RASĀYAN *J. Chem.*
Vol. 19 | No. 3 | 1692-1701 | July-September | 2026
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

POTENT ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF SARACA INDICA BARK WITH MINIMAL CYTOTOXICITY

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ABSTRACT

This study contributes to the growing field of phytopharmacology by providing systematic evidence supporting the therapeutic potential of *Saraca indica* bark, particularly its ethanolic extract, as a source of bioactive compounds with antioxidant and anti-inflammatory properties. The study aimed to investigate the antioxidant, anti-inflammatory, and cytotoxic properties of various solvent extracts of *Saraca indica* bark. The Soxhlet extraction technique was utilized with solvents of varying polarity, and the extraction yield revealed ethanol as the most efficient solvent for extraction. A wide range of phytoconstituents—namely alkaloids, flavonoids, tannins, phenols, saponins, glycosides, steroids, and terpenoids were predominantly present in the ethanolic and aqueous extracts, whereas non-polar extracts contained only trace amounts. Among the extracts tested, the ethanolic extract (AE) showed the highest scavenging activity through the NO radical scavenging assay with an IC_{50} value of 51.68 µg/mL, followed by the ethyl acetate extract, while the hexane extract showed negligible activity. Anti-inflammatory activity was assessed via NO production inhibition in LPS-stimulated RAW 264.7 macrophages. Concentration-dependent reduction in NO production was observed with AE, indicating significant anti-inflammatory potential, though less potent than the standard dexamethasone. Through MTT assay, it was confirmed that AE was non-toxic to RAW 264.7 cells, with cell viability remaining above 75% at the highest concentration and an IC_{50} value greater than 100 µg/ml. Overall, this research addresses key challenges in natural product research, including validation, safety assessment, and reproducibility, thereby contributing to the rational development of plant-derived therapeutics with potential applications in managing oxidative stress and inflammatory disorders.

Keywords: *Saracaindica*, Antioxidant, Anti-Inflammatory, Cytotoxicity, Nitric Oxide.

RASĀYAN*J. Chem.*, Vol. 19, No.3, 2026

INTRODUCTION

Medicinal plants play a crucial role in traditional healthcare systems across the globe and act as a significant source for the identification of emerging therapeutic agents.[1] Natural products and bio actives derived from medicinal plants are known to possess several bio actives with significant therapeutic and pharmacological potentials, including antioxidant, anti-inflammatory, and anticancer activities.[2] Scientific interest in investigating safer, more affordable, and physiologically effective plant-based treatments has increased due to growing worries about the side effects, toxicity, and resistance linked to synthetic medications.[3] Oxidative stress is a key contributor to the pathophysiology of several chronic disorders, including cancer, inflammatory conditions, cardiovascular diseases, neurodegenerative disorders, and diabetes. These disease states develop due to an imbalance between the excessive generation of reactive oxygen species (ROS) and the body's antioxidant defense systems. Elevated ROS levels can cause extensive damage to essential cellular macromolecules such as DNA, proteins, and lipids, ultimately leading to cellular dysfunction and the progression of disease.[4] In such a scenario, the antioxidants derived from natural herbs or medicinal plants can neutralize the free radicals, reduce oxidative damage, and thereby contribute to disease prevention and management. Moreover, inflammation is a protective physiological response to injury and infection; however, uncontrolled or continued progression of inflammation can result in damage to tissues that ultimately results in the emergence of diverse chronic

