RESEARCH ARTICLE

Phytochemical Characterization of the Leaves of *Saraca indica* and Investigation of the Antimicrobial and the Antioxidant Properties of the Saponin-rich Extract of the Leaves

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Abstract

Saraca indica, commonly referred to as the Ashoka tree in the Indian subcontinent, is screened for potential antimicrobial and antioxidant properties of the phytochemicals present in the leaves. The leaves are rich in saponins, as shown by the preliminary testing of phytochemicals. The phytochemical profile of the saponin-rich extract of the leaves was obtained by using the gas chromatography-mass spectra (GC-MS) data, which revealed a total of 30 bioactive compounds. The total saponin content was represented by 11 of these phytochemicals and they accounted for 73% of the total of the 30 bioactive compounds. The extract of these phytochemicals with the predominant presence of saponins exhibited *in-vitro* antimicrobial and antioxidant properties. Saponins are known to have properties to treat skin-related ailments and the leaves of *S. indica* can serve as an abundant herbal source of saponins.

Keywords: *Saraca indica*, Saponins, Antimicrobial, Antioxidant, Phytochemicals, free Radicals, Extract.

Highlights

- GC-MS analysis of the leaves of *Saraca indica* was carried out and it revealed that the leaves were predominantly rich in Saponins.
- The extract of leaves of *S. indica* exhibited *in-vitro* antimicrobial activity.
- The extract of leaves of *S. indica* also exhibited antioxidant properties.
- Nanovariants of these saponins can prove to be beneficial for skin-related ailments.

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Introduction

Saraca indica is one of the most sacred and popular trees in India with ethnobotanical 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. It belongs to the family Fabaceae (earlier: Caesalpinaceae). Ashoka plant has been named as one of the endangered plants by the National Medicinal Plant Board in India (Smitha & Thondaiman, 2016), (*S. indica*, n.d.). Indigenous knowledge about various medicinal plants has been proven to be beneficial for the prevention and treatment of a wide range of diseases (Aziz *et al*., 2018). The use of the traditional system of medicines offers advantages such as fewer side effects, better patient tolerance, more affordability and no resistance to drugs (Ekor, 2013). 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 the discovery and standardization of new herbal medicines. In the realm of Ayurveda, *S. indica* is known to cure many diseases pertaining to cancer, uterine disorders, inflammation, and diabetes, besides exhibiting anti-tumor, antimicrobial 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 (Tabassum & Hamdani, 2014). The leaves of *S.indica* have high saponin content which can be useful for skincare purposes (Parham *et al*., 2020). 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 *S.indica*, the present study is focused on the identification of various active bio-chemicals present in the leaves using the technique of GC-MS.

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Material And Methodology

Sampling and Processing

The leaves of *S. indica* plant were 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). Fig. 1 shows the photograph of the *S. indica* plant. There is an advantage in the fact that the leaves are the only

parts that keep on shedding and newer ones keep on arriving at a higher rate. The leaves were washed under running tap water, air dried and then homogenized into a fine powder by using a stainless-steel blender. The powdered leaves were stored in air-tight bottles at room temperature for further analysis.

Chemicals

All the chemicals such as cyclohexane, petroleum ether, methanol, chloroform, n-butanol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), acetone, nutrient agar were bought from Loba Chemie, Sigma & Himedia and were of analytical grade.

Preliminary Phytochemical Screening of leaves of *S. indica*

The preliminary tests are standard tests to detect the presence of phytochemicals. The tests were carried out as given in Table 1. As preliminary phytochemical testing of *S. indica* leaves extract showed the presence of a rich amount of saponins in the saponin-foam test, saponin-specific extraction was carried out with *S. indica* leaves extract for GC-MS analysis

Fig. 1: *S. indica* plant

Table 1: Preliminary tests to determine the presence of phytochemicals in the leaves of *S. indica*. "-" denotes the absence of that particular class of phytochemicals, "+", "++", "+++" and "++++" denotes the relative detection or presence of that particular class of phytochemicals

