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RESEARCH ARTICLE

Phytochemical characterization of *Saraca indica* bark and investigation of antimicrobial and antioxidant properties of the saponin-rich extract

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Abstract

Saraca indica, commonly known as *Ashoka* in the Indian subcontinent is well known for its ethnobotanical significance. This study delves into the phytochemical profile of *Saraca indica* bark and elucidates its antimicrobial and antioxidant properties. Utilizing analytical techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Thin Layer Chromatography (HPTLC), we conducted a comprehensive analysis of the bark's bioactive compounds. This study revealed a rich presence of secondary metabolites including flavonoids, tannins, glycosides, and saponins, which are known for their anti-inflammatory, antimicrobial, and antioxidant activities. The absence of certain compounds like reducing sugars and proteins was noted, which frames the specific biochemical profile of the bark. The saponin-rich extract, prepared using rigorous extraction methods, was analysed through GC-MS, revealing a complex mixture of compounds with varied saponin content. The antioxidant capabilities of the extract were quantified using the DPPH assay, showcasing a dose-dependent scavenging activity that underscores the extract's potential as a natural antioxidant source. Furthermore, antimicrobial tests against pathogens such as *E. coli*, *S. pyogenes*, *S. aureus*, and *P. aeruginosa* demonstrated significant inhibitory effects, suggesting the extract's usefulness in combating bacterial and fungal infections. Cytotoxicity assays affirmed the non-toxic nature of the extract, highlighting its safety for potential therapeutic use. The findings of this study not only corroborate the traditional uses of *Saraca indica* in Ayurvedic medicine but also open avenues for its application in modern pharmaceutical and cosmetic industries. By bridging traditional knowledge and contemporary scientific approaches, this research contributes to the evolving landscape of natural product research and their integration into evidence-based practices.

Keywords: *Saraca indica*; Bark Extract; Phytochemical; Antimicrobial, Antioxidant; Saponin; Cytotoxicity; Immunomodulatory Assay

1. Introduction

This work investigates how magnesium-enriched phytochemicals from plants like *Saraca indica* can interact with skin cells to potentially improve autoimmune skin conditions (Bhalerao et al., 2014). Utilizing advanced bioanalytical methods such Gas Chromatography Mass Spectrometry, the study aims to evaluate the biological effects of these phytochemicals. The research suggests a multi-faceted approach could yield more effective treatments with fewer side effects, combining ancient herbal wisdom with contemporary scientific approaches. *Saraca indica* (family Caesalpiniaceae) is one of the most ancient sacred plants widely distributed throughout the Indian subcontinent (Chakka et al., 2021; Fathima et al., 2023; Gupta et al., 2014; Yadav et al., 2015). Various medicinal uses of *Saraca indica* had been reported in Charaka Samhita, 100 A.D. (Singh et al., 2015). Different parts of the plant exhibit a number pharmacological effects like antihyperglycemic, antipyretic, antibacterial, anthelmintic, activity, and so forth, which are well described in literature (Khare et al., 2018). A traditional drug *Asoka Aristha* used for the treatment of menorrhagia originated from *Saraca indica* (Shahid et al., 2018). Secondary metabolites like flavonoids, terpenoid, lignin, phenolic compounds, tannins, and so forth are reported from *Saraca indica* stem bark extracts and found responsible for their therapeutic action (Nair et al., 2023; Singh et al., 2015).

Saraca indica is one of the most sacred and popular trees in India with ethno-botanical importance and it is commonly called as *Ashoka* tree in the Indian subcontinent. It is a medically valued plant for its ability to treat various infections (Nyeem et al., 2017). *Ashoka* plant has been named as one of the endangered plants by national medicinal plant board. The indigenous knowledge about various medicinal plants has been proven to be beneficial for prevention and treatment of a wide range of diseases (Aziz et al., 2018). The use of traditional system of medicines is accepted widely due to fewer side-effects, better patient tolerance, relatively less expensive, long history of use and growing problem of drug resistance towards modern drugs (Ekor, 2014). Besides, herbal medicines provide rational means for the treatment of many diseases that are obstinate and incurable in other systems of medicine. For checking safety and improving efficiency and quality, there is a need for discovery and standardization of new herbal medicines. In the realm of Ayurveda, *Saraca indica* is known to cure many diseases pertaining to cancer, uterine disorders, inflammation, and diabetes besides exhibiting anti-tumor, anti-microbial and analgesic activities. Our skin guards the underlying muscles, bones, ligaments and internal organs. Skin diseases are of several kinds and a commonly occurring health problem affecting people of all ages from the neonates to the elderly. The most common dermatological problems include fungal infections, acne, eczematous conditions, bacterial infections, contact dermatitis and psoriasis (Richard et al., 2022). The leaves of *Saraca indica* have high saponin content which can be useful for skin-care purposes (Mohan et al., 2016). This plant is not known to be used widely for developing formulations for treating skin problems. Therefore, there is scope for developing products beneficial for skin care purposes. Keeping in view the medicinal value of *Saraca indica*, the present study is focused on the identification of various active bio-chemicals present in the leaves using the technique of GC-MS. Studies such as antimicrobial,

Figure 1 (A) *Saraca indica* tree and (B) bark of *Saraca indica*

antioxidant, toxicity, and immunomodulatory essay are carried out to establish the therapeutic potential of the bark of *Saraca indica*.

