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Screening of active phytochemicals in stem bark and leaves of *Saraca indica* L.

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Abstract: *Saraca indica L. (Ashok) is an evergreen tree of Caesulpinaceae. Their leaves are paripinnate with orange coloured flowers. 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. This plant 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. It is used as spasmogenic, oxytocic, uterotonic, antibacterial, anti implantation, anti tumour, anti progestational, anti estrogenic activity against menorrhagia and anti cancer agent, dyspepsia, fever, burning sensation, colic, ulcer, menorrhagia, leucorrhoea, pimples. 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.*

In the present investigation a number of active phytochemicals from methanol and n-hexane extracts of leaves and n- hexane, methanol and aqueous methanol extracts of bark of S. indica have been isolated and screened employing HPTLC and HPLC techniques. The results revealed the presence of gallic acid in n- Hexane and Methanolic extracts of leaves. The stem bark of Saraca indica yielded l- oleo-diplmitin, triterpinoids, viz. ursolic acid, lupeol, glochidiol and sterols, viz. campesterol, β-sitosterol, stigmasterol. Out of these, ursolic acid, l-oleo-dipalmitin, lupeol, glochidiol have been isolated for the first time. The bark extracts showed the presence of leucopelargonidin, leucocyanidin, 5,3'-dimethoxy-(-)-epicatechin, (-)-epicatechin, (+)-catechin, (-)-3'-deoxyepicatechin-3-O-β-D-glucopyranoside, lyoniside, (+)-3'-deoxycatechin-3-O-α-L-rhamnopyranoside, (-)-epigallocatechin, (-)-gallocatechin. The present study contributes to the current knowledge of the presence of various active phytochemical compounds in stem bark and leaves of Saraca asoca which possess significant drug yielding molecules for treatment of various diseases.

Key Words: *Saraca indica, active phytochemicals, HPTLC, HPLC, n-hexane, methanol, gallic acid*

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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 indica* dried bark has been used for menorrhagia in India (Middelkoop and Labadie, 1986; Bhandari et al. 1995) [8, 9]. In India *Saraca indica* 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] (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] (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] (Ashok Gomashe *et al*., 2014).

Saraca asoca is reported to contain glycoside, flavonoids, tannins and saponins [16] (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] (Pradhan *et al*., 2010; Mollik *et al*., 2010; 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] (Swamy *et*

al., 2013). The Ashok plant also has specific analgesic properties and it is said to improve the complexion of skin [20] (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] (Singh *et al*., 2015). Dried flower buds are reported to have antibacterial activity [22] (Pradhan *et al*., 2009). Aqueous suspension of *Saraca asoca* flower has antiulcer activity in albino rats [23] (Maruthappan *et al*., 2010). *Saraca indica* bark and flowers exhibit antitumour activity against DLA, S-180 and Ehrlich ascites carcinoma tumour cell lines [24] (Cibin and D. G. Devi, 2012). Larvicidal activity has also been recorded [25] (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.

Ayurvedic physicians use ashoka in treatment of various diseases, especially the gynaecological disorders. It is popularly used in the pharmaceutical preparations like asokarishta and asokagirtha, which are prescribed against leucorrhoea, haematuria, menorrhagia and other diseases of genitourinary system of women [27, 28] (Mishra, 2013; Pradhan *et al*., 2009). Phenolic glycosides from S. asoca have shown antioxidant activity due to the presence of phenolics and flavonoids. The methyl alcohol extract has been shown to exhibit antibacterial and antifungal activities [29] (Sainath *et al*., 2009). It has been reported that the methanol extract reduced paw swelling, increased body weight, reduced level of lysosomal enzymes, decreased protein bound carbohydrates, urinary collagen and serum cytokines as well as normalized histopathology of joints [30] (Saravanan *et al*., 2013) and did not show any toxicity [31] (Mukhopadhyay, 2011).

Earlier, the scantly pursued phytochemical studies on the stem bark of S. asoca have shown the presence of campesterol, β- sitosterol, stigmasterol, leucocyanidin, leucopelargonidin, procyanidin B1, procyanidine B12, catechin, epicatechin, gallaocatechin [32, 33, 34, 35, 36] (Sen, 1963; Behari *et al*., 1977; Duggal and Mishra, 1980; Middelkoop and Labadie, 1985; Mittal *et al*., 2013) and antioxidant and DNA topoisomerase active ligan glycosides [37, 38] (Sadhu *et al*., 2007; Mukherjee *et al*., 2012). The presence of anti- inflammatory activity, flavon glycosides and pinitol from S. asoca bark have been reported by [39, 40] (Ahmad *et al*., 2015; Ahmad *et al*., 2016).

In the present investigation a number of active phytochemicals from methanol and n-hexane extracts of leaves and n- hexane, methanol and aqueous methanol extracts of bark of *S. indica* have been isolated and screened employing HPTLC and HPLC techniques.

II. Materials and Methods

The stem barks and leaves of *Saraca indica* were collected from the campus of Gaya College, Gaya and the samples were washed with water and air-dried at room temperature for 7 days, then oven-dried at 40ºC to remove the residual moisture. The dried stem barks and leaves were pulverized and stored in air-tight container for future use. Methanol (polar solvent) and n-hexane (non polar solvent) were used as solvent for phytochemical screening. Equivalent amount of powdered samples of stem barks and leaves were extracted with methanol and n-hexane at room temperature for 3 days. Water extraction was done in water bath at 60ºC. The filtrates were separately concentrated in water bath at 45ºC and evaporated under reduced pressure and then the percent extract yield (%) was calculated.

