	**62.65±0.15**	**13.75±0.15**
	Leaf	**24.36±0.20**	**3.85±0.08**	**32.35±0.61**	**11.37±0.20**	**12.70±0.14**
Distilled water	**Bark**	**42.65±0.31**	**5.45±0.37**	**119.76±0.71**	**72.35±0.19**	**65.45±0.19**
	Leaf	**40.45±0.16**	**6.75±0.27**	**147.37±0.62**	**64.75±0.16**	**55.27±0.22**

Mean ± SD of five measurements

Phenols are expressed as Gallic acid equivalent (GAE) and Flavonoids are expressed as Quercetin equivalents (QE) in mg/100 gm

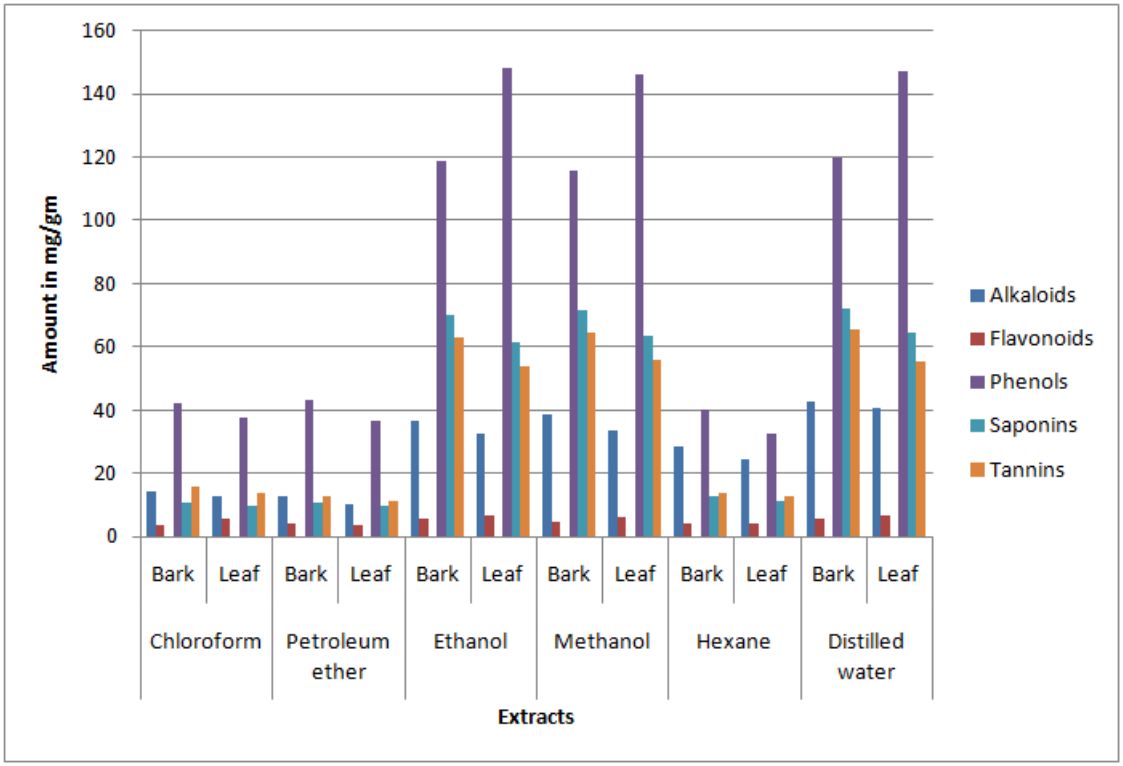

Fig- 2: Comparison o phytochemicals in six different solvent extracts from bark and leaves of *Saraca indica*