2. Material and method

2.1 Sampling and Processing

The bark of the *Saraca indica* plant was collected from Ramnarain Ruia Autonomous College (Latitude 19.02372° or 19° 1' 25" north; Longitude. 72.85011° or 72° 51' 0" east.), where the species was authenticated by the department of Botany (Specimen No. BGX-789-PLT-2024). A photograph of the *Saraca indica* plant and bark of the same plant is shown in Figure 1.

2.2. Chemicals

All chemicals such as Cyclohexane, Petroleum ether, Methanol, chloroform, n-butanol, 1, 1 diphenyl-2-picrylhydrazyl (DPPH), Acetone, and nutrient agar were bought from Loba Chemie, Sigma and Himedia. The chemicals used were of analytical grade.

2.3. Preparation of extract

10 grams of finely grounded sample parts were placed in 250 mL of round bottom flask. 100 mL of 50% aqueous methanol was added to this flask. These extracts were used for phytochemical tests as shown in Figure 4. The contents of the flask were stirred overnight at room temperature. Thereafter, the contents were centrifuged at 3000rpm for 10 min. The supernatant liquid was collected and stored in an amber coloured bottle. The process was repeated, and both the supernatant liquids were combined. The supernatant liquid was filtered through Whatman paper No.41 to remove any solid particles. The methanol evaporated with rotary-evaporator. The resulting aqueous phase was centrifuged at 300 rpm for 10 min (Ezeonu and Ejikeme, 2016). The aqueous phase was transferred to a separating funnel. Using an equal volume of chloroform, the pigments are removed from the aqueous phase. In the final step of preparing the saponin-rich extract, the aqueous phase was treated with n-butanol using a separating funnel. This process was repeated two times. The n-butanol was evaporated with rotary-evaporator. To record the GC-MS data, this extract was reconstituted in Petroleum ether.

2.4. High-Performance Thin Layer Chromatography (HPTLC)

The mobile phase composition for developing the HPTLC fingerprints was Toluene, Ethyl acetate, Ethanol, and Formic acid in a ratio of 4.5:3.5:1:1 (v/v/v/v). We have optimized the mobile phase with respect to standards and extract such that there is visible separation of the bands. Standards such as Gallic acid, Catechin, Quercetin, and B-Sitosterol were employed. All solvents were of analytical grade and the standards were purchased from Sigma Aldrich (Mumbai, India). HPTLC was performed on silica gel 60f 254, 20 × 10 cm HPTLC plates (Merck, Darmstadt, Germany). The separations were documented, and profile images were captured at 253 nm and 366 nm.

2.5. Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The saponin-rich extract obtained from the bark of *Saraca Indica* was analysed using Shimadzu GCMS-QP2010 Ultra for identification of various compounds present in the sample. Column flow was volumetric at 1.5 mL/min using ultra purified helium (499.999%) as the carrier gas. The split injection port was maintained at 260°C. Injection was in split mode with a split ratio of 40:1. The transfer line temperature was set at 290°C. The mass analyser was set at 70 eV, electron impact source temperature of 230°C, electron-multiplier voltage of 1588 mV and solvents delay of 3 min. All the data was obtained by collecting the full-scan mass spectra within the scan range of 50–600 amu. The temperature program was as follows: initial from 200 to 270°C at ramp rate of 25°C/min, and from 270 to 290°C at ramp rate of 11°C/min (Rai et al., 2023).

2.6. Estimation of total saponin content

The peaks in the GC-MS spectra were assigned by consulting the library of National Institute of Standard and Technology (NIST). The phytochemical profile of the bark was built up in this way by identification of the compounds by their name and chemical structure and duly tabulated. The phytochemicals were assigned to

their chemical class in order to identify the total saponin content. The amount of phytochemical corresponds to the percentage area under the curve. This percentage area was tabulated for every phytochemical.

2.7. Antioxidant activity

To measure the DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant activity, first prepare the solutions: dissolve 10 mg of ascorbic acid in 100 ml of ethanol for the standard, and 10 mg of DPPH in 250 ml of ethanol for the DPPH solution. Prepare sample solutions by diluting plant extracts with methanol. Add these solutions into cuvettes, along with 4 ml of DPPH solution, and adjust the total volume with methanol. Incubate the cuvettes in the dark at room temperature for 30 minutes. Measure the absorbance at 540 nm using a UV-Vis spectrophotometer. Include a control with only methanol and DPPH solution to establish a baseline (A0) (Baliyan et al., 2022). Calculate the antioxidant activity as the percentage of DPPH scavenging, using the formula:

$$\text{DPPH scavenging effect (\%)} = \left\{\frac{A0-A}{Ao}\right\}\times 100$$

Where *A*0 is the control absorbance and *A* is the sample absorbance. This method is reliable for assessing the free radical scavenging capacity of antioxidants in various samples.