HPTLC analysis *Chemicals and standard gallic acid:* Gallic acid was obtained from Titan Biotech Ltd. and Methanol, Toluene, Ethyl Acetate, Formic Acid were used of analytical grade E-Merck. Silica gel 60 F_{254} precoated Thin Layer Chromatography (TLC) aluminium plate was used of E-Merck.

Preparation of standard and sample solution: 5 mg Gallic acid was dissolved in 3 ml of methanol and n-hexane separately. It was then sonicated for 5 min and the final volume was made up to 5 ml with the same solvent to obtain stock solution containing 1 mg/ml. Air dried samples (0.5g) was extracted with 10 ml of methanol and 10 ml of n-hexane. Extracts were concentrated, filtered and the final volume made up to 10 ml with methanol and 10 ml of n-hexane separately prior to HPTLC analysis to get stock solution containing 50 mg/ml.

Chromatographic conditions: Chromatography was performed on precoated silica gel 60 F_{254} HPTLC plates (10.0 x 10.0 cm). Methanolic solutions of standard compound (gallic acid) and samples of known concentrations were applied to the plate positioned at 10 mm from the bottom and 19 mm from the side of the plate having 8 mm bandwidth using a CAMAG Linomat 5 automated TLC applicator with the nitrogen flow providing a delivery speed of 150 nl/s from the syringe.

Detection of Gallic acid: Plate was eluted in pre-saturated CAMAG twin trough glass tank with the mobile phase Toluene: Ethyl Acetate: Formic Acid: Methyl alcohol (6:6:1.6:0.4 v/v/v/v) to a distance of 86.2 mm at room temperature. After drying, the spots were visualized under CAMAG UV cabinet (254 and 280 nm). Then

the plate was scanned using CAMAG TLC scanner 3 equipped with WINCATS software (CAMAG). The identification of gallic acid in methanolic solution of leaf of *Saraca indica* was confirmed by superimposing the UV spectra of samples and standards within the same retention factor (Rf value).

The air dried bark of *Saraca indica* (1 kg) was extracted with 1.5L of n- hexane, 1.5L of methanol and 1.5L of water- methanol (3:2) three times each for 24 hr at room temperature. The extract was filtered and solvent evaporated under reduced pressure to give crude extract of hexane (1.8g), methyl alcohol (60g) and aqueous methyl alcohol (60%) extract (30g).

The n- hexane extract (1.1g) was chromatographed over silica gel column with n- hexane as mobile phase. Elution was carried out in n- hexane and ethyl acetate as solvent gradient. The polarity was increased by sequentially adding 2- 40% ethyl acetate in n- hexane with every 2% and 5% increase step. The fractions (100ml each) were collected and pooled into eight fractions on the basis of similar TLC pattern which were visualized after spraying with anisaldehyde- sulphuric acid spraying reagent.

The methyl alcohol extract (50g) was chromatographed over silica gel column with n- hexane as mobile phase and then elution was carried out in n- hexane, ethyl acetate and methanol as solvent gradient. The polarity was increased by sequentially adding 10- 90% ethyl acetate in n- hexane with every 10% increase step, then pure ethyl acetate and finally5, 10, 15 and 20% methanol was added in ethyl acetate.

Aqueous methyl alcohol extract (20g) was chromatographed on Polyamide 6 column with methyl alcohol as mobile phase. Elution was carried out in methyl alcohol and water with solvent gradient. The polarity was increased sequentially in the range of 5- 30% H2O in methyl alcohol with every 5% increase step.

The IR was recorded with FT- IR Perkin- Elmer instrument and optical rotations were measured with a Harobia SEPA- 300 polarimeter. ^{1}H and ^{1}3C NMR spectra were recorded on a Bruker FT- NMR 300MHz. equipped with a 5 mm ^{1}H and ^{13}C (ATP) probe operating at 300 and 75 MHz, respectively, with TMS as internal standard. Chemical shifts were reported in δ (ppm) and coupling constants (*J*) were measured in Hz. The HRMS was recorded on a JMS- T100 LC (AccuTof) atmospheric pressure ionization time of- flight mass spectrometer (Jeol, tokio, Japan) fitted with a DART ion source. The DART ion source was iperated with helium gas flowing at approximately 4.0 L/min. The gas heater was set to 300^{0}C. The potential on the discharge needle electrode of the DART source was set to 3000V; electrode 1 was 100 V and the grid was at 250 V. The mass spectrometer was operated in positive–ion mode with a resolving powere of 6000 (full-width at half-maximum). Flash chromatography was performed with a Buchil Pump manager C- 615 flash model operating with pump modules C-605 on silica gel (230- 400 mesh) column. Silica gel (60- 120 and 230- 400 mesh), aluminium backed TLC silica gel 60, Prep. TLC silica gel 60 and all other chemicals were purchased from Merck. TLC spots were visualized after spraying with anisaldehyde- sulphuric acid in methanol spraying reagent followed by heating. Polyamine 6, deurterated solvents and reference compounds were purchased from Sigma Aldrich St, Louis MO, USA.

HPLC Analysis of Solvent extracts: HPLC analysis was performed on a Waters (Milford, MA, USA) PDA (model 996) and separations were achieved using a Waters reversed phase Sunfire ^{TM} make C178 column (250 X 4.6 mm; i. d. 5µm) subjected to binary gradient elution. The two solvents used for the analysis consisted of water containing 0.1% triflouroacetic acid (A) and methanol (B). The equilibrium time required during the gradient elution was 20 min. Gradient programming of the solvent system was carried out at 27^{0}C and was; initially at 75%A, changed to 65%A in 25 min, 65% to 50% in 25 min, 50% to 20% in 10 min, and then 20% to 0.0% in 10min at a flow- rate of 0.8mL/min for a total period of 70 min. All the gradient segments were linear (curve type 6, Waters Empower Softwere).The wavelength scan range of PDA was set to 200- 400nm and chromatograms were recorded at 278.6nm. The precision (%) of the method was evaluated by adding different concentrations of reference compounds to the samples and comparing amounts determined from their chromatogram (by applying the respective regression equation to the increase in peak area) with the amount actually added. Similarly, recovery (%) was estimated by spiking samples by adding the marker compounds to the extracts prior to sample preparation. The results obtained have been presented in Table- 1, 2 and 3; Figure- 1, 2, 3, 4, 5 and 6.