S. No.	*Secondary metabolites*	*Phytochemical tests*	*Relative detection*
1	Alkaloids	Mayer's test	++
2	Carbohydrates	Benedicts test	+++
3	Reducing sugars	Fehling's test	-
4	Flavonoids	Alkaline reagent test	++
5	Saponins	Foam test	+++++
6	Tannins	Braymer's test	++
7	Steroids	Salkowski's test	+
8	Proteins	Millon's test	-
9	Glycosides	Keller killioni's test	+
10	Phenols	Ferric chloride test	++
11	Amino acids	Ninhydrin test	-
12	Terpenoids	Salkowski's test	++
13	Phytosterols	Libermann burchard test	+++
14	Anthocyanins	2M NaOH test	-

Preparation of Saponin-specific Extract

About 10 grams of finely ground *S. indica* leaves were placed in a 250 mL round bottom flask. In 100 mL of 50% aqueous methanol was added to this flask. The contents of the flask were stirred overnight at room temperature. Thereafter, the contents were centrifuged at 3000 rpm for 10 minutes. The supernatant liquid was collected and stored in an amber-colored 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 was evaporated with a rotary-evaporator. The resulting aqueous phase was centrifuged at 300 rpm for 10 minutes. The aqueous phase was transferred to a separating funnel. Using 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 a rotary-evaporator. In order to record the GC-MS, this extract was reconstituted in petroleum ether.

Gas Chromatography-Mass Spectra (GC-MS) Analysis

The saponin-rich extract obtained from the leaves of *S. indica* was analyzed using Shimadzu GCMS-QP2010 Ultra for the 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. The injection was in split mode with a split ratio of 40:1. The transfer line temperature was set at 290°C. The mass analyzer was set at 70 eV, electron impact source temperature of 230°C, electron-multiplier voltage of 1588 mV and solvents delay of 3 minutes. All the data was obtained by collecting the full-scan mass spectra within the scan range of 50 to 600 amu. The temperature program was as follows: initial from 200 to 270°C at a ramp rate of 25°C/min, and from 270 to 290°C at a ramp rate of 11°C/min.

Estimation of total Saponin content

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

Antioxidant Assay with DPPH

The assay is based on the measurement of the scavenging capacity of antioxidants towards the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. The odd electron of the nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine. This is the scavenging mechanism wherein a phytochemical or an antioxidant reduces the DPPH as a substrate. The DPPH assay is based on the principle of color change due to the reduction of the stable free radical DPPH by antioxidants. In the presence of antioxidants, the dark purple DPPH radical is converted to a yellow non-radical form, resulting in reduced absorption at certain wavelengths,

which can vary between 515 nm to 520 nm depending on the nature of the solvent used for the phytochemical (Baliyan *et al*., 2022). By measuring this change in absorbance at that particular wavelength, the antioxidant activity of a substance can be measured. The antioxidant assay was performed by measuring absorbance for varying concentrations of extract of leaves of *S. indica* from 0 to 1-mg/mL in 40% methanol, in which 0.135 mM DPPH has been added. Each mixture was kept at room temperature for 30 min. Optical density was recorded at 517 nm using a UV-visible spectrophotometer (LAB India UV-vis spectrophotometer UV3200). Ascorbic acid was used as a standard antioxidant material.

Estimation of percentage reduction of concentration of DPPH (Q)

The DPPH (α, α-diphenyl-β-picrylhydrazyl) is a stable free radical with the free electron extensively delocalized over the molecule. This delocalization imparts a deep violet color to a solution of DPPH. Depending on the nature of the solvent, the absorption maximum of the solution can vary between 517 to 520 nm. Among the different methods that are available to interpret the data with DPPH, we have chosen to calculate the percentage reduction of concentration of DPPH, denoted by Q. Q is proportional to the amount of DPPH quenched under given conditions. Q is given by:

$$Q = \frac{\text{Absorbance of DPPH (Ao)} - \text{Absorbance of sample (A)}}{\text{Absorbance of DPPH}} \text{X100}$$

The greater the value of Q, the greater is the amount of DPPH quenched by the sample and the greater is the antioxidant property of that sample. We prepared samples of varying concentrations by dissolving the saponin-rich extract in 40% methanol.