2.8. Anti-microbial activity

Petri dishes were sterilized, and the nutrient agar was poured in them. The agar was allowed to solidify by leaving the petri dishes undisturbed for some time. It was ensured that the agar was evenly spread and solidified. The four microorganisms used were *E. coli, S. pyogenes, S. aureus and P. aeruginosa*. Of these, the first two are bacteria and the latter two are fungi. Each petri dish was labelled accordingly. With sterile cotton, each of the microorganisms was deposited on the agar plates. It was ensured that there was a uniform lawn of growth on each of the four plates. After this Inoculation procedure, wells were created in the agar plates. Wells were created with a help of sterile corkscrew such that they were evenly spaced and that their creation did not damage the agar surface. In each well, a specific amount of extract was added. The agar plates were turned lid-side down to prevent condensation from falling onto the agar surface. The plates were incubated at 37 C in CO2 incubator. The recommended incubation times for bacterial strain (24 hours) and for fungi strain (48 hours) were followed (Sanders, 2012).

Zone of Inhibition refers to the area which is unaffected by the microbes due to the protective effect of the test samples. Larger surface areas or zones of inhibition indicate stronger antibacterial activity against the specific microorganism. Zone of inhibition around each well was measured using vernier calipers. It was measured from the edge of the well to the edge of the clear zone.

2.9. Cytotoxicity study

The MTT-based cytotoxicity assay for the bark sample was performed with HaCaT skin epithelial cell line. The Cytotoxicity MTT Assay is a colorimetric assay which measures cell viability, proliferation and cytotoxicity. It involves the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial dehydrogenases in metabolically active cells to form purple formazan crystals. Cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37°C in a 5% CO_2-in-air atmosphere, in the absence of antibiotics. Cells were seeded into 96-well plates at a density of 5 x 10^3 cells per well and were incubated for 48 hours without addition of sample extract of bark of *Saraca indica*. The stock concentration of the extract of bark of *Saraca indica* was prepared in following manner: The extract was prepared in 50% Methanol. The phytochemicals were extracted by using 10 g of the *Saraca indica* bark. The series of the sample solutions is made from this stock solution using the medium of 1% FBS (Table 1 shows the series). Sample A is control and from samples B to F the concentration of the extract increases proportionately. 0 mg/ml was treated as Control in which no extract was added. The "% cell viability" (% of cells survived) is 100% in this Control sample. % Cell viability refers to the number of live, healthy cells in a sample. Cells were treated with extracts for 72 h. MTT was dissolved in Dulbecco's Phosphate Buffered Saline (DPBS) at a concentration of 5mg/ml. This solution of MTT was added to each well and plates were incubated at 37°C for 4 h to allow reduction of MTT by viable cells to insoluble formazan product. Well supernatants were aspirated and cellular formazan was solubilized by addition of 100 µl of isopropanol with 1N HCl, after which absorbance in each well was read on a plate reader at 570 nm. The OD value was expressed in terms of %Cell viability by using the following formula: %Cell Viability = [OD (sample) / OD (control)] x 100

Table 1. The MTT-based cytotoxicity assay for the bark sample

Sample	Extract of Stock (in ml)	1% FBS (in ml)	Dilution Factor
A (Control)	0.0000	1.000	--
B	0.0015	0.999	666.600
C	0.0030	0.997	333.000
D	0.0060	0.994	166.600
E	0.0120	0.988	83.330
F	0.0250	0.975	40.000

Table 2. Preliminary tests to determine the presence of phytochemicals in the bark of Saraca indica ("0" denotes the absence of that class of phytochemicals, "1" denotes the presence of that class of phytochemicals).

Sl. No.	Secondary Metabolites	Phytochemical Tests	Ashoka Bark
1	Alkaloids	Mayer's Test	1
2	Carbohydrates	Benedicts Test	1
3	Reducing Sugars	Fehling's Test	0
4	Flavonoids	Alk Reagent Test	1
5	Saponins	Foam Test	1
6	Tannins	Braymer's Test	1
7	Steroids	Salkowski's Test	0
8	Proteins	Millon's Test	0
9	Glycosides	Keller Killioni's Test	1
10	Phenols	Ferric Chloride Test	1
11	Amino Acids	Ninhydrin Test	0
12	Terpenoids	Salkowski's Test	1
13	Phytosterols	Liebermann B. Test	1
14	Anthocyanins	2M NaOH Test	0

2.10. Immunomodulatory assay

The effect of phytochemicals on proliferation of U937 cells derived from human monocytes was carried out in this assay. These cells were cultured and maintained in 10% FBS (fetal bovine serum) containing RPMI media with 100 U/ml penicillin-streptomycin (antibiotics). Cells were maintained at 37 C inside 5% CO_2 incubator and further stimulated with 100ng/ml of lipopolysaccharides (LPS) for 24 h to induce inflammatory cytokine secretions (Nakadai et al., 2006). The Control sample in this study was 1%FBS without the Bark extract. Sample was the "Bark extract" of concentration of 0.625 mg/ml and it was introduced in cultured cells for 24 h to determine the drug mediated response against the cytokine secretion. The concentration of 0.625 mg/ml was selected from Cytotoxicity studies. After 24 h the cells were further processed for RNA extraction (using Trizol reagent from Sigma Aldrich as per manufacturer's instructions), cDNA conversion (Aura cDNA synthesis kit as per the manufacturer's instructions) and followed