Table- 1: Phytochemical constituents identified from stem bark of three different solvents extracts of *Saraca indica*

Compounds	Hexane extract	Methanol extract	Aqueous methanol extract
1	**Ursolic acid**	**Ursolic acid**	-
2	**1-Oleodipalmitin**	-	-
3	**Lupeol**	**Lupeol**	-
4	**Compesterol**	-	-
5	**β-Sitosterol**	-	-
6	**Stigmasterol**	-	-
7	**Glochidiol**	-	-
8	-	**Leucopelargonidin**	-

9	**-**	**Leucocyanidin**	**-**
10	**-**	**5,3'-Dimethoxyepicatechin**	**-**
11	**-**	**Epicatechin**	**Epicatechin**
12	**-**	**Catechin**	**Catechin**
13	**-**	**3'-Deoxyepicatechin-3-O-β-D- glucopyranoside**	**3'-Deoxyepicatechin-3-O-β-D-glucopyranoside**
14	**-**	**Lyoniside**	**Lyoniside**
15	**-**	**3'-Deoxycatechin-3-O-α-L-rhamnopyranoside**	**3'-Deoxycatechin-3-O-α-L-rhamnopyranoside**
16	**-**	**Epigallocatechin**	**Epigallocatechin**
17	**-**	**Gallocatechin**	**Gallocatechin**

Table- 2: HPLC identification of phytochemical compounds of Saraca indica stem bark methanol extract

Compounds	Retention Tme (RT)	% Area
Tannic Acid	**5.083**	**9.87**
(-)-Gallocatechin	**7.183**	**5.72**
(+)-Catechin and (-)-Epigallocatechin	**12.905**	**9.11**
(-)-Epigallocatechin gallate	**16.740**	**0.64**
(+)-3'Deoxycatechin-3-O-α-L-rhamnopyranoside	**18.508**	**19.37**
(-)-3'Deoxyepicatechin-3-O-β-D-glucopyranoside	**19.389**	**8.14**
(-)-Epicatechin	**20.684**	**7.07**
(-)-Gallocatechin gallate	**25.029**	**2.90**
Lyoniside	**30.323**	**13.77**
(-)-Catechin gallate	**34.595**	**3.99**
(-)-Epicatechin gallate	**36.790**	**8.41**
5,3'-Dimethoxy-(-)-epicatechin	**45.410**	**7.22**
Leucocyanidine	**61.997**	**1.40**
Leucopelargonidin	**64.981**	**2.40**

Table- 3: HPLC identification of phytochemical compounds of *Saraca indica* bark aqueous methanol (3:2) extract

Compounds	Retention Time (RT)	% Area
Tannic Acid	**5.095**	**17.16**
(-)-Gallocatechin	**7.176**	**2.87**
(+)-Catechin and (-)-Epigallocatechin	**10.938**	**6.32**
(-)-Epigallocatechin gallate	**17.067**	**2.89**
(+)-3' Deoxycatechin-3-O-α-L-rhamnopyranoside	**18.789**	**30.86**
(-)-3'Deoxyepicatechin-3-O-β-D-glucopyranoside	**20.544**	**7.51**
(-)-Epicatechin	**21.840**	**9.51**
(-)-Gallocatechin gallate	**25.307**	**2.57**
Lyoniside	**29.593**	**9.11**
(-)-Catechin gallate	**36.526**	**1.30**
(-)-Epicatechin gallate	**36.526**	**1.30**
5,3'Dimethoxy-(-)-epicatechin	**37.191**	**4.51**
Leucocyanidin	**60.786**	**1.07**
Leucopelargonidin	**65.116**	**0.98**

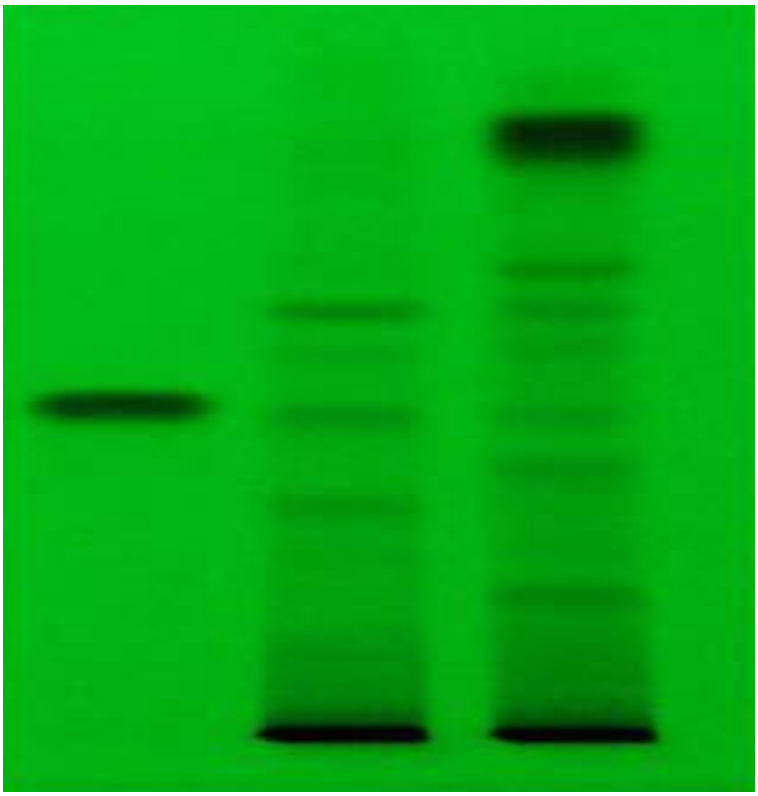

Fig- 1: Photograph of chromatograms obtained at 280nm from standard Gallic acid (1) n-hexane leaf extract (2) and methanol leaf extract (3) of *Saraca indica*

