Phytochemical screening, antioxidant and anticancer properties of *Saraca asoca* (Roxb.) Willd

Sija S.L.*, Reshmi S. and Athira T.S.
Department of Biotechnology, Sree Narayana College, Kollam-691 001, Kerala, INDIA
*sijasl007@gmail.com

Abstract

The current research investigates the phytochemical composition, antioxidant capabilities and anticancer effects of methanol extracts from different parts (flowers and leaves) of Saraca asoca. Phytochemical screening indicated the presence of carbohydrates, alkaloids, saponins, resins, phenols, steroids, glycosides and flavonoids in the methanol extracts. Quantitative analysis revealed higher levels of alkaloids and total phenols in the methanol extracts from the leaves compared to those from the flowers. The extracts from Saraca asoca leaves exhibited stronger reducing power activity than those from flowers, as measured spectrophotometrically using quercetin (10mg/mL DMSO) as a standard compound. Both extracts showed a dose-dependent decrease in cell viability when tested against the human ovarian cancer cell line SKOV-3.

This study underscores Saraca asoca's richness in phytochemicals, highlighting its potential as a natural source of antioxidants and its promising anticancer properties. These findings support its traditional use in treating various human ailments, suggesting potential for future isolation and medical application of its phytochemicals.

Keywords: *Saraca asoca*, antibacterial activity, antioxidant activity, anticancer activity.

Introduction

Medicinal plants are invaluable resources, providing compounds that are not only used directly in therapies but also serve as crucial ingredients for developing effective drugs. They are often employed alongside conventional treatments, catering to diverse ailments due to their affordability and widespread availability. One such example is the Ashoka tree (*Saraca asoca* or *Saraca indica*), a venerable member of the Caesalpinaceae family in India. The name 'ashoka' symbolically refers to its bark's reputation for preserving women's health and youthfulness, translating to "without sorrow."

The stem bark of this tree is rich in various chemicals like glycosides, flavonoids, tannins, saponins, alkanes, esters and primary alcohols which are integral to traditional medicine practices separate from Ayurveda in India[14]. Ashoka holds cultural and religious significance in Buddhism and Jainism, recognized as a sacred tree in both traditions. Its applications in traditional medicine are extensive, particularly in treating women's health issues such as menorrhagia, leucorrhoea, hemorrhoids and uterine bleeding disorders[7]. Globally, the market for herbal medicines has seen substantial growth, with its value expected to increase from USD 165.66 billion in 2022 to USD 347.50 billion by 2029[9]. Ashoka is widely distributed across India, thriving notably in regions like the Himalayas, Kerala, Bengal and southern India, where its medicinal properties continue to be utilized and valued.

TAXONOMIC POSITION

Kingdom	-	Plantae
Division	-	Magnoliophyte
Class	-	Magnoliopsida
Order	-	Fabales
Family	-	Caesalpinaceae
Genus	-	Saraca

Indian research groups frequently investigate the diverse applications of ethanobotanicals, focusing particularly on the pharmacological properties of *S.asoca*. These properties include its roles as an oxytocin, uterine tonic, antidiabetic[16] and antimenstrual agent. *S.asoca*'s dried bark, roots and flowers are traditionally used to manage various uterine disorders such as menorrhagia, ammenorhea, endometriosis and menstrual irregularities[13]. Numerous studies have reported on the phytochemical composition of *S.asoca* extracts from different parts of the plant, highlighting the bark as the most significant component followed by the flowers.

Given their established anticancer properties, these plant extracts and phytochemicals hold potential for both preventive and therapeutic approaches in cancer chemoprevention[4]. Therefore, this study aims to explore the phytochemical screening, antioxidant capacity and anticancer potential of *S.asoca*, aiming to validate its traditional medicinal uses.

Material and Methods

Source of plant materials: Fresh leaves and flowers of *S.asoca* were gathered from Umayanallur in the Kollam district of Kerala, India. These plant materials were then transported to the laboratory.

Preparation of plant extracts: The dried plant material was ground and used to prepare extracts. The powdered samples were soaked in methanol and chloroform using the maceration method at a ratio of 5 grams of plant material per 100 milliliters of solvent. After 24 hours at room temperature, the homogenized samples were centrifuged at

10,000 rpm for 15 minutes and the resulting supernatants were combined. These extracts were then filtered through Whatmann no. 1 filter papers and each extract was concentrated using a rotary evaporator to remove methanol. The residue obtained was dissolved in methanol and chloroform and stored at 4-8ºC in a refrigerator for subsequent analysis[8].