conditions. In such a case, plant-derived anti-inflammatory agents are therefore of great therapeutic interest.[5] Cancer is the leading cause of mortality throughout the globe, and the search for safer and more effective anticancer agents continues to be a major focus of biomedical research.[6] The capacity of cytotoxic chemicals derived from medicinal plants to specifically limit the proliferation of cancer cells while showing less toxicity toward normal cells has drawn interest. A thorough grasp of the therapeutic efficacy of plant extracts and their phytoconstituents is provided by the assessment of cytotoxicity in addition to antioxidant and anti-inflammatory properties.[7]*Saraca indica* L. (Family: Fabaceae), widely known as the Ashoka tree, is a plant of considerable cultural and therapeutic importance, commonly used in Indian traditional medicine, especially Ayurveda.[8] The bark of S. indica has long been incorporated into traditional medicinal preparations for managing gynecological conditions, including menorrhagia, dysmenorrhea, and inflammatory disorders of the uterus. Beyond its ethnomedicinal applications, the bark has been documented to exhibit a range of pharmacological activities, such as antimicrobial, analgesic, anti-ulcer, and hepatoprotective effects.[9] These therapeutic effects are largely attributed to the occurrence of a wide range of phytochemical constituents, such as flavonoids, tannins, phenolic compounds, glycosides, saponins, and sterols.[10] Despite its long-standing traditional use, systematic scientific evaluation of the phytochemical profile and biological activities of *Saraca indica* bark remains limited. It has been observed that although several medicinal plants have been extensively investigated for antioxidant and anti-inflammatory potential, there is still a lack of comprehensive studies integrating phytochemical profiling with simultaneous evaluation of antioxidant, anti-inflammatory, and cytotoxic activities specifically for *Saraca indica*bark. This represents a critical research gap, particularly in correlating solvent-dependent extraction efficiency with biological activity.[9.10] Phytochemical extraction and qualitative identification are essential steps in understanding the phytoconstituent profile of medicinal plants and correlating specific phytoconstituents with observed pharmacological effects.[11] The choice of extraction method and solvent polarity significantly affects both the yield and the range of bioactive compounds obtained from plant sources. Recent studies suggest that phenolic and flavonoid-rich plant extracts exhibit strong antioxidant activity due to their potential to act as hydrogen or electron donors and to bind metal ions.[12] These compounds may also reduce inflammatory responses by targeting pro-inflammatory molecules, enzymatic activity, and signaling pathways. Furthermore, several plant-derived phenolics and flavonoids have been reported to exert cytotoxic effects on cancer cell lines via apoptosis induction, inhibition of proliferative capacity, and modulation of oxidative stress responses.[13] In alignment with the United Nations Sustainable Development Goal 9 (*SDG-9: Industry, Innovation, and Infrastructure*), which emphasizes ensuring healthy lives and promoting well-being for all, the exploration of plant-based therapeutics such as *Saraca indica*contributes toward the development of safer, affordable, and accessible healthcare solutions. The identification of bioactive compounds with antioxidant and anti-inflammatory potential supports global efforts to reduce the burden of chronic diseases associated with oxidative stress and inflammation. In view of this context, the present investigation aims to undertake phytochemical extraction and qualitative identification of bioactive constituents present in the bark of *Saraca indica*. Specifically, this study seeks to fill the identified research gap by systematically comparing different solvent extracts and establishing a relationship between phytochemical composition and biological activities. Additionally, the study evaluates the antioxidant potential using established in vitro assays, investigates the anti-inflammatory activity through relevant experimental models, and assesses cytotoxicity to explore its possible anticancer relevance. By integrating phytochemical screening with biological activity evaluation, this research seeks to establish a scientific basis for the indigenous use of *Saraca indica* bark and contribute to the identification of potential natural therapeutic agents for oxidative stress–related disorders, inflammation, and cancer.

EXPERIMENTAL

Material and Methods

Saraca indica bark was purchased from Yucca Enterprises, Mumbai, INVOICE NO. YE/23-24/757 Dated 17-2-2024. Hexane, Ethyl acetate and Chloroform, Ethanol was procured from SRL Chemicals, Mumbai. All other reagents and chemicals used for qualitative estimation and related analyses were obtained from Merck Limited, Mumbai.

Collectionand Authentication of Plant Species

The dried barks of the *Saraca indica* were obtained from Krishna Vishwa Vidyapeeth's Central Park, Malkapur, Karad. The plant was verified by Dr. A. Benniamin, Scientist Fand Head of Office BSI Western Regional Centre, Pune, India, as *Saraca indica* (Family-Fabaceae) in the provided Voucher Specimen Number: JVD-78.