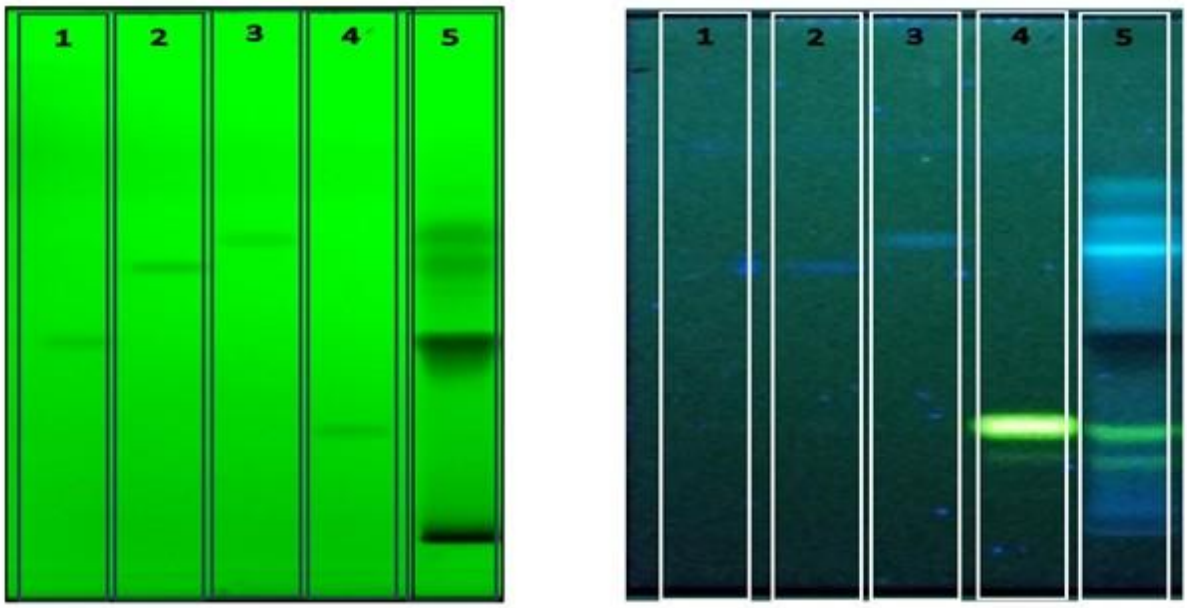

Figure 2. HPTLC Fingerprint at 254 nm and 366 nm

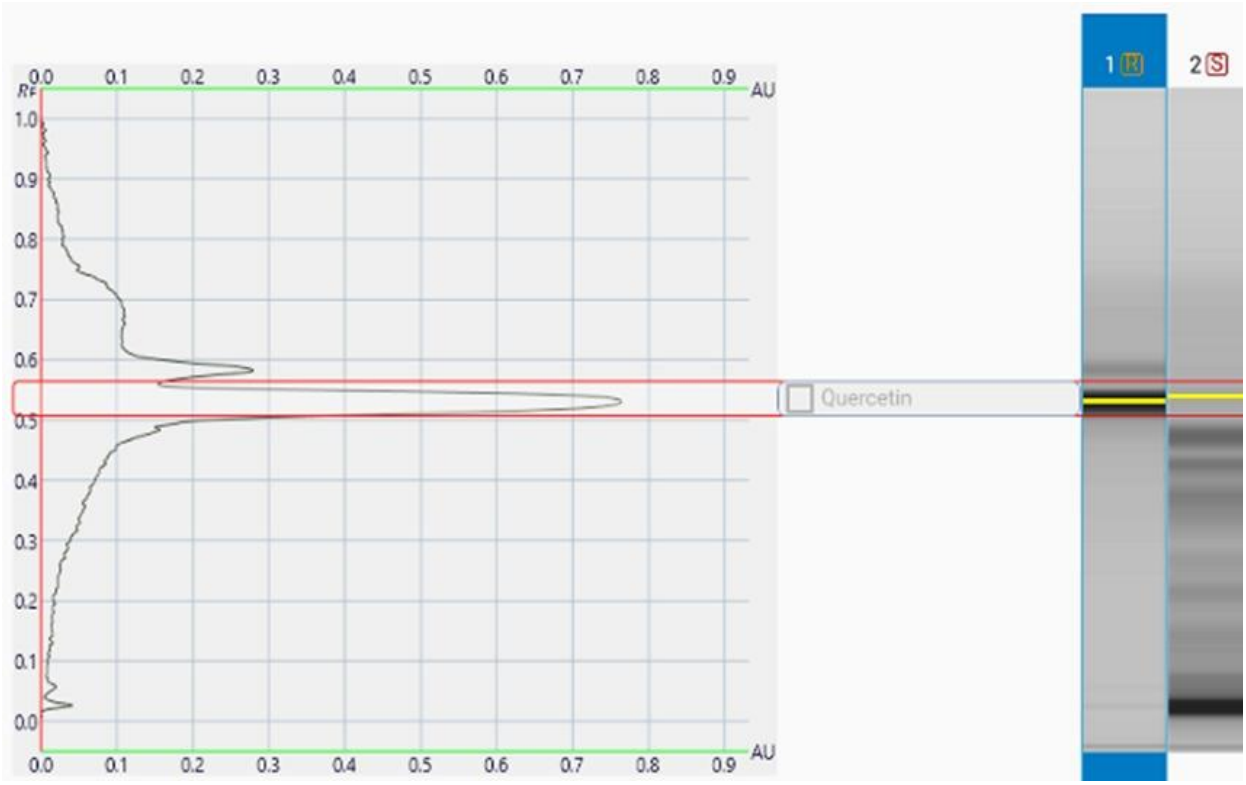

Figure 3. HPTLC Spectra Comparison of Quercetin and Sample

by real time PCR (Agilent AriaMX system, using Sybr green method) based gene expression analysis for two inflammatory cytokines:

TNF-α: Forward: CCTCTCTCTAATCAGCCCTCTG,
TNF-α: Reverse: GAGGACCTGGGAGTAGATGAG
IL-6: Forward: ACTCACCTCTTCAGAACGAATTG,
IL-6: Reverse: CCATCTTTGGAAGGTTCAGGTTG

β-actin is used as a housekeeping gene. Results were expressed in terms of "Fold change in mRNA expression" based on ddCt method.

3. Result and discussion

3.1. Preliminary phytochemical test

The results of preliminary tests are summarized in Table 2. The presence of a wide variety of phytochemicals such as alkaloids, carbohydrates, flavonoids, saponins, tannins, glycosides, phenols, terpenoids, and phytosterols indicates that *Saraca indica* bark possesses significant bioactive potential. These compounds are known for their various therapeutic properties, including anti-inflammatory, antioxidant, and antimicrobial activities. The