III. Results and Discussion

Phytochemical analysis conducted on the *Saraca indica* leaves and bark extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. The phytochemical screening of the bark and leaves of *Saraca indica* was done with chloroform, petroleum ether, ethanol, methanol, hexane and distilled water. Among the six solvent extracts studied the carbohydrate was present in aqueous extract of bark as evidenced by positive Fehling's test. All the six solvent extracts of bark showed positive Benedict's test for carbohydrate. Only the chloroform and hexane extracts of bark showed positive Iodine test for carbohydrate (Table- 1). The phenols and tannins were detected only in chloroform, ethanol, methanol and hexane extracts but not in petroleum ether and aqueous extracts. All the six solvent extracts of bark showed negative alkaline reagent test which indicated the absence of flavonoids. Saponin was detected in four solvent extracts of bark viz. ethanol, methanol, hexane and distilled water as evidenced by positive froth foam test. Glycosides were not detected in any of the six solvent extracts of bark in Libermann's test, but in Salkowski and Killer- Kilani tests all the six solvent extracts showed the presence of glycosides. Phenolic compounds were detected in all extracts except petroleum ether. The steroids were recorded in all the six solvent extracts of bark. Among other phytochemicals phlobatannin, terpenoid, diterpinoid, anthraquinones, carotenoids, anthocyanin, coumarin, fatty acids, proteins and amino acids were detected in all the six solvent extracts. Emodins were detected in all except petroleum ether extract of bark. Similarly the phytosterols were present in all extracts except ethanol and methanol extracts of bark of *Saraca indica* (Table- 1).

In the solvent extracts of leaves of *Saraca indica* carbohydrates were detected in all the six solvent extracts. Fehling's test was positive in chloroform, petroleum ether, ethanol and methanol extract but negative in hexane and aqueous extracts. Benedict's test was positive in all except petroleum ether extract. Similarly, Iodine test for carbohydrates was positive in all except chloroform extract (Table- 2). Phenols and tannins were detected in all except chloroform and aqueous extracts. Flavonoid was detected in all except chloroform, and saponins in all except chloroform and petroleum ether extracts. Petroleum ether extract showed negative results for the presence of glycosides with all the three tests viz. Libermann's, Salkowski and Killer- Kilani. In petroleum ether and hexane extract of leaves glycosides were not detected. Similarly, glycosides were also not detected in chloroform and petroleum ether extracts as Salkowski test was found to be negative. Killer- Kilani test for glycosides was positive with chloroform, ethanol and methanol extracts but negative with petroleum ether, hexane and distilled water extracts. Phenolic compounds and steroids were detected in all the solvent

extracts of leaves except hexane and distilled water extracts. Among other phytochemicals phlobatannin, terpenoid, diterpinoid, anthraquinones, carotenoids, anthocyanin, coumarin, fatty acids, proteins and amino acids were detected in all the six solvent extracts. Emodins were detected in all except petroleum ether extract of leaves. Similarly the phytosterols were present in all extracts except ethanol and methanol extracts of leaves of *Saraca indica* (Table- 2).

The Phytochemical study conducted by Aditya *et al*., 2013 [28] shows the presence of various chemical constituents of Ashoka Bark. The non polar solvents viz. chloroform, petroleum ether, ethanol, methanol and hexane dissolve many hydrophilic and lipophilic components of plants. Distilled water is universal solvents for a large number of phytochemicals and is largely used for bioassay because of its low toxicity. It is a very useful extractant, especially for antimicrobial studies where more phenolic compounds are required to be extracted. A study reported by Das *et al*., 2010 [29] revealed that extraction of tannins and other phenolics was better in aqueous acetone than in aqueous methanol. The higher activity of the ethanolic extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. It means that they are more efficient in cell walls and seed degradation which have unpolar character and cause polyphenols to be released from cells. More useful explanation for the decrease in activity of aqueous extract can be ascribed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts, whereas in methanol and ethanol they are inactive. The higher concentrations of more bioactive flavonoid compounds were detected with ethanol 70% due to its higher polarity than pure ethanol. By adding water to the pure ethanol up to 30% for preparing 70% ethanol the polarity of solvent was increased (Bimakr.,2010) [30]. Additionally, ethanol was found easier to penetrates the cellular membrane to extract the intracellular ingredients from the plant material (Wang 2010) [31]. In the bark and leaves of *Saraca indica* maximum results such as carbohydrates, phenols, flavanoids, saponins, glycosides and steroids were seen in ethanol in the present study (Table- 1 and 2).