Sample Preparation

A good quality dried bark was screened and subjected to a size reduction process by Mortar, pestle, ball mill and electric grinder and with the help of a sieve shaker to get fine powder of *Saraca indica* bark. The obtained powder was weighed, stored in an air-tight container and used for extraction.

Extraction of *Saraca Indica*

Dried and powdered barks were loaded into the Soxhlet extraction sleeve and overlaid with glass wool. Powdered barks (250g) were successively extracted using nonpolar to polar solvents like (Hexane, Chloroform, Ethyl acetate, Ethanol and Water) using a Soxhlet apparatus for 24 h. Filtration of the extract was carried out using Whatman No. 1 filter paper; the filtrate was evaporated by a rotary evaporator, and the residual the extract was subjected to drying. The dried extract was stored at 5 °C in a refrigerator until further use.[14]

Characterization of *Saraca indica* Bark

Organoleptic Properties

The organoleptic properties like *color, odor*, appearance, touch and taste were determined manually.

Physicochemical Parameters

Physicochemical parameters like pH, LOD, total ash value, alcohol-soluble extractive content, acid-insoluble ash value, aqueous extractive value and extraneous matter were determined as per official procedures reported in literature.[15]

Characterization of Extracts

Yield of Extract

The yield of an extract was determined by accurately weighing the dried extract obtained after solvent removal and comparing it with the initial weight of the raw plant material used for extraction.[16]

Preliminary Phytochemical Profiling (Qualitative)

Preliminary phytochemical screening of the extracts was carried out to identify the presence of alkaloids, flavonoids, saponins, tannins, phenolic compounds, cardiac glycosides, steroids, terpenoids, quinones, and proteins. All qualitative tests were conducted in accordance with standard procedures described in the literature.[17]

Evaluation of In Vitro Antioxidant Potential Using Nitric Oxide Radical Neutralization Assay

The ability to scavenge nitric oxide radicals was determined by test samples (AH, AEA, AC, AE, and AW) was evaluated following a previously reported method with minor modifications.[18] The plant extracts were initially formulated as stock solutions at a concentration of 10 mg/mL and subsequently diluted with purified water to obtain working concentrations ranging from 20 to 800 µg/ml. The same concentration range was maintained for the standard compound, gallic acid. All prepared solutions were maintained at 4 °C until use. Immediately before the assay, the Griess reagent was freshly formulated by mixing equal volumes of 1% sulphanilamide dissolved in 2.5% phosphoric acid with 0.1% naphthyl ethylenediamine dihydrochloride prepared in the same acidic medium. For the assessment of nitric oxide scavenging activity, 0.5 mL of 10 mM sodium nitroprusside prepared in phosphate-buffered saline was combined with 1 ml of the ethanolic extract at different concentrations (20–800 µg/mL). The resulting reaction mixtures were subsequently incubated at 25 °C for 3 hours. After incubation, a comparable volume of freshly formulated Griess reagent was added to each reaction mixture. Control preparations were conducted under the same conditions by replacing the test extract with an equal volume of buffer solution. Color control tubes contained the corresponding concentrations of plant extracts without the addition of sodium nitroprusside. Following the reaction, 150 µL of each mixture was dispensed into a

96-well microplate, and the absorbance was measured at 546 nm using a Robotnik microplate reader. Gallic acid served as the positive reference standard.[18] The percentage scavenging of nitric oxide radicals by the extracts and the standard was computed using the following formula:

$$\text{Nitric Oxide Scavenging (\%)} = [(A_{control} - A_{test}) / A_{control}] \times 100$$