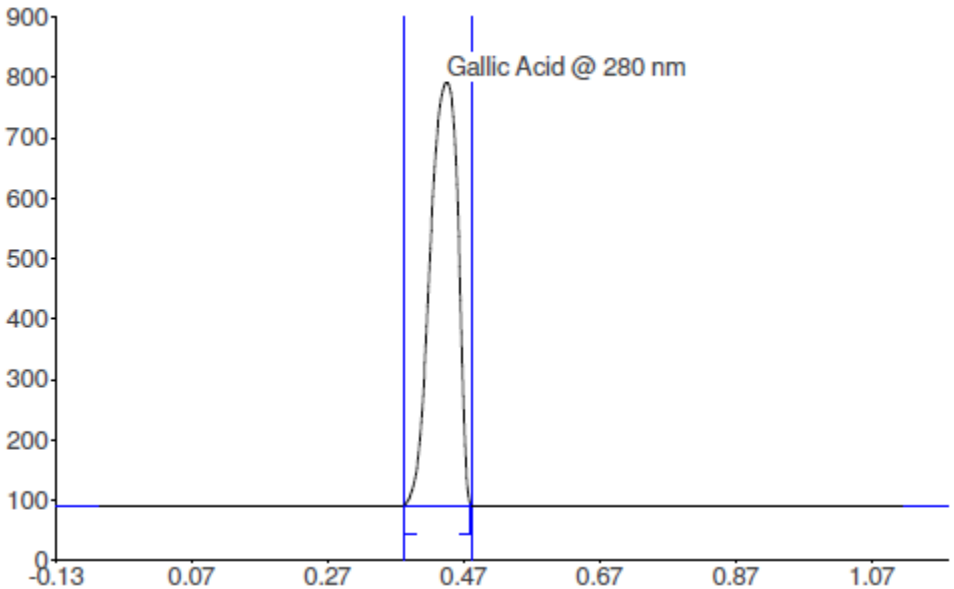

Fig- 2: Chromatogram of standard Gallic acid

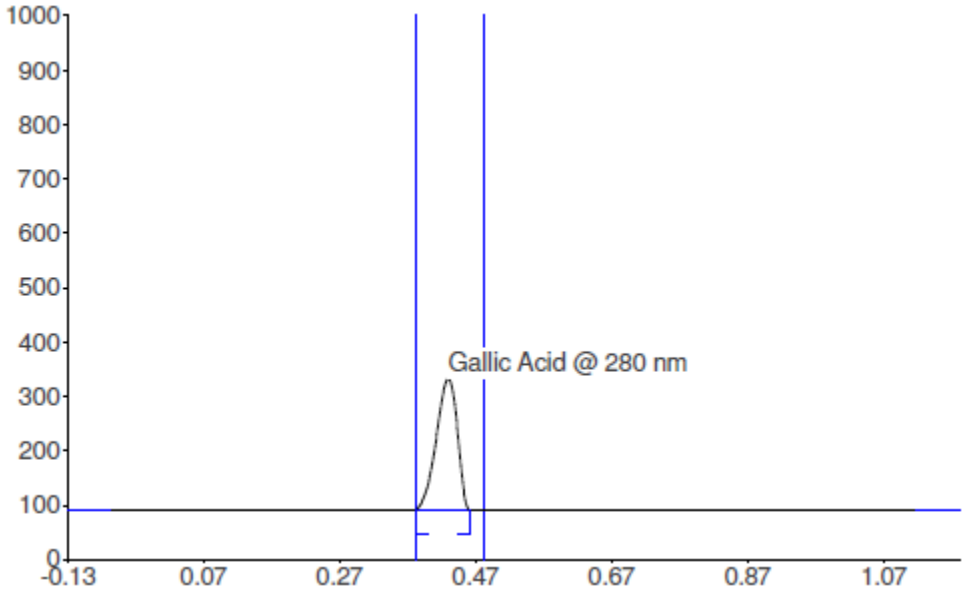

Fig- 3: Chromatogram of Gallic Acid in the n-hexane extract of leaf of S. *indica*