Preliminary phytochemical studies: The initial investigation of crude methanolic and chloroform extracts from leaves and flowers involved standard color reaction tests to analyze phytochemical content. The extracts were evaluated for alkaloids, tannins, saponins, terpenoids, steroids, phenolics, quinine, starch, carbohydrates, reducing sugars and flavonoids using established methods with slight adjustments[15].

Test for Alkaloid: 1ml sample of extract was taken in a test tube and mixed with few drops of Dragendroff reagent. The sample reacts with the reagent and produces an orange or orange red coloration or precipitate indicating the presence of alkaloids.

Test for Flavonoids: Few ml of extract is added to concentrated sulphuric acid. The presence of flavonoids can be identified by the development of orange colour.

Test for glycosides: 1ml of extract mixed with 1ml of glacial acetic acid was treated with 0.05ml of 5% ferric chloride solution and few drops of concentrated sulphuric acid. Formation of a dark blue green colour indicates the presence of glycosides.

Test for phenolic compounds: The formation of intense blue color in the extract, on adding 2ml of distilled water and 1ml of 10% ferric chloride solution to the extract is considered as a positive reaction test.

Test for quinones: Adding 1 ml of concentrated sulfuric acid to 1 ml of the extract and thorough mixing reveals the presence of quinones when a red color appears.

Saponins test: Foaming occurred when 1 ml of extract was mixed with 1 ml of distilled water and shaken in a test tube for 15 minutes, indicating the presence of saponins.

Test for starch: When 1 ml of extract was combined with 5 ml of 5% KOH solution, the appearance of a Cinary coloration indicates the presence of starch.

Test for steroids: When 1 ml of extract was mixed with 1 ml of chloroform, 1 ml of glacial acetic acid and a few drops of concentrated sulfuric acid, the development of a green color indicates the presence of steroids.

Test for Tannins: Mix 1 ml of the extract with a few drops of 10% ferric chloride solution. If a dark green color develops, it indicates the presence of tannins.

Test for terpenoids: For the Leibermann's test to detect terpenoids, mix 1 ml of the extract with 1 ml of glacial acetic acid. Then, carefully add concentrated sulphuric acid alongside. The formation of a red coloration suggests the presence of terpenoids.

Quantitative estimation of alkaloids: The alkaloid content was determined using a spectrophotometric method involving Dragendorff's reagent[17]. Initially, 1 mg of each extract was precisely weighed and diluted to 1 ml with DMSO. Subsequently, 0.5 ml of the sample was mixed with 1 ml of 0.1 N HCl followed by the addition of 0.25 ml of Dragendorff's reagent to induce precipitation.

The resulting precipitate was separated by centrifugation at 3000 rpm for 5 minutes and washed with 0.25 ml of ethanol. After discarding the supernatant, the residue was treated with 0.25 ml of 1% w/v disodium solution, yielding a brownish black precipitate upon further centrifugation. The precipitate was dissolved in 0.2 ml of concentrated nitric acid. Then, 0.1 ml of this solution was taken and combined with 0.5 ml of a 3% w/v thiourea solution.

The absorbance of the resultant solution was measured at 435 nm using a UV-Visible spectrophotometer (Agilent, Cary 60) against a blank solution containing 0.1 ml of concentrated nitric acid and 0.25 ml of 3% w/v thiourea solution. The obtained absorbance values were then interpreted using a standard caffeine graph to determine the milligram equivalents of caffeine present in the samples.

Estimation of total phenolic content: Phenolic content was assessed using the spectrophotometric Folin-Ciocalteu method. Each extract (1 mg) was dissolved in 1 ml of DMSO. To 0.2 ml of the sample, 0.5 ml of Folin-Ciocalteu reagent was added and left to incubate for 5 minutes. Subsequently, 5 ml of 7.5% sodium carbonate solution was added and the mixture was then incubated at room temperature for 40 minutes. Absorbance was measured at 750 nm using a UV-VIS spectrophotometer (Shimadzu, UV-1900i). Results were compared with a standard graph of gallic acid to determine milligram equivalents of gallic acid.

Determination of reducing power activity: The reducing power of the extract was evaluated by preparing different concentrations ranging from 125 µg/mL to 2000 µg/mL derived from a stock solution of 10 mg/mL. Each sample was mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1% potassium ferric cyanide solution followed by boiling at 50°C for 20 minutes. A control sample containing only distilled water was also prepared. After incubation, 2.5 mL of 10% trichloroacetic acid (TCA) was added to each mixture and then centrifuged at 650g for 10 minutes. The upper layer (5 mL) was combined with 5 mL of distilled water and 1 mL of 0.1% ferric chloride solution. Finally, the absorbance was measured at 700 nm using quercetin (10 mg/mL in DMSO) as a standard reference.