Inhibition of Nitric Oxide (NO) Production

RAW 264.7 macrophage cells were plated in 96-well culture plates at a seeding density of 1×10^5 cells per well and maintained for 24 hours at 37 °C in a humidified incubator with 5% CO_2 . Test samples at concentrations of 25 and 50 µg/mL, along with the reference drug dexamethasone (5 µg/mL), were formulated in serum-free DMEM, and the final treatment volume in each well was adjusted to 100 µl. Following a pre-treatment period of 1 hour, the cells were stimulated with lipopolysaccharide (LPS) at a concentration of 1 µg/mL and incubated for an additional 24 hours. For the determination of nitric oxide levels, 100 µL of the culture supernatant was combined with an equal volume of Griess reagent in a 96-well plate and incubated at room temperature for 10 minutes. The absorbance was then measured at 540 nm using a microplate reader. Nitrite concentrations in the culture medium were quantified by reference to a sodium nitrite ($NaNO_2$) standard calibration curve.[19]

MTT Assay

For the MTT assay, the test samples were subjected to a two-fold serial dilution to obtain concentrations ranging from 6.25 to 100 µg/ml. The RAW 264.7 macrophage cell line was obtained from the National Centre for Cell Science (NCCS). Stock cultures were propagated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 IU/ml), and streptomycin (100 µg/mL), and maintained at 37 °C in a humidified incubator with 5% CO_2 until confluence was attained. The confluent monolayer was harvested by trypsinization, and the cell concentration was adjusted to 1.0×10^5 cells/mL using the appropriate growth medium supplemented with 10% FBS. Thereafter, 100 µL of the cell suspension (1×10^4 cells per well) was dispensed into each well of a 96-well microtiter plate. After a 24-hour incubation period to allow partial monolayer formation, the culture medium was gently aspirated, and the cell layer was washed once with fresh medium. Subsequently, 100 µL of test samples at different concentrations were added to the respective wells containing the partial monolayer. The plates were subsequently incubated for an additional 24 hours at 37 °C in a 5% CO_2 atmosphere. Following incubation, the treatment media were removed, and 20 µL of MTT solution (2 mg/mL prepared in phosphate-buffered saline) was added to each well. The plates were then incubated for a further 4 hours at 37 °C in a humidified 5% CO_2 environment to facilitate formazan crystal formation. The supernatant was carefully discarded, and 100 µL of DMSO was added to each well to dissolve the formed formazan crystals, followed by gentle shaking of the plate. Absorbance was measured at 570 nm using a microplate reader.[20] Cell viability was expressed as a percentage and calculated using the formula:

$$\text{\% viability} = (\text{Sample absorbance} / \text{Control absorbance}) \times 100$$

RESULTS AND DISCUSSION

Characterisation of *Saraca Indica* Bark

Organoleptic Characteristics

An assessment of the sensory characteristics of *Saraca indica* bark was carried out. This study, performed on *Saraca indica* bark, demonstrated distinct as well as characteristic features useful for its preliminary identification and quality assessment. The bark samples were found to be dark brown in color, possessing a distinctive odor and taste, indicating the presence of inherent phytoconstituents typical of the plant material. Its woody character was reflected in its solid look and stiff surface roughness. Overall, the bark's consistent and well-defined organoleptic characteristics supported its authenticity and eligibility for more phytochemical and pharmacological research.

Physicochemical Parameters

It has been observed through physicochemical evaluation of *Saraca indica* bark that all physicochemical parameters were found to be within acceptable ranges, showing excellent quality and purity of the bark.

The pH of the bark was found to be 6.7, suggesting a slightly acidic to near-neutral nature, which is quite suitable for the development of herbal dosage forms.[21] The LOD of the bark was observed to be very low (0.80%), demonstrating lower moisture retention with reduced risk of microbial growth and degradation.[22] The total ash content (6.31%) as well as acid-insoluble ash (0.20%) were found to be within acceptable limits, demonstrating very low contamination of inorganic and siliceous matter. The Alcohol-extractable constituents (6.50%) and aqueous extractive value (2.5%) clearly demonstrated the presence of a moderate concentration of alcohol- and water-soluble phytoconstituents, respectively. Foreign matter was absent in bark samples, further confirming its purity. Overall, the physicochemical parameters supported the authenticity, stability, and suitability of *Saraca indica* bark for further phytochemical and pharmacological investigations. This confirms the quality and purity of the plant material, ensuring the reliability of further studies and supporting the standardization of *Saraca indica* for pharmaceutical use. The physicochemical characteristics evaluated are summarised in Table-1.