Cowan (1999) [31] reported that ether is commonly used selectively for the extraction of coumarins and fatty acids. The extracts were subjected to preliminary phytochemical analysis using standard chemical methods which mainly revealed the presence of carbohydrates, flavonoids, tannins and saponins. The present observations are supported by Divya et al. (2017)[33] who have screened a more or less similar phytochemicals from bark, leaves and flowers of *Saraca asoca*. The present findings also gain support from the work of Athiralakshmy et al. (2016) [34] who analyzed qualitatively a more or less similar phytochemicals from the leaves of *Saraca asoca*.

The present study revealed that the various phytochemical components such as carbohydrates, flavonoids, saponins, phenols, tannins, glycosides and steroids that are present in the bark and leaves of *Saraca indica*. Ashoka have many medicinal uses and is a nontoxic traditional medicinal plant. Numerous medicinal therapies treat their patients with herbal medicines for its extraordinary influence, though relatively little knowledge about their mode of action is available. In the Ayurvedic system of medicine, herbal extracts instead of purified compounds have been used since centuries because many constituents with more than one mechanism of action are considered essential for the required holistic therapeutic action. Ashoka is one of the most legendary and sacred trees. *Saraca indica/ asoca* is highly regarded as an universal panacea in the ayurvedic medicine. It is one of the universal plant having medicinal activities and is the source of various types of compounds. The present study revealed that the various phytochemical components such as carbohydrates, flavonoids, saponins, phenols, tannins, glycosides and steroids, are present in the bark, leaves and flower of *Saraca asoca/ indica*. Ashoka have many medicinal uses and is a nontoxic traditional medicinal plant. The use of phyto compounds of Asoka against diseases is a challenge in the development of modern drug discovery. This versatile plant is the source of various types of compounds. In the present scenario many plant are used to treat many diseases. But *Ashoka* is ancient and reliable source of medicine so Ashoka is used in many pharmacological activities. It has many uses like to treat skin infections, CNS function, genitor-urinary functions. As the global scenario is now changing towards the use of nontoxic plant product having traditional medicine use, development of modern drug from *Saraca asoca* should be emphasized for the control of various diseases. The present work can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future prospective study.

From the results (Table- 3; Fig- 1) it is evident that the bark and leaves of *Saraca indica* contain a significant amount of phytochemicals viz. alkaloid, flavonoids, phenolic, saponins and tannin. The bark of *Saraca indica* contained relativelely higher amounts of phytochemicals than leaves. The amount of flavonoids in bark was maximum (45.65mg/gm) followed by phenols (25.85mg/gm), alkaloids (20.75mg/gm), saponins (17.65mg/gm) and tannins (16.35mg/gm). The amount of phytochemicals in leaves also showed a similar trend i.e. the amount of flavonoids was maximum (38.75mg/gm), followed by phenol (21.67mg/gm), saponins (18.75mg/gm), alkaloids (18.35mg/gm) and tannins (14.45mg/gm) (Table- 3; Fig- 1).

The comparative analysis of phytochemicals viz. total alkaloids, flavonoids, phenols, saponins and tannins in six different solvent extracts from bark and leaves of *Saraca indica* has been presented in Table- 4

and Fig- 2. In all the six solvent extracts it was found that the bark of *Saraca indica* contained higher amount of phytochemicals in comparison to leaves. The concentration of total alkaloids in bark was maximum in distilled water extract (42.65mg/gm), followed by ethanol extract (38.45mg/gm), methanol extract (36.35mg/gm), hexane extract (28.65mg/gm), chloroform extract (14.35mg/gm), petroleum ether extract (12.75mg/gm). Similarly, the concentration of total alkaloids in leaves also showed a similar trend, being maximum in distilled water extract (40.45mg/gm), followed by methanol extract (33.35mg/gm), ethanol extract (32.65mg/gm), hexane extract (24.36mg/gm), chloroform extract (12.65mg/gm) and petroleum ether extract (10.25mg/gm). In all the six solvent extracts it was found that the bark contained relatively less amount of total alkaloids in comparison to leaves. In 4.12mg/gm and hexane extract contained 4.15mg/gm of total alkaloids. Similarly, the amount of total alkaloids was maximum in ethanol, methanol and distilled water extracts (5.86- 6.85mg/gm). Hexane, chloroform and petroleum ether extracts of leaves contained less amount of total alkaloids. The amount of total phenols was highest in ethanol, methanol and distilled water extracts of both bark and leaves. The distilled water extracts of bark and leaves contained 119.76/gm and 147.37mg/gm respectively; of ethanol extracts contained 118.75mg/gm and 148.35mg/gm respectively and of methanol extracts 115.98mg/gm and 146.37mg/gm respectively of total phenols. Other extracts contained 40.28 to 43.35mg/gm and 32.35 to 37.65mg/gm of total phenols respectively in bark and leaves. The concentration total saponin and total tannins was maximum in ethanol, methanol and distilled water extracts of both bark and leaves in comparison to non polar solvents viz. chloroform, petroleum ether and hexane which contained less amount of total saponins and tannins in both bark and leaves. The total saponin concentration in bark and leaves was maximum in distilled water extract (72.35mg/gm and 64mg/gm respectively) followed by methanol extract (71.45mg/gm and 63.35mg/gm respectively) and ethanol extract (70.25mg/gm and 61.37mg/gm respectively). Other extracts contained relatively very low amount of total saponins in both bark and leaves. A more or less similar pattern for total tannins was recorded in all the six solvent extracts, being maximum in polar solvents viz. distilled water, ethanol and methanol extracts and lesser in non polar solvents i.e. chloroform, petroleum ether and hexane extracts (Table- 4; Fig- 2).