In-vitro Antimicrobial Activity of Saponin-rich extract

Petri dishes were sterilized and the nutrient agar was poured into 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 labeled accordingly. With sterile cotton, each of the microorganisms was deposited on the agar plates. It was ensured that there was 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 the help of a 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 a CO_2 incubator. The recommended incubation times for bacterial strain (24 hours) and for fungi strain (48 hours) were followed.

Estimation of Zone of Inhibition

Zone of inhibition refers to the area that 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. The zone of inhibition around each well was measured using a vernier caliper. It was measured from the edge of the well to the edge of the clear zone.

Results and Discussion

The GC-MS data

The saponin-rich extract was analysed with the technique of GC-MS in order to establish the phytochemical profile of *S. indica* leaves. The results are tabulated in Table 2, which gives the percentage of each phytochemical as represented by the area under the curve.

The table shows the numerical order in which the peaks were detected during the analysis. Each peak corresponds to a specific compound in the sample. Retention time is a crucial parameter in chromatography and is used to identify and quantify compounds based on the time they spend in the chromatographic column. The area under the curve is a measure of the quantity or concentration of the compound present. The percentage of the total area under the curve that each peak occupies is a relative measure of the abundance of each compound in the sample as compared to others. A larger percentage of areas under the curve indicate a higher concentration of that compound. A total of thirty phytochemicals were identified and arranged chronologically according to their peak numbers as they appeared in the GC-MS spectra. The classes of phytochemicals identified were saponins, sterols and polyenes. Oleic acid constitutes nearly 42% of the total phytochemicals. Oleic acid is an important member of emulsifying agents and excipients in pharmaceutical formulations. The sodium salt of Oleic acid functions as a soap. It forms a major component of Olive oil. As a monounsaturated fat, Oliec acid is an important part of the human diet. Table 3 gives us the relative amounts of saponins.

Saponins are a large family of amphiphilic glycosides of steroids and triterpenes found in plants and some marine organisms. Oleic acid constitutes nearly 57% of all the saponins present in the extract of leaves of *S. indica*. This data is valuable for characterizing the phytochemical composition of the sample and can be used for further analysis and interpretation, such as identifying the potential health benefits or biological activities associated with these compounds. The specific significance of these compounds would depend on the context and purpose of the analysis, such as in the study of plant extracts for pharmaceutical or nutritional purposes.

Antioxidant Activity

Antioxidants are compounds that protect biological systems from oxidative damage caused by free radicals and reactive oxygen species (ROS). These highly reactive molecules are produced as natural by-products of metabolic processes or ingested from external sources such as environmental pollutants and unhealthy diets (Lobo *et al*., 2010). When the balance between antioxidants and free radicals is disrupted, oxidative stress occurs, which causes cellular damage and contributes to the development of various chronic diseases, including cancer, cardiovascular disease, and neurodegenerative conditions. Therefore, there is a need to evaluate the antioxidant activity in potential health benefits of various natural and synthetic compounds. A widely used and reliable method for determining

Table 2: Phytochemicals as detected in the GC-MS spectra and identified using the NIST library