Table-1: Evaluation of Physicochemical Properties of *Saraca indica* Bark

S. No.	Parameter	*Saraca indica* bark	Normal Range
1	pH	6.7	5.5 to 7.5
2	LOD	0.80	Not more than 7.0%
3	Total ash value	6.31	Not more than 30.0%
4	Alcohol-extractable constituents	6.50	Within a limit of 10%
5	Acid-insoluble residue	0.20	Within a limit of 1.0%
6	Aqueous extractive value	2.5	Within a limit of 9.0%
7	Foreign matter	-	Not more than 2.0%

Characterisation of Extracts

Yield of Extracts

Soxhlet extraction of *Saraca indica* bark was carried out employing different solvent systems. Wide variation was observed in the extraction yield of *Saraca indica* (Ashoka) bark powder as determined by the chemical properties and polarity of the solvent system used, demonstrating the influence of solvent selection on the efficiency of phytochemical extraction. Among all solvents used in extraction, ethanol showed the highest yield with an extract quantity of 8.238 g, demonstrating that a higher proportion of phytoconstituents present in the bark are polar to moderately polar in nature. The high yield obtained with ethanol suggests its suitability as an effective extraction solvent for comprehensive phytochemical recovery. It is a well-known fact that ethanol has a greater ability to solubilize a wide range of phytoconstituents, including tannins, flavonoids, phenols, glycosides, and saponins, which are commonly reported in *Saraca indica* bark. Aqueous extraction demonstrated a moderate yield of 2.156 g, which may be due to the water-soluble bio actives, such as inorganic salts, carbohydrates, glycosides, and certain phenolics. Ethyl acetate also demonstrated a moderate extraction yield of 2.022 g, indicating the presence of semi-polar bioactives like flavonoid aglycones and certain phenolic derivatives.

Lower extraction yield was observed in the case of non-polar and less polar solvents like hexane (0.800 g) and chloroform (0.733 g), indicating a relatively lower amount of non-polar bio actives like lipids, waxes, and terpenoids in the bark matrix. Ethanol turns out to be the most effective extraction solvent, and the extraction yield profile shows that *Saraca indica* bark is rich in polar and moderately polar phytochemicals. These results validate the choice of ethanolic extracts for further phytochemical screening and biological activity assessment, including cytotoxic, anti-inflammatory, and antioxidant investigations.

These results highlight the importance of solvent polarity, with ethanol proving most effective for extracting bioactive compounds, supporting its use in future studies and formulations. The summary of the extraction yield with various solvents is presented in Table-2.

Preliminary Phytochemical Profiling (Qualitative)

The initial phytochemical profiling of the extracts clearly demonstrated the marked impact of solvent polarity on the extraction of phytoconstituents.[23] The AH extracts lesser constituents like phenols, steroids,

and terpenoids, while other constituents were absent. Lipophilic constituents like steroids and terpenoids were primarily extracted in nonpolar solvents.

Table-2: Extraction Yield with Various Solvents

Sample	Extraction solvent composition	Weight of Powdered Sample (g)	Yielded extract mass (g)
Ashoka bark powder	Hexane	250	0.800
	Ethyl acetate	250	2.022
	Chloroform	250	0.733
	Ethanol	250	8.238
	Water	250	2.156

On the other hand, AEA exhibited a broad spectrum of phytoconstituents, including alkaloids, flavonoids, tannins, phenolic compounds, cardiac glycosides, steroids, terpenoids, and quinones, demonstrating the extraction of moderately polar compounds. AC demonstrated a narrow range of bio actives like steroids and terpenoids, indicating efficacy to extract non-polar to slightly polar phytoconstituents. AE and AW extract exhibited a rich bioactive profile, comprising alkaloids, flavonoids, saponins, tannins, phenols, cardiac glycosides, steroids, terpenoids, and quinones.