Table 3. Total Saponin Content

Sr. No.	Total Saponin	Category	Name
1	1.85	Saponins	1-Bromoeicosane
2	0.74	Saponins	Naphthalene, decahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1S-(1. alpha
3	2.16	Saponins	3-Chloropropionic acid, heptadecyl ester
4	1.05	Saponins	2-Pentene, 3-(chloroethylboryl)-2-(chlorodimethylsilyl)-, (E)-
5	0.96	Saponins	2,6-Lutidine 3,5-dichloro-4-dodecylthio-
6	0.57	Saponins	Di-n-decylsulfone
7	0.61	Saponins	Ginsenol
8	0.44	Saponins	Ginsenol
9	1.57	Saponins	1,2-Bis(trimethylsilyl)benzene
10	3.84	Saponins	6-Dimethyl(chloromethyl)silyloxytetradecane
11	0.54	Saponins	2-[3-(4-tert-Butyl-phenoxy)-2-hydroxy-propylsulfanyl]-4,6-dimethyl-nic
12	1.61	Saponins	Tetrasiloxane, decamethyl-
13	48.40	Saponins	1,54-dibromo-Tetrapentacontane,
14	21.22	Saponins	Di-n-decylsulfone
15	7.30	Saponins	1,2-Propanediol, 3-(octadecyloxy)-, diacetate
16	7.15	Saponins	1-Bromoeicosane
	100.00		

Table 4. Q values of the samples by DPPH method

Test tube number	*Concentration (mg/ml)*	*Absorbance at 517nm*	*Q*
1	0	0.6988	0
2	0.200	0.4913	29.69
3	0.400	0.3892	44.30
4	0.600	0.3006	56.98
5	0.800	0.2333	66.61
6	1.000	0.1575	77.46