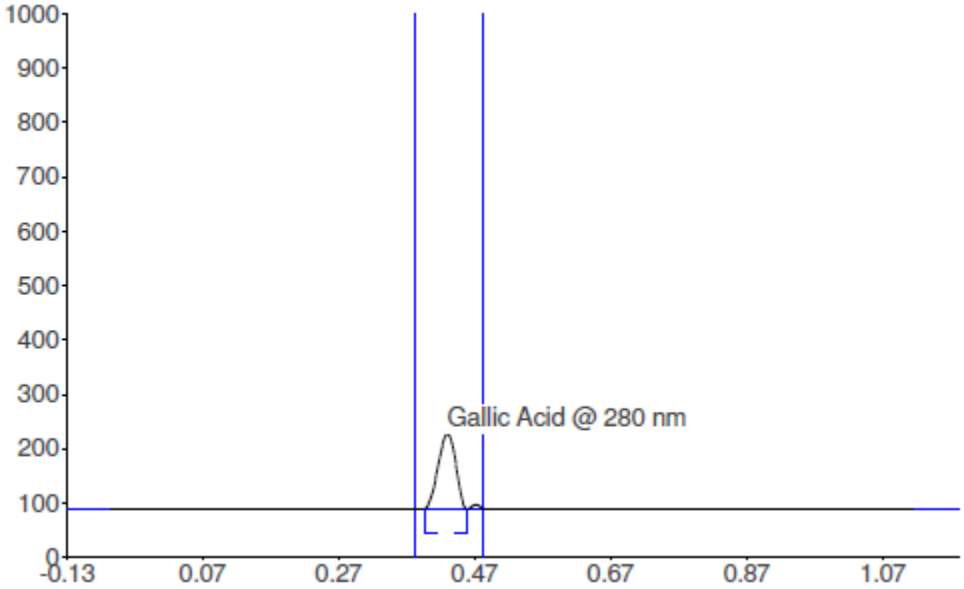

Fig- 4: Chromatogram of Gallic Acid in the methanolic extract of leaf of *Saraca asoca*