The polar solvent extracts' high concentration of phenolic compounds, flavonoids, and tannins is especially noteworthy as these phytochemical groups are frequently linked to cytotoxic, anti-inflammatory, and antioxidant properties.[24] Overall, the findings show that water and ethanol are the best solvents for removing a variety of bioactive phytoconstituents from *Saraca indica* bark, which might explain the pharmacological potential shown in later biological analyses. The presence of phenolics and flavonoids explains the observed biological activities and supports their role in plant-based therapeutic effects. The comparative phytochemical screening of various extracts is reported in Table-3.

Table-3: Preliminary Qualitative Phytochemical Evaluation of Various Extracts

Specimen	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Cardiac glycosides	Steroids	Terpenoids	Quinones	Proteins
AH	-	-	-	-	+	-	+	+	-	-
AEA	+	+	-	+	+	+	+	+	+	-
AC	-	-	-	-	-	-	+	+	-	-
AE	+	+	+	+	+	+	+	+	+	-
AW	+	+	+	+	+	+	+	+	+	-

Present; - Absent; AH = Hexane extract; AEA = Ethyl acetate–derived extract; AC = Chloroform isolate;
AE = Ethanol based extract; AW= water extract

In vitro antioxidant Activity by Nitric Oxide Radical Scavenging Assay

In vitro evaluation of the free radical–scavenging potential of *Saraca indica*bark isolates was performed using the nitric oxide (NO) radical scavenging assay. A clear concentration-dependent improvement in scavenging potential across all tested extracts (AH, AEA, AC, AE, and AW) (as presented in Table-4 and Fig.-1) was observed when compared with the standard antioxidant gallic acid. The strong NO scavenging activity supports the potential of *Saraca indica* as a natural antioxidant for managing oxidative stress-related conditions.

The standard Gallic acid demonstrated the highest NO scavenging potential, while the percentage inhibition was increased from 30.99% at 25 µg/mL to 96.59% at 800 µg/mL and a substantially lower IC_{50} value of 18.84 µg/mL, confirming the sensitivity and validity of the test. Among all tested plant extracts, the ethanol extract (AE) and ethyl acetate extract (AEA) demonstrated the highest in *vitro*neutralisation potential in comparison to the remaining extracts. The AE extract showed excellent concentration-dependent NO radical inhibition potential, increasing from 40.39% to 90.87%, with an IC_{50} of 51.68 µg/mL, suggesting potent antioxidant efficacy. Similarly, the AEA extract also showed concentration-dependent inhibition ranging from 36.98% to 90.72% with an IC_{50} of 63.67 µg/ml. The chloroform (AC) and aqueous (AW) extracts demonstrated moderate neutralisation potential, reflected by IC_{50} values of 302.32 µg/mL and 279.78 µg/mL, respectively.

Table-4: Comparative in Vitro NO Radical Neutralisation Activity

Conc.(µg/ml)	Gallic acid	AH	AEA	AC	AE	AW
25	30.99	34.16	36.98	34.96	40.39	32.81
50	47.11	36.57	42.30	38.42	48.19	37.28
100	64.94	39.30	54.89	40.80	57.62	40.97
200	82.63	43.00	73.47	47.14	72.64	46.87
400	90.86	46.70	81.66	58.76	82.45	53.39
800	96.59	48.81	90.72	69.42	90.87	58.14
IC 50	18.84	>800	63.67	302.32	51.68	279.78

The hexane extract (AH) showed the weakest antioxidant activity, failing to reach 50% inhibition even at the uppermost concentration tested (IC_{50}> 800 µg/mL). Qualitative phytochemical screening revealed that the ethanol and ethyl acetate extracts' higher concentration of phenolic substances, flavonoids, and tannins may be responsible for their improved antioxidant efficacy. Overall, the findings indicate that *Saraca indica* bark has strong nitric oxide radical scavenging activity, especially in polar and semi-polar extracts, supporting its potential to act as a naturally derived source of antioxidants pertinent to the treatment of inflammatory conditions linked to oxidative stress.