The extraction of various phytochemicals was seen to be more effectively done in polar solvents (ethanol, methanol and distilled water) than the non polar (chloroform, petroleum ether and hexane) solvents. Especially, distilled water, ethanolic and methanolic extracts of bark and leaves showed presence of most of the tested phytochemicals. Hence, it can be reported that alcoholic and aqueous extracts were the best for extracting the active principle than other solvents. Flavonoids are water-soluble polyphenolic compounds which are extremely common and widespread in the plant kingdom as their glycosides. The flavonoids are known to act through scavenging or chelating process.

The present study contributes valuable information of bioactive compounds in *S. indica.* Qualitative analysis of plant different extract (bark and leaves) was carried out for Alkaloids, Flavonoids, Glycosides, Saponins, Phenols, Steroids, Tannins and terpenoids, diterpinoids etc. Methanol, ethanol and aqueous extract of bark and leaves had all the phytochemicals like flavonoids, glycosides, saponins, phenols, steroids, tannins and terpinoids. The present findings are in agreement with the works of Nayak *et al.,* 2011, Ghatak *et al.,* 2014; Gayathri *et al.,* 2013 and Ch. Mohan, 2016; Ravindran Jaganath et al. 2017) [35, 36, 37, 38, 39] who also analyzed the same phytochemicals in the bark, flowers and leaves of *Saraca asoca*.

IV. Conclusions

The present study revealed that the various phytochemical components such as carbohydrates, flavonoids, saponins, phenols, tannins, glycosides and steroids that are present in the bark and leaves of *Saraca indica*. Ashoka have many medicinal uses and is a nontoxic traditional medicinal plant. Numerous medicinal therapies treat their patients with herbal medicines for its extraordinary influence, though relatively little knowledge about their mode of action is available. In the Ayurvedic system of medicine, herbal extracts instead of purified compounds have been used since centuries because many constituents with more than one mechanism of action are considered essential for the required holistic therapeutic action. Ashoka is one of the most legendary and sacred trees. *Saraca indica/ asoca* is highly regarded as an universal panacea in the ayurvedic medicine. It is one of the universal plant having medicinal activities and is the source of various types of compounds. The present study revealed that the various phytochemical components such as carbohydrates, flavonoids, saponins, phenols, tannins, glycosides and steroids, are present in the bark, leaves and flower of *Saraca asoca/ indica*. Ashoka have many medicinal uses and is a nontoxic traditional medicinal plant. The use of phyto compounds of Asoka against diseases is a challenge in the development of modern drug discovery. This versatile plant is the source of various types of compounds. In the present scenario many plant are used to treat many diseases. But *Ashoka* is ancient and reliable source of medicine so Ashoka is used in many pharmacological activities. It has many uses like to treat skin infections, CNS function, genitor-urinary functions. As the global scenario is now changing towards the use of nontoxic plant product having traditional medicine use, development of modern drug from *Saraca asoca* should be emphasized for the control of various

diseases. The present work can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future prospective study.

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