Sr. No.	*Peak No.*	*Retention time (min)*	*Area% under the curve*	*Class of the phytochemical*	*Name of the phytochemical*
1	1	7.889	0.79	Phenol	Phenol, 2,4-bis(1,1-dimethylethyl)
2	2	9.380	0.39	Fatty acid (saponin)	Tetradecanoic acid
3	3	9.882	0.59	Phenol	Phytol, acetate
4	4	10.225	0.22	Alkane	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
5	5	10.985	3.45	Fatty acid (saponin)	n-Hexadecanoic acid
6	6	12.247	0.21	Fatty acid (saponin)	6-Octadecenoic acid, methyl ester, (Z)-
7	7	12.556	42.19	Fatty acid (saponin)	Oleic Acid
8	8	12.690	4.90	Fatty Acid (saponin)	Octadecanoic acid
9	9	13.253	0.47	Ester	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)
10	10	13.908	0.77	Sulphurous acid	Sulfurous acid, octadecyl 2-propyl ester
11	11	14.100	0.26	Fatty Acid (saponin)	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate
12	12	14.233	3.01	Fatty Acid (saponin)	Oleoyl chloride
13	13	14.472	0.63	Fatty Acid (saponin)	9,12-Octadecadienoic acid (Z,Z)
14	14	14.525	2.39	Alkene	2-Methyl-E-7-octadecene
15	15	14.864	0.37	Halogen	erythro-7,8-Bromochlorodisparlure
16	16	15.474	0.45	Polyene	E, E, Z-1,3,12-Nonadecatriene-5,14-diol
17	17	16.309	0.82	Alkene	Acenaphthylene, octachloro
18	18	17.514	0.81	Complex ring structure	Tricyclo[5.4.3.0(1,8)]tetradecan-3-ol-9-one, 4-ethen
19	19	17.922	1.41	Sterols	Stigmast-5-en-3-ol, oleate
20	20	18.372	1.25	Sterols	22,23-Dibromostigmasterol acetate
21	21	18.551	1.66	Sterols	beta. -Riboside, 2′,3′-acetone
22	22	18.727	4.69	Sterols	Stigmasta-5,22-dien-3-ol, acetate, (3. beta.)-
23	23	18.985	3.52	Sterols	Stigmastan-3,5-diene
24	24	19.158	1.76	Complex ring structure	cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl
25	25	19.275	1.04	Longifolene	1,4-Methanoazulene-9-methanol, decahydro-4,8,8
26	26	19.398	13.26	Steroidal saponin	Stigmastan-3,5-diene
27	27	19.541	3.58	Aldehyde	7-Hexadecenal
28	28	21.692	0.70	Steroidal saponin	Stigmasterol
29	29	22.647	4.20	Steroidal saponin	gamma. -Sitosterol
30	30	23.314	0.21	Amide	N-(4-Bromo-1H-pyrazol-3-yl)-formamide
			100.00		

Table 3: List of phytochemicals from Table 2, which are categorized as saponins and their relative amounts in percentage

S. No.	*Total saponin content (%)*	*Class of the phytochemical*	*Name of the phytochemical*
1	0.53	Fatty Acid (Saponin)	Tetradecanoic acid
2	4.71	Fatty acid (saponin)	n-Hexadecanoic acid
3	0.29	Fatty acid (saponin)	6-Octadecenoic acid, methyl ester, (Z)-
4	57.64	Fatty acid (saponin)	Oleic Acid
5	6.69	Fatty acid (saponin)	Octadecanoic acid
6	0.36	Fatty acid (saponin)	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate
7	4.11	Fatty acid (saponin)	Oleoyl chloride
8	0.86	Fatty acid (saponin)	9,12-Octadecadienoic acid (Z, Z)-
9	18.11	Steroidal saponin	Stigmastan-3,5-diene
10	0.96	Steroidal saponin	Stigmasterol
11	5.74	Steroidal saponin	Gamma-Sitosterol
	100.00		

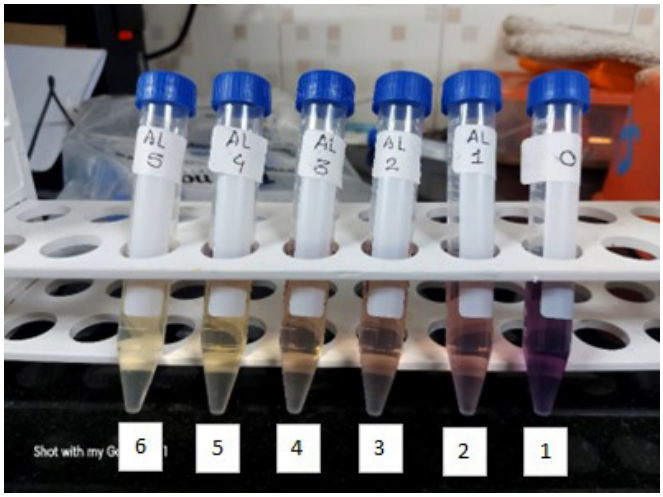

Fig. 2: Test tubes 1-6 show the antioxidant testing by DPPH reagent using a colorimeter. The DPPH radical in its native, unreduced state is a purple-colored solution in methanol, as shown in Test tube number 1. Test tubes 2-6 have varying concentrations of the extract, as given in Table 4. On being oxidized by the samples in test tubes 2-6, the solution turns yellow in color