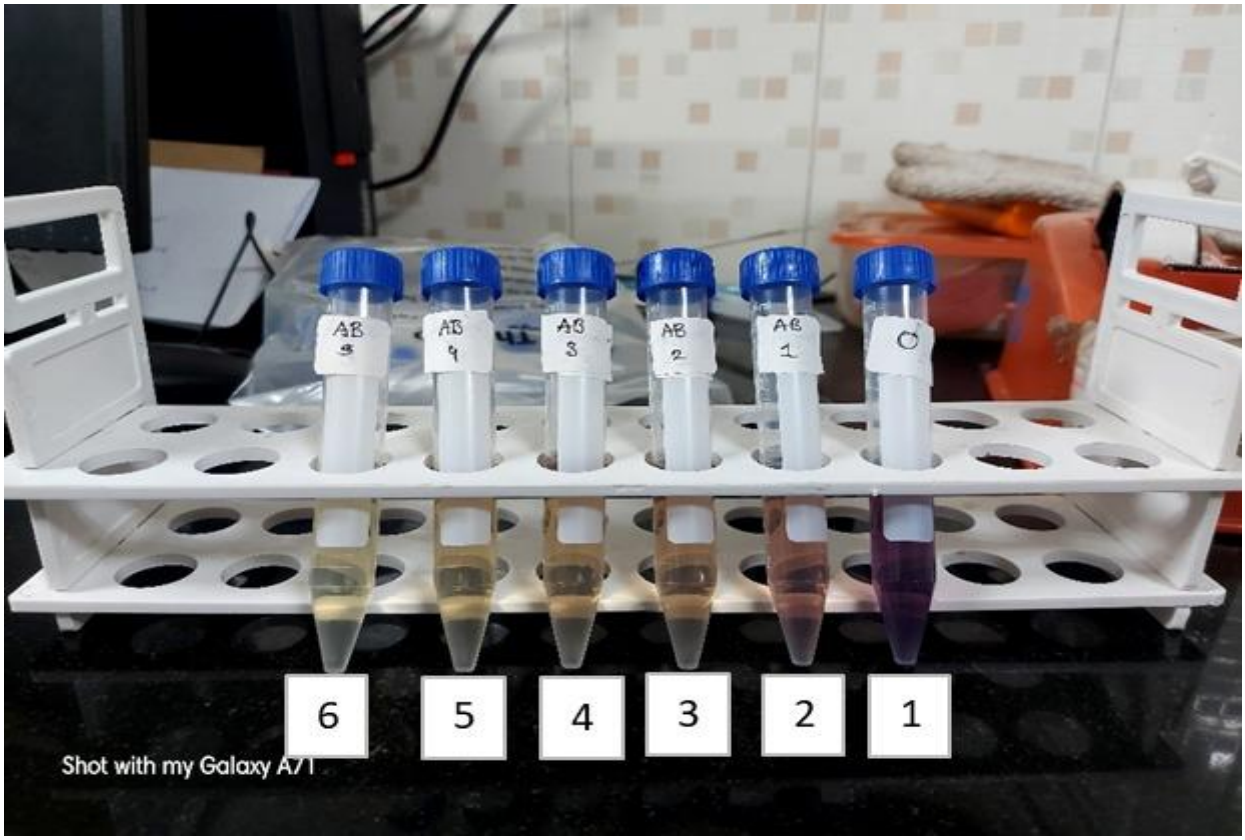

Figure 4. Test tubes 1-6 show the anti-oxidant testing by DPPH reagent using colorimeter.

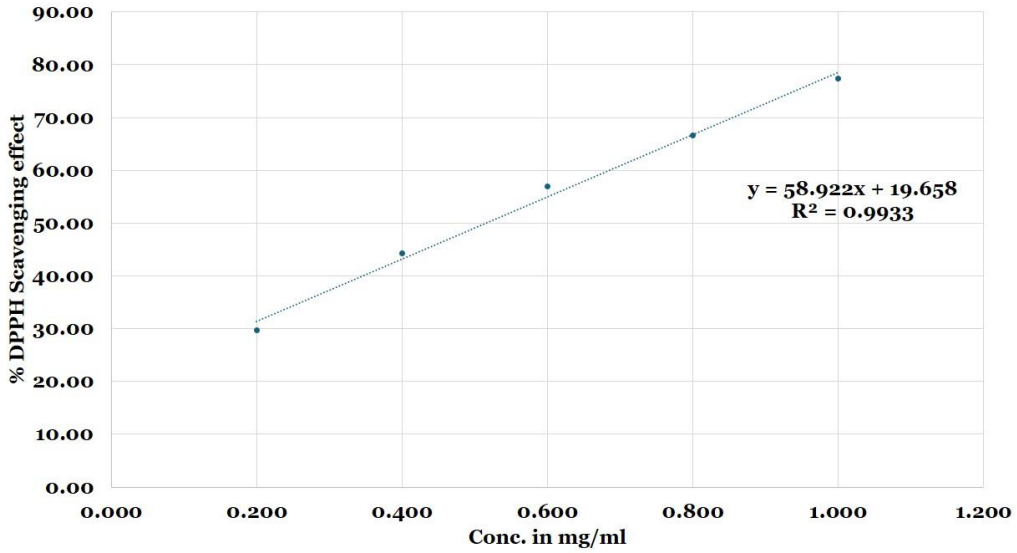

Figure 5. Graphical representation of the DPPH activity.