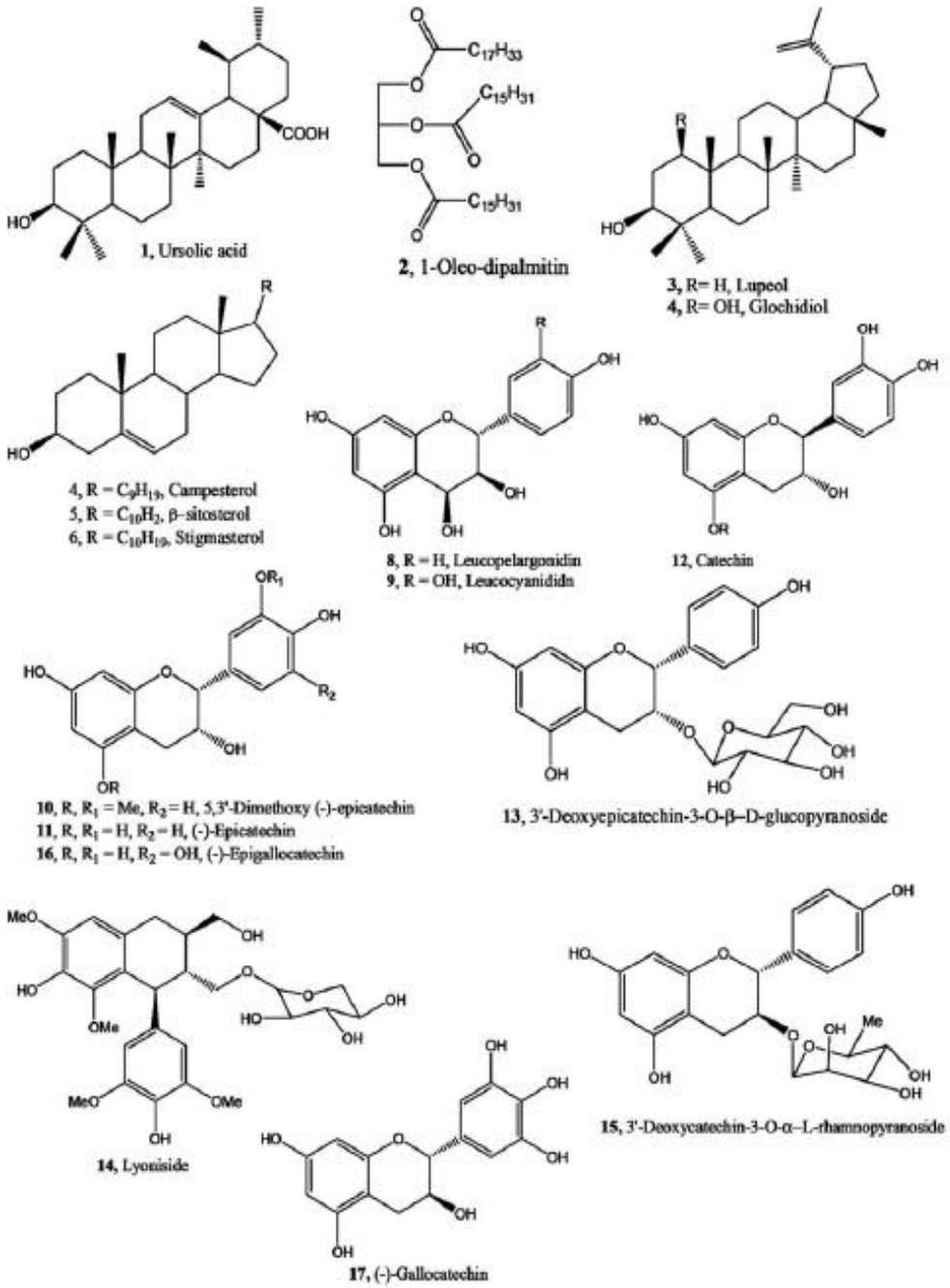

Fig- 5: Chemical structure of isolated compounds from *Saraca indica* bark

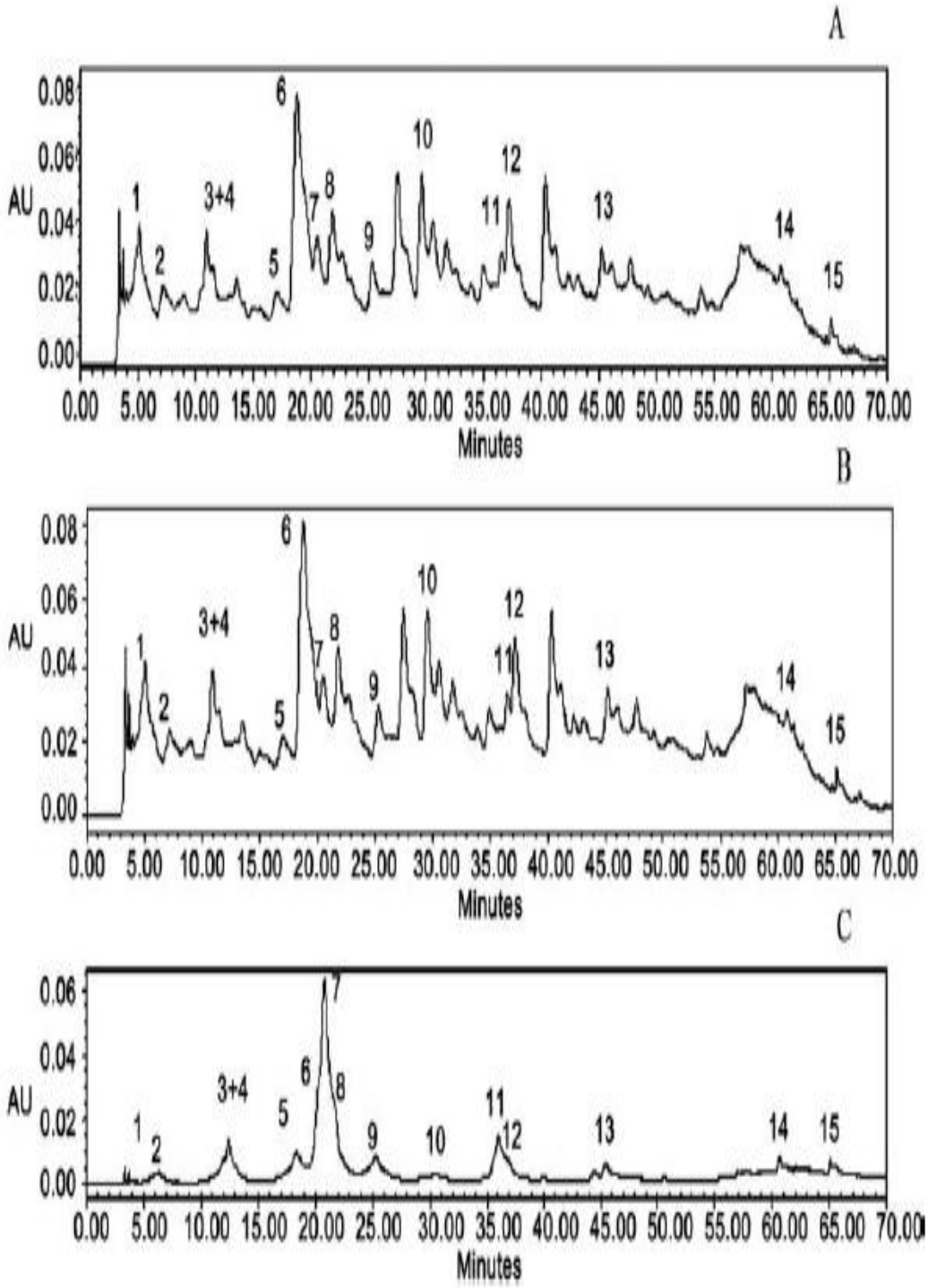

Fig- 6: (A) HPLC chromatogram of identified references of methanol extract. (B) HPLC chromatogram of identified references of aqueous methanolic extract (3:2), (C) HPLC chromatogram of identified reference markers:

1. Tannic acid; 2. (-)-Gallocatechin; 3. (+)-Catechin; 4. (-)-Epigallocatechin; 5. (-)-Epigallocatechin gallate; 6. (+)-3'- deoxycatechin- 3- O- α-L-rhamnopyranoside; 7. (-)-3'- deoxyepicatechin-3-O-β-D-glucopyranoside; 8. (-)-Epicatechin; 9. (-Gallactocatechin gallate; 10. Lyoniside; 11. (-)-Catechin gallate; 12. (-)-Epicatechin gallate; 13. 5, 3' – dimethoxy-(-)-epicatechin; 14. Leucocyanidin, 15. Leucopelargonidin

III. Results and Discussion

The present study by Sabita *et al*., (2018) [41] 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; Mohan. Ch, 2016; Ravindran Jaganath *et al.,* 2017) [42, 43, 44, 45, 46] who also analysed the same phytochemicals in the bark, flowers and leaves of *Saraca indica.*

In the present investigation a number of active phytochemicals from methanol and n-hexane extracts of leaves and n- hexane, methanol and aqueous methanol extracts bark of *S. indica* have been isolated and screened employing HPTLC and HPLC techniques. These results show that leaves of *Saraca indica* contain a number of chemical ingredients, which may be responsible for the various pharmacological actions although their specific roles remain to be investigated. It has been observed that most active principles present in the leaves are flavonoid, steroids, tannins and glycosides. These phytoconstituents may be responsible for various pharmacological actions of this plant part, like antibacterial, antiulcer, anticancer, larvicidal and chemo protective activities [27, 28, 47, 48, 49] (Misra, 2013; Pradhan *et al*., 2009; Satyavati *et al*., 1970; Saha *et al*., 2013; Saravanan *et al*., 2011). n- Hexane and Methanolic extracts of leaves confirmed the presence of gallic acid using HPTLC assay. This is the first report of the presence of gallic acid in *Saraca indica* leaf. Hence, the amount of gallic acid in *Saraca indica* leaf can be quantified further for proper utilization of this age old plant. The physicochemical evaluation of this plant is an essential parameter for the detection of adulterant and improper handling of drugs. 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 study or application.