Table 4: Absorbance and calculated Q values, which represent percentage reduction in the concentration of DPPH in methanol for varying concentrations of extract of leaves of *S. indica* from 0 to 1.000 mg/mL. The A0 value is the value of the optical density of the 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

Test tube number	*Concentration (mg/mL)*	*Optical density at 517 nm*	*Q (percentage reduction in DPPH)*
1	0	0.6988	0
2	0.200	0.5205	25.52
3	0.400	0.4265	38.97
4	0.600	0.3586	48.68
5	0.800	0.2452	64.91
6	1.000	0.1632	76.65

the antioxidant capacity of natural products, including plant extracts, fruits, vegetables, and various bioactive compounds (Jan *et al*., 2013) is the DPPH assay (Kedare & Singh, 2011). These results help identify and characterize potential sources of antioxidants, allowing the development of functional foods, nutraceuticals, and pharmaceutical formulations with improved antioxidant properties. Fig. 2 shows the purple unreduced state of DPPH in methanol as solvent, and the yellow reduced state of the leaves extract in methanol.

We investigated the antioxidant activity of *S. indica* leaves using a DPPH assay. DPPH is a stable free radical in methanol solution. The results are given in Table 4.

The activity of the extract is represented as a graph in Fig. 3. The Q values are represented as a function of the concentration of the extract in mg/mL. The varying concentrations of extract of leaves of *S. indica* are from 0 to 1.000 mg/mL.

As the concentration of the sample increases, the optical density

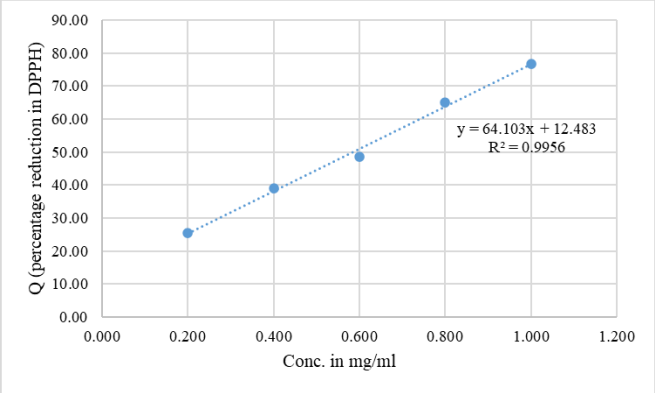

Fig. 3: Graphical representation of the DPPH activity with saponin leaf extract

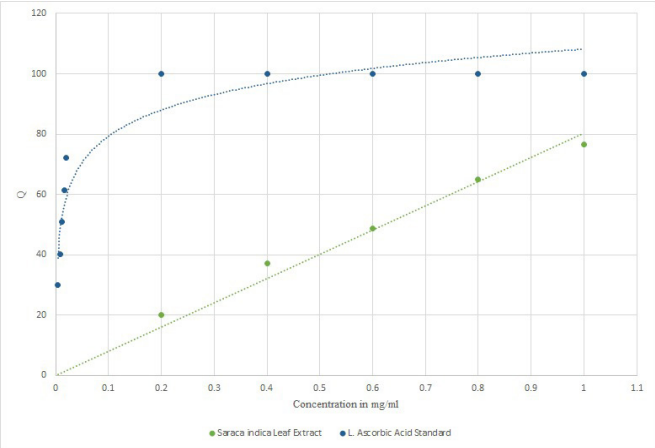

Fig. 4: Comparison of DPPH activity with ascorbic acid

decreases, indicating an increase in DPPH scavenging activity. It starts at 0% for the 0 mg/ml sample and reaches 76.65% for the 1.000 mg/mL sample. The increasing trend in antioxidant activity with increasing concentration can be used to arrive at the potency of the extract for particular applications.