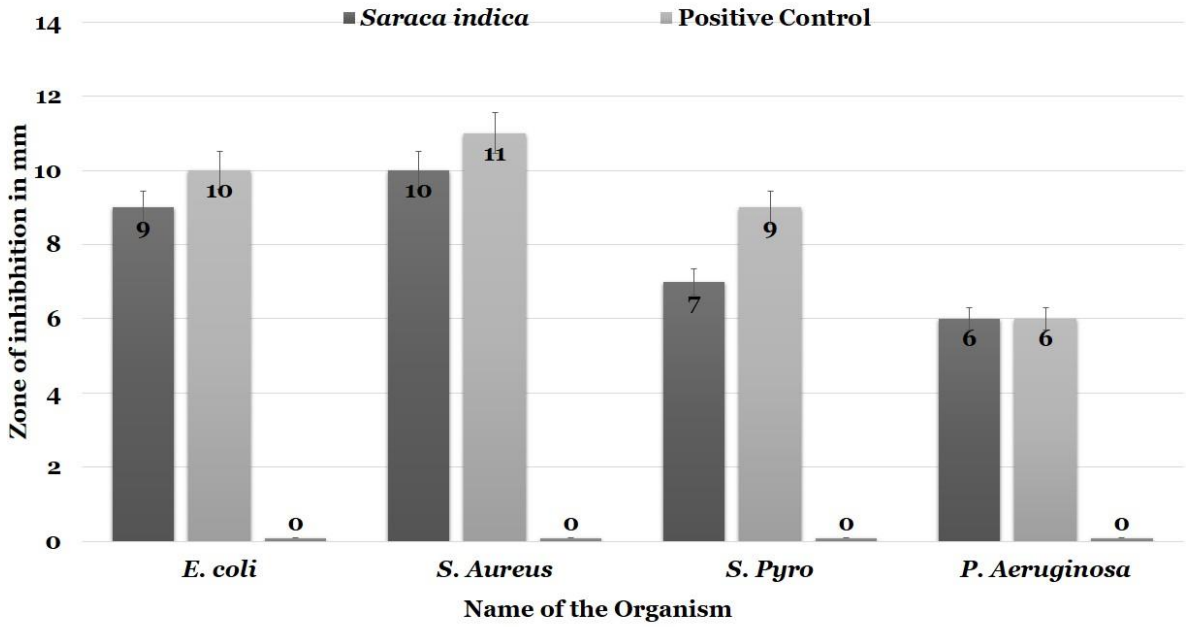

Figure 6. Anti-Microbial activity of bark extract of *S. indica* is compared with Positive control for each microorganism. The dark-shaded bars represent values for *S. indica*, while the light-shaded bars represent values for the positive control.

absence of reducing sugars, steroids, proteins, and anthocyanins might limit certain functionalities, but the overall phytochemical richness suggests substantial medicinal value. These findings support the traditional use of *Saraca indica* bark in herbal medicine and justify further detailed studies to explore specific pharmacological activities.

3.2. HPTLC fingerprint data

The HPTLC fingerprint method was carried out using standards such as Quercetin, Catechin, B-Sitosterol and Gallic acid corresponding to Tracks 1-4 in Figure 2. The HPTLC plate was observed in absorption and fluorescence mode as given in Figure 2. The plate shows several bands as visualized in the UV box of the instrument. There are a total of five tracks. Track 1-4 are standards and track 5 is the sample of Ashoka bark. This visualized data of Figure 2 is plotted in form of graph for all the standards. We have shown this plot only for Quercetin in Figure 3. On the right-hand side of Figure 3 there are 4 tracks corresponding to 4 samples. Track 1 is for The Standard Quercetin and Track 3 is for Ashoka bark. Please ignore Track 2 and Track 4 which are for other samples.

In Figure 3 the x-axis represents the area under curve (AU) and the y-axis represents the Retention factor **(**Rf). The area under curve for the standard is 0.02421 and for sample is 0.00300. This indicates that the sample contains Quercetin-like compounds. Quercetin has anti-cancer, anti-inflammatory and anti-fungal medicinal qualities.

3.3. The GC-MS data

The chromatogram displayed peaks of various compounds such as n-Hexadecenoic acid, Propylene glycol monoleate, and Nonacosane. The retention time varied from 2.0 to 13.9 minutes. Each peak was identified with a corresponding compound name, molecular formula. Table 3 gives the Saponin profile of the bark of *Saraca indica*.

1,54-dibromo-Tetrapentacontane, showed the highest saponin content, markedly higher than others, at 48.40. Di-n-decylsulfone and 1,2-Propanediol, 3-(octadecyloxy)-, diacetate also exhibited relatively high saponin concentrations at 21.22 and 7.30, respectively. Compounds such as 6-Dimethyl(chloromethyl) silyloxytetradecane and 1-Bromoeicosane had moderate contents at 3.84 and 7.15. Lower concentrations were noted in compounds like Ginsenol, with both entries showing 0.44 and 0.61 respectively. The compound with the least content was 2,6-Lutidine 3,5-dichloro-4-dodecylthio- with a saponin content of 0.44. This comprehensive analysis of saponin content in various chemical compounds illustrates the diverse potential of these substances across multiple sectors. High saponin levels in compounds like Tetrapentacontane, 1,54-dibromo-, suggest excellent utility as natural surfactants or immune boosters, crucial for industries ranging from healthcare to agriculture. On the other hand, compounds with substantial but not excessive saponin contents, such as Di-n-decylsulfone and 1,2-Propanediol, 3-(octadecyloxy)-, diacetate, emphasize their value in pharmaceuticals and cosmetics for their broad-spectrum antimicrobial properties. Conversely, those with lower concentrations could be particularly beneficial in scenarios where less intense saponin activity is desired, thus avoiding potential adverse effects from high dosages. This variability in saponin content highlights the need for targeted research to optimize their application in medicine, industry, and environmental management, ensuring that each compound's properties are fully leveraged for appropriate uses.

3.3. Antioxidant activity

The antioxidant activity was determined using the DPPH method. Figure 4 shows the oxidation of the samples.