Photograph of chromatograms of the standard Gallic acid at 280 nm obtained in the n-hexane and methanolic leaf extract of *Saraca indica* are given in Figure- 1. The Gallic acid bands in sample chromatogram of *Saraca* sp. are identified and confirmed by comparing the chromatogram obtained from the reference standard solution (Figures- 2, 3 and 4) and by comparing retention factor (Rf) of Gallic acid from sample and standard solution. The Rf value of standard gallic acid is 0.44, whereas the Rf value of n-hexane extract and methanolic extract of the leaf of Saraca asoca is 0.43 which almost coincides with standard Rf value of gallic acid (Figure- 1).

The stem bark of *Saraca indica* after successive extractions by non-polar (n-hexane) to polar solvents (methanol and aqueous methanol) has yielded three extracts, the n-hexane, methanol and 60% aqueous methanol. The n-hexane extract after chromatographic separations afforded a triglyceride, viz. l- oleo-diplmitin (**2**), triterpinoids, viz. ursolic acid (**1**), lupeol (**3**), glochidiol (**7**) and sterols, viz. campesterol (**4**), β-sitosterol (**5**), stigmasterol (**6).** Out of these, ursolic acid (**1**), l-oleo-dipalmitin (**2**), lupeol (**3**), glochidiol (**7**) have been isolated for the first time (Table- 1; Fig- 5) from *Saraca indica*. The structures of these compounds were confirmed by extensive use of spectroscopic methods, especially NMR and comparison with the data available in our library and reported in the literature [50, 51, 52, 53, 54, 55, 56] (Hsu and Turk, 2010; Seebacher *et al*., 2003; Burns *et al*., 2000; Choi *et al*., 2007; Zhang *et al*., 2005; Furkan Ahmad *et al*., 2016; Puapairoj *et al*., 2005).

The methanol extract after rigorous chromatographic separations, has yielded terpinoids, ursolic acid (**1**) and lupeol (**3**) along with several flavan-3-ol derivatives, viz. leucopelargonidin (**8**), leucocyanidine (**9**), 5, 3'-dimethoxy epicatechin (**10**), epicatechin (**11**), catechin (**12**), 3'-deoxyepicatechin-3-O-β-D-glucopyranoside (**13**), 3'-deoxycatechin-3-O-α-L-rhamnopyranoside (**15**), epigallocatechin (**16**), gallocatechin (**17**) and lyoniside (**14**). Among them, 5, 3'-dimethoxy epicatechin (**10**), 3'-deoxyepicatechin-3-O-β-D-glucopyranoside (**13**), 3'-deoxycatechin-3-O-α-L-rhamnopyranoside (**15**) and epigallocatechin (**16**) are now being reported from *Saraca indica* (Ahmad *et al*., 2015) (Table- 3; Figure- 6). The structures of these compounds were confirmed by the interpretation of their IR, NMR and mass spectra which were also compared with the data available in the literature [51, 52, 57, 59, 60, 61, 37, 58] (Seebacher et al. 2003; Burns et al. 2000; Hetter et al. 1985; Morimoto et al. 1985; Davis et al. 1996; Morimoto et al. 1988; Sadhu et al. 2007; Drewes et al. 1992). Aqueous methanol extract afforded flavan-3-ol derivatives, viz. epicatechin (**11**), catechin (**12**), 3'-deoxyepicatechin-3-O-β-D-glucopyranoside (**13**), epigallocatechin (**16**), gallocatechin (**17**), lyoniside (**14**), 3'-deoxycatechin-3-O-α-L-rhamnopyranoside (**15**). Among them, 3'-deoxyepicatechin-3-O-β-D-glucopyranoside (**13**), epigallocatechin (**16**) gallocatechin (**17**) and 3'-deoxycatechin-3-O-α-L-rhamnopyranoside (**15**) are being reported for the first time (Table- 1; Figure- 5). The structures were proved by recording their IR, NMR and mass spectra and compared with those reported in the literature (Moromoto *et al*., 1985; Davis *et al*., 1996; Morimoto *et al*., 1988; Sadhu *et al*., 2007; Drewwes *et al*., 1992) [59, 60, 61, 37, and 58].

The bark extracts were too complex and developing its HPLC chemical profiling happened to be a bit difficult. After several repeated attempts, it has been possible to develop suitable conditions for the HPLC profiling by using the isolated and identified references, e.g. leucopelargonidin (**7**), leucocyanidin (**8**), 5,3'-

dimethoxy-(-)-epicatechin (**9**), (-)-epicatechin (**10**), (+)-catechin (**11**), (-)-3'-deoxyepicatechin-3-O-β-D-glucopyranoside (**12**), lyoniside (**13**), (+)-3'-deoxycatechin-3-O-α-L-rhamnopyranoside (**14**), (-)-epigallocatechin (**16**), (-)-gallocatechin (**17**). Some of the references could not be properly purified, and therefore were procured from Sigma Aldrich, St. Louis MO, USA, viz. tannic acid, epigallocatechin gallate, gallocatechin gallate, epicatechin gallate, catecin gallate.

The HPLC profile of tannin removed methanol extract showed that (+)-3'-deoxycatechin-3-O-α-L-rhamnopyranose, lyoniside, (-)-catechin gallate, (+)-3'-deoxycatechin-3-O-α-L-rhamnopyranoside and tannic acid were the major components of the methanol extract of bark of Saraca indica (Table- 32; Fig- 32A). Similarly, the tannin removed aqueous methanol (3:2) extract in its HPLC profile (Table- 3; Figure- 6B) showed that (-)-epigallocatechin gallate and tannic acid constitute about half of the percent of the identified compounds. The other important identified compounds were catechin, (-)-3'deoxyepicatechin-3-O-β-D-glucopyranoside and (-)-gallocatechin gallate. The retention time (RT) matched well with the HPLC chromatogram of reference markers (Figure- 32C) mixed together, each with 1 mg/mL concentration. Chemical structure of isolated compounds has been shown in Figure- 31. HPLC chromatogram and its analysis for the methanol and aqueous methanol extracts have been given in Figure- 6A and Figure- 6B and Table- 2 and 3. The percentage of identified compounds has been given in Table- 2 and 3.