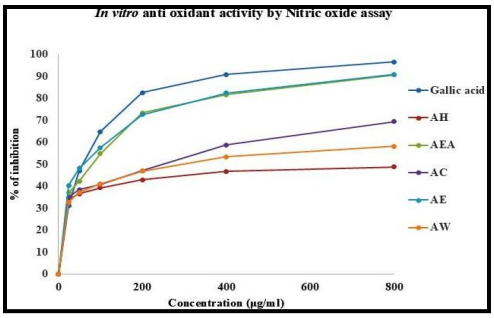

Fig.-1: Comparative in vitro NO Radical Neutralisation Activity

Suppression of Nitric Oxide (NO) Synthesis (In vitro Anti-inflammatory potential)

An ethanol-based extract (AE) of *Saraca indica* bark was examined for its potential to attenuate nitric oxide (NO) synthesis in RAW 264.7 macrophage cells stimulated with lipopolysaccharide (LPS). The suppression of NO generation was found to be concentration-dependent. The comparative NO production inhibition is presented in Table-5 and Fig.-2.

Table-5: Comparative Reduction of NO Production

Sample	% NO production
LPS control	100
AE (25 µg/ml)	85.345003
AE (50 µg/ml)	62.955424
Dexamethasone (5 µg/ml)	14.9196

The LPS control group demonstrated 100% NO production, indicating effective induction of the inflammatory response. The effect of AE at 25 µg/mL showed a significant reduction in NO production to 85.35%, demonstrating a modest but measurable inhibitory effect. Furthermore, the effect of AE at 50 µg/mL resulted in a tremendous reduction of NO levels to 62.96%, showing enhanced anti-inflammatory activity with increasing dose. In comparison, dexamethasone (5 µg/mL), which is the standard anti-inflammatory drug, significantly suppressed NO production to 14.92%, demonstrating the responsiveness of the assay. The findings suggested that ethanol-derived extract possessed marked anti-inflammatory potential, though its inhibitory effect was lower than that of standard dexamethasone. Phenolic compounds, flavonoids, and tannins found in the phytochemical screening may be responsible for this action since they are known to downregulate inducible nitric oxide synthase (iNOS) and reduce the generation of inflammatory mediators.[25] Overall, the findings point to *Saraca indica* bark ethanol extract's potential as a natural anti-inflammatory drug that can reduce NO-mediated inflammatory reactions. The

reduction in NO production suggests potential modulation of inflammatory pathways, supporting its use as a safer natural anti-inflammatory agent.

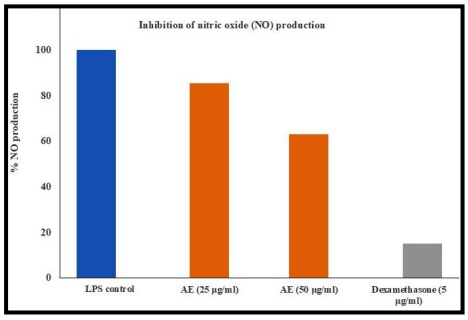

Fig.-2: Comparative Inhibition of NO Production

MTT Assay

The cytotoxicity of the AE was assessed using an MTT-based assay employing RAW 264.7 macrophage cells. It has been observed that AE showed very low cytotoxicity across the concentrations ranging from 6.25–100 µg/ml. As presented in Table-6 and Fig.-3, 100% cell viability was observed in the control group, demonstrating normal cell growth. The treatment with AE showed a dose associated with a gradual reduction in the viability of cells. At lower concentrations, loss in cell viability was found to be minimal. At concentrations 6.25 and 12.5 µg/mL, the viability of cell was remained above 95%, while at 50 µg/mL it was found to be above 90%, indicating excellent cellular tolerance. At the highest concentration of 100 µg/mL, the viability of the cells was noted to be nearly 75.9%, which was well above the cytotoxic threshold generally considered significant (<70%). Furthermore, IC_{50} of AE was found to be more than 100 µg/mL, demonstrating a nontoxic nature towards RAW 264.7 cells within the tested range. The microscopic observation was also further supported by the quantitative findings. No marked morphological changes or extensive cell deaths were observed at lower and intermediate concentrations, as can be seen from Fig.-3. The modest cell density reduction was observed at 100 µg/ml. Overall, the MTT assay confirms that AE is relatively safe for RAW 264.7 macrophages, validating its suitability for subsequent in vitro anti-inflammatory and antioxidant evaluations without confounding effects from cytotoxicity. Low cytotoxicity indicates a favorable safety profile, supporting further pharmacological and in vivo investigations.