The DPPH activity was standardized against Ascorbic acid. We carried out this experiment and came to the conclusion that for a given value of Q, as seen in Fig. 4, the values of concentration for saponin-rich extract lie much lower than for Ascorbic acid. For instance, for the Q value of 76%, the concentration of Ascorbic acid is less than 100 ppm, while for the same value of Q, the concentration of the extract is 1000 ppm.

In-vitro Antimicrobial Activity

The antibacterial effect was evaluated by comparing the inhibition area of each extract against different microorganisms. Fig. 5 shows such an effect for one of the microorganisms. Positive control in the form of sterlium (a sanitizer) and negative control in the 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

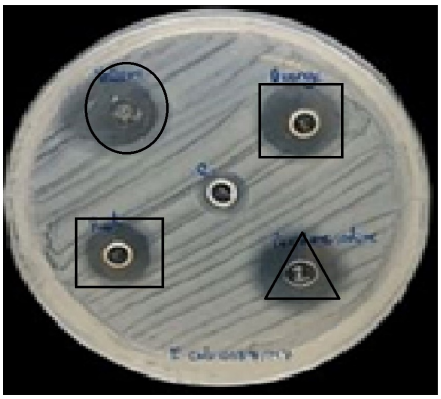

Fig. 5: Extract of *S. indica* leaves is tested with four different microorganisms. The area marked with a circle belongs to *S. indica*. The area marked with squares belongs to samples, which can be ignored for the present study. The area marked with a triangle is the positive control. The spot in the center is the negative control

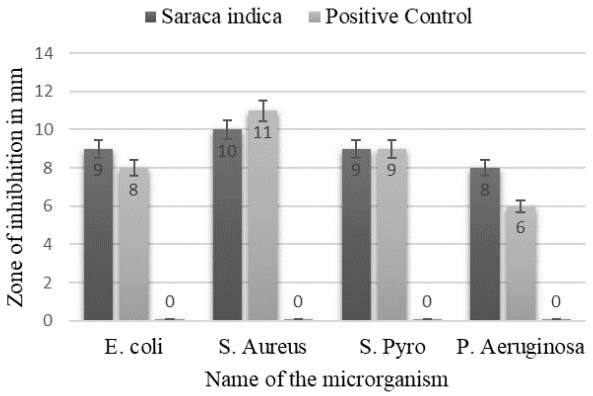

Fig. 6: Antimicrobial activity of leaves extract of *S. indica* is compared with positive control for each microorganism. The dark-shaded bars represent values for *S. indica*, while the light-shaded bars represent values for the positive control

experiment's variables. In this case negative control was sterile saline water.

The bar diagram of Fig. 6 shows that *S. indica* exhibited excellent antimicrobial activity against all tested microorganisms, with the highest inhibition zone observed against S. aureus. The positive control demonstrated significant antimicrobial activity against all tested microorganisms, confirming the validity of the assay. The negative control showed no inhibition zones, validating the absence of any antimicrobial effect.

Conclusion

S. indica, commonly known as the Ashoka tree, is native to the Indian subcontinent and was selected for the present study due to its ethno-medical uses. The aim of the present study was to establish the phytochemical profile of its leaves by using the technique of GC-MS and to assess its antibacterial and antioxidant properties. Our present study revealed that the leaves of *S. indica* are rich in saponins – a class of phytochemicals that exhibit soap-making ability. The saponin-rich extract exhibited excellent *in-vitro* antimicrobial properties. The leaves also exhibited antioxidant properties in the DPPH assay. Saponins and saponin derivative compounds are proven to be beneficial for the prevention and treatment of various skin diseases. Therefore, *S. indica* plant leaves, due to naturally present bioactive compounds, namely the saponins, have the potential for the development of skincare products. Furthermore, the nano-variants of these bioactive phytochemicals have the potential for increased bioavailability and penetration through the skin barrier.

Acknowledgments

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Authors' Contribution

Mr. Siddhesh Pote carried out the experimental work, recorded the experimental data, and managed references using Mendeley. Dr Gaganjyot Kaur contributed to the analysis of the data collected. Dr. Sonali Kokane guided the experimental work and organized and prepared the manuscript.

Conflict of Interest

None.

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