In the present investigation air dried bark of *Saraca indica* (1 Kg) was extracted with three solvents viz. 1.5 L of n- hexane, 1.5 L of methanol and 1.5 L of water-methanol (3:2) three times each for 24 hr at room temperature. The extract was filtered and solvent evaporated under reduced pressure to give crude extract of hexane (1.8 g), methanol (60 g) and aqueous methanol (605) extract (30 g).

When n-hexane extract (1.1 g) was chromatographed over silica gel with n-hexane as mobile phase eight fractions were visualized after spraying with anisaldehyde-sulphuric acid reagent on TLC plate. Fraction 2 (120 mg) after prep-TLC n-hexane-EtOAc (Ethyl acetate) (17:3) afforded ursolic acid (**1**, 39.3 mg) and 1-oleo-dipalmitin (**2**, 30 mg). Fraction 4 (70 mg) after prep-TLC n-hexane-ethyl acetate (4:1) gave lupeol (**3**, 26.3 mg). Fraction 6 (80 mg) after prep-TLC n-hexane-ethyl acetate (37:13) yielded campesterol (**4**, 6.4 mg), β-sitosterol (**5**, 15.0 mg) and stigmasterol (**6**, 9.8 mg). Fraction 7 (30 mg) after prep-TLC n-hexane- ethyl acetate (2:3) afforded golchidiol (**7**, 11.2 mg).

When methanol extract (50 g) was chromatographed over silica gel with n-hexane as mobile phase twelve fractions were observed on TLC plate. Fraction 2 (100 mg) on flash chromatography yielded ursolic acid (**1**, 10 mg) and lupeol (**3**, 12.2 mg). Fraction 3 (35 mg) after prep-TLC gave leucopelargonidin (**8**, 2.3 mg) and leucocyanidin (**9,** 5.5 mg). Flash chromatography of fraction 5 (1 g) was done using chloroform as solvent A and methanol, as solvent B. The percentage of B in A was gradually increased by adding 1-15%B in A with every 2% increase step with a flow rate of 3 mL/min. Flash chromatography gave 5,3'-dimethoxy- (-)-epicatechin (**10**, 17.5 mg), (-)-epicatechin (**11**, 35.3 mg) and catechin (12, 15.0 mg). Fraction 7 (2 g) was subjected to flash chromatography using ethyl acetate as solvent A and methanol as solvent B. The percentage of B in A was gradually increased by adding 1-15%B in A with every 2% increase step with a flow rate of 3 mL/min which gave four subfractions. Further, flash chromatography of subfraction 2 (300 mg) with chloroform as solvent A and methanol as solvent B. The polarity was gradually increased as described above giving 3'-deoxyepicatechin-3-O-β-D-glucopyranoside (**13**, 25.8 mg). Subfraction 3 (100 mg) was purified by flash chromatography to obtain lyoniside (**14**, 32.6 mg). Fraction 8 (1 g) was subjected to flash chromatography with chloroform as solvent A and methanol as solvent B.The percentage of B in A was gradually increased by adding 1-20% B in A with every 2% increase step with a flow rate of 3 mL/min which gave 5 subfractions. Subfraction 3 (350 mg) after further flash chromatography under similar conditions yielded (+)-3'-deoxycatechin-3-O-α-L-rhamnopyranoside (Ahmad *et al*., 2015) [39] (**15**, 90 mg). Fraction 9-10 (2 g) after crystallization gave (-)-epigallocatechin (**16**, 500 mg) and (-)-gallocatechin (**17**, 450 mg).

Aqueous methanol extract (20 g) when chromatographed on Polyamine 6 column with methanol as mobile phase six fractions were visualized on TLC gel after spraying with anisaldehyde-sulphuric acid and $FeCl_3$-$K_3[Fe(CN_6)]$ spraying reagent. A part of fraction 3 was chromatographed over silica gel column with chloroform as mobile phase and elution was carried out in chloroform and methanol with solvent gradient. The polarity was increased by sequentially adding 5-25% methanol in chloroform with every 5% increase step, six fractions were observed. Further flash chromatography of subfraction 1 (500 mg) gave (-)-epicatechin (**11**, 25.0 mg) and catechin (**12**, 14.6 mg). Subfraction 2 (300 ,g) was further purified by flash chromatography yielding 3'-deoxyepicatechin-3-O-β-D-glucopyranoside (**13**, 10.7 mg). Subfraction 3 (350 mg) was purified by flash chromatography to yield lyoniside (**14**, 25.1 mg). Subfraction 4 (200 mg) after flash chromatography yielded (+)-3'deoxycatechin-3-O-α-L-rhamnopyranoside (**15**, 20.5 mg). Fraction 6 (1.2 g) after crystallization yielded (-)-epigallocatechin (**16**, 100 mg) and (-)-gallocatechin (**17**, 80 mg).

IV. Conclusions

Medicines derived from plants have made immense contribution towards the betterment of human health and act as a source of inspiration for novel drug compounds. From the above research it can be concluded that this plant has immense potential to be used in the area of pharmacology and as a prospective source of valuable drugs.

The present study contributes to the current knowledge of the presence of various active phytochemical compounds in stem bark and leaves of *Saraca asoca* which possess significant drug yielding molecules for treatment of various diseases. Further fractionation and purification will elucidate the potential compound for therapeutic uses.

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