Table-6: Summary of MTT Assay Results

Sample	Concentration	% Viability (Mean)	SD	IC50 (µg/ml)
Control	0	100	0	
AE	6.25	98.011	0.34	>100
	12.5	96.687	1.09	
	25	94.974	1.69	
	50	90.561	1.05	
	100	75.891	0.26	

Overall, the study establishes a clear link between the extraction method, phytochemical content, and the biological activity of *Saraca indica.* The findings highlight its potential as a safe natural antioxidant and anti-inflammatory agent. This work aligns with SDG 3 (Good Health and Well-being) by supporting the development of affordable plant-based therapeutics.

CONCLUSION

The current research work shows that *Saraca indica* bark is rich in various pharmacologically potent bioactives, especially in polar and moderately polar solvents. Ethanol was found to be the most effective solvent for the extraction of a wide spectrum of phytoconstituents, which showed superior antioxidant and anti-inflammatory activities. Moreover, the ethanolic extract demonstrated potent NO radical scavenging activity and significant NO inhibition production in activated macrophages, while retaining lower cytotoxicity toward RAW 264.7 cells.

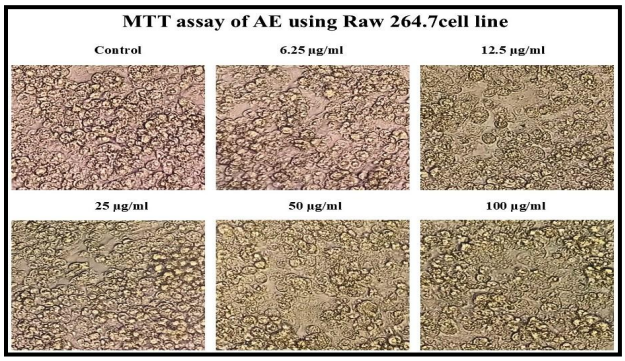

Fig.-3: MTT Assay of AE on Raw 264.7 Cell Line

The present findings clearly confirmed the safety and efficacy of the ethanolic extract for *in vitro* biological applications. Collectively, the present research work demonstrates that *Saraca indica* bark possesses inherent antioxidant and anti-inflammatory agents with clear scientific justification for its conventional medicinal application. Further studies focusing on bioactive compound isolation and in vivo validation are warranted. Overall, the study establishes a clear relationship between solvent extraction, phytochemical composition, and biological activity, providing a concise scientific basis for its pharmacological potential. In alignment with Sustainable Development Goal 3 (Good Health and Well-being), these findings support the development of safe, affordable, and plant-based therapeutic alternatives for managing oxidative stress and inflammatory disorders. Further studies focusing on bioactive compound isolation, mechanistic insights, and in vivo validation are recommended to advance its clinical applicability.

ACKNOWLEDGMENTS

The authors want to thank Vels Institute of Science, Technologyand Advanced Studies, Pallavaram, Chennai, Tamil Nadu.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-9: Industry, Innovation, and Infrastructure*. All the authors contributed significantly to this manuscript, participated in reviewing, editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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Cite this article as: Pooja. B. Kale and J. Swaminathan, *Rasayan J. Chem.*, 19(3), 1692-1701(2026), https://doi.org/10.31788/RJC.2026.1939808

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[RJC-9808/2026]