The DPPH radical in its native, unreduced state is purple coloured solution in methanol as shown in Test tube number 1. Test tubes 2-6 have varying concentration of the extract as given in Table 4. On being oxidized by the samples in test tubes 2-6, the solution turns yellow in colour. Absorbance and calculated Q values which represent percentage reduction in concentration of DPPH in methanol for varying concentration of extract of leaves of *S. indica* from 0 mg/mL to 1.000 mg/ml are given in Table 4. The A0 value is the value of optical density of DPPH solution in which there is no extract given in test tube number 1. The absorbance of test tubes 2-6 are taken as A values and substituted in the formula given in the previous section to arrive at Q values.

As the concentration of the sample increases from 0.200 mg/ml to 1.000 mg/ml, the optical density at 540nm decreases, indicating higher free radical scavenging activity. Specifically, the percentage of DPPH scavenging activity progressively increases, starting from 29.69% at 0.200 mg/ml and reaching up to 77.46% at 1.000 mg/ml, demonstrating the dose-dependent efficacy of the antioxidant. This is plotted as a graph in Figure 5.

As the concentration of the sample increases, the optical density decreases, indicating an increase in DPPH scavenging activity. It starts at 0% for the 0 mg/ml sample and reaches 77.46% for the 1.000 mg/ml sample. The increasing trend in antioxidant activity with increasing conc. can be used to arrive at the potency of the extract for particular application.

3.4. In vitro anti-microbial activity

The antibacterial effect was evaluated by comparing the inhibition area of each extract against different microorganisms. Positive control in the form of sterlium (a sanitizer) and negative control in form of saline water was used to ascertain that the experiment proceeded with accuracy. The positive control sample authenticates the anticipated outcome and assists the scientist in determining whether the experiment was carried out correctly. Negative controls are specific samples that are included in the experiment and are given the same treatment as the others but are not thought to be affected by any of the experiment's variables. In this case negative control was sterile saline water. The zone of inhibition for the four selected strains of bacteria is given in Figure 6.

3.5. Cytotoxicity activities

The result of the cytotoxicity study is expressed as a graph as shown in Figure 7. The y-axis shows % cell viability for increasing concentrations from 0 mg/ml to 2.5 mg/ml.

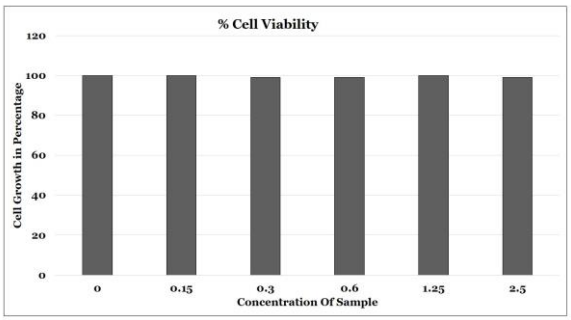

Figure 7. Toxicity activities of the bark of *Saraca indica*

The extract of the sample shows 100% cell viability in the chosen range of concentration. Since the sample is not toxic in this range of concentration, for our Immunomodulatory Assay we selected a concentration of 0.625 mg/ml of extract. This is the median value of concentrations selected for Cytotoxicity Study.

3.6. Immunomodulatory assay

Our sample exhibited anti-inflammatory property by reducing TNF-α expression in comparison to the Control sample. The graph in Figure 8 shows that the sample of bark marked S is able to prohibit cell proliferation by approximate 30%.

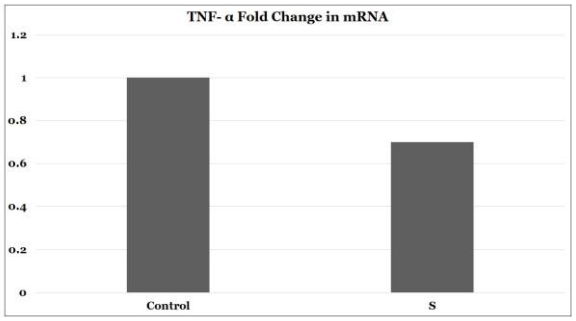

Figure 8. TNF-α expression is reduced by sample S

4. Conclusion

This research provides a detailed phytochemical profile of bark of *S. indica* and evaluates its antimicrobial, antioxidant, cytotoxicity and immunomodulatory activities. The results indicate that the *S. indica* have paving potential applications in the pharmaceutical and cosmetic industries. The study highlights the bark's rich saponin content, which contributes significantly to its therapeutic efficacy. The antimicrobial tests reveal its capability to inhibit common pathogens, supporting its use in treating infections. Moreover, the antioxidant assay confirms its potential in oxidative stress mitigation, a crucial aspect in the prevention of various chronic diseases. The non-toxic nature of the bark's extract, as evidenced by cytotoxicity studies, further underscores its suitability for therapeutic uses. The immunomodulatory essay proves the anti-inflammatory property of the bark. Overall, the study reinforces the integration of traditional herbal medicine with

modern scientific research, emphasizing the potential of *Saraca indica* as a valuable resource for health and medicine.

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Authors' contributions

SP framed the research design and carried out experimental work, GK contributed to the interpretation of the results and SK contributed to preparation of the manuscript and communication with the journal.

Conflict of interest

The authors declare that there is no conflict of interest.

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