***In vitro* anticancer effect determination by MTT assay:** SKOV-3, a human ovarian cancer cell line, was originally obtained from the National Centre for Cell Sciences (NCCS) in Pune, India. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) from Sigma Aldrich (USA) in 25 cm^2 tissue culture flasks. The culture medium was supplemented with 10% fetal bovine serum (FBS), L-glutamine, sodium bicarbonate (Merck, Germany) and an antibiotic solution containing penicillin (100 U/ml), streptomycin (100 µg/ml) and amphoteracin B (2.5 µg/ml). Cell cultures were incubated at 37ºC in a humidified atmosphere containing 5% CO_2 using an NBS Eppendorf incubator from Germany. Cell viability was assessed by direct observation under an inverted phase contrast microscope and confirmed using the MTT assay method.

Cells seeding in 96 well plate: Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^3$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

Preparation of compound stock: 1mg of sample was weighed and dissolved in 1ml 0.1% DMSO using a cyclomixer. The sample solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

Anticancer Evaluation: After 24 hours, the growth medium was removed, DMEM was five times serially diluted by two-fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 500µl of DMEM) and concentration of 100µl was added in triplicate to the respective wells and incubated at 37ºC in humidified 5% CO_2 incubator. Non treated control cells were also maintained.

Anticancer Assay by Direct Microscopic observation: Entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observations were recorded as images. Detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Anticancer Assay by MTT Method: 15 mg of MTT (Sigma, M-5655) was dissolved in 3 ml of PBS until completely dissolved and then sterilized by filter sterilization. After a 24-hour incubation period, the contents of the wells were aspirated and replaced with 30 µl of the reconstituted MTT solution. The plate was gently shaken and incubated at 37ºC in a humidified 5% CO_2 environment for 4 hours. Following the incubation, the supernatant was removed and 100 µl of MTT solubilization solution (Dimethyl sulphoxide, DMSO, Sigma Aldrich, USA) was added to each well. The wells were gently mixed by pipetting to solubilize the formazan crystals. Absorbance values were measured using a microplate reader at a wavelength of 540 nm. The percentage of growth inhibition was calculated using the following formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples x 100}}{\text{Mean OD of control group}}$$

IC 50: Half-maximal inhibitory concentration (IC50) is the most widely used and informative measure of a drug's efficacy. It indicates how much drug is needed to inhibit a biological process by half, thus providing a measure of potency of an antagonist drug. The IC 50 values were calculated using the equation for slope (y= mx + C) obtained by plotting the average absorbance of the different concentrations of the test sample (6.25 µg, 12.5 µg, 25 µg, 50 µg and 100 µg) in Microsoft Excel.

Data analysis: The data consisted of means and standard deviations (SD) from three repeated measurements. Statistical analysis was conducted using SPSS software (version 17, 2012, SPSS Inc., Chicago, IL). Treatment means were compared using One-way Analysis of Variance (ANOVA) and Duncan's new multiple range test was applied at a significance level of 0.05 ($p < 0.05$).

Results and Discussion

Preliminary qualitative analysis of phytochemicals: Recent studies on *S.asoca* have identified active plant compounds with bioactive properties. Qualitative analysis of phytoconstituents was performed separately on different parts of the plant (flowers and leaves). Methanol and chloroform extracts were obtained from these plant parts using the maceration method (Fig.1). The dried powders of flowers and leaves of *S.asoca* were extracted with methanol and chloroform to obtain crude extracts. The initial analysis of extracts from the flowers and leaves of *S.asoca* indicated the presence of diverse bioactive secondary compounds such as saponins, carbohydrates, flavonoids, alkaloids, phenols, cardiac glycosides, tannins, starch and steroids, as summarized in table 1.

The results of the phytochemical analysis of *S.asoca* leaves revealed that the methanolic extract contained saponins, carbohydrates, flavonoids, alkaloids, phenols, cardiac glycosides, tannins, starch and steroids, indicating significant phytochemical activity. In contrast, the chloroform extract showed lower levels of phytochemicals.

Similarly, the phytochemical screening of *S.asoca* flower extracts showed that the methanolic extract had moderate levels of saponins, carbohydrates, terpenoids, flavonoids, alkaloids, phenols, quinones, tannins and reducing sugars with starch, steroids and cardiac glycosides absent.

The chloroform extract of the flowers exhibited moderate levels of saponins, carbohydrates, terpenoids, flavonoids, alkaloids, phenols, quinones and tannins (Fig. 2). These findings align with previous studies on the same plant, confirming its medicinal potential due to the diverse secondary metabolites known for their antioxidant and antimicrobial properties[2,12].

Fig. 1: Various plant parts of *S.asoca* and its extraction by maceration method
F-Flower and L-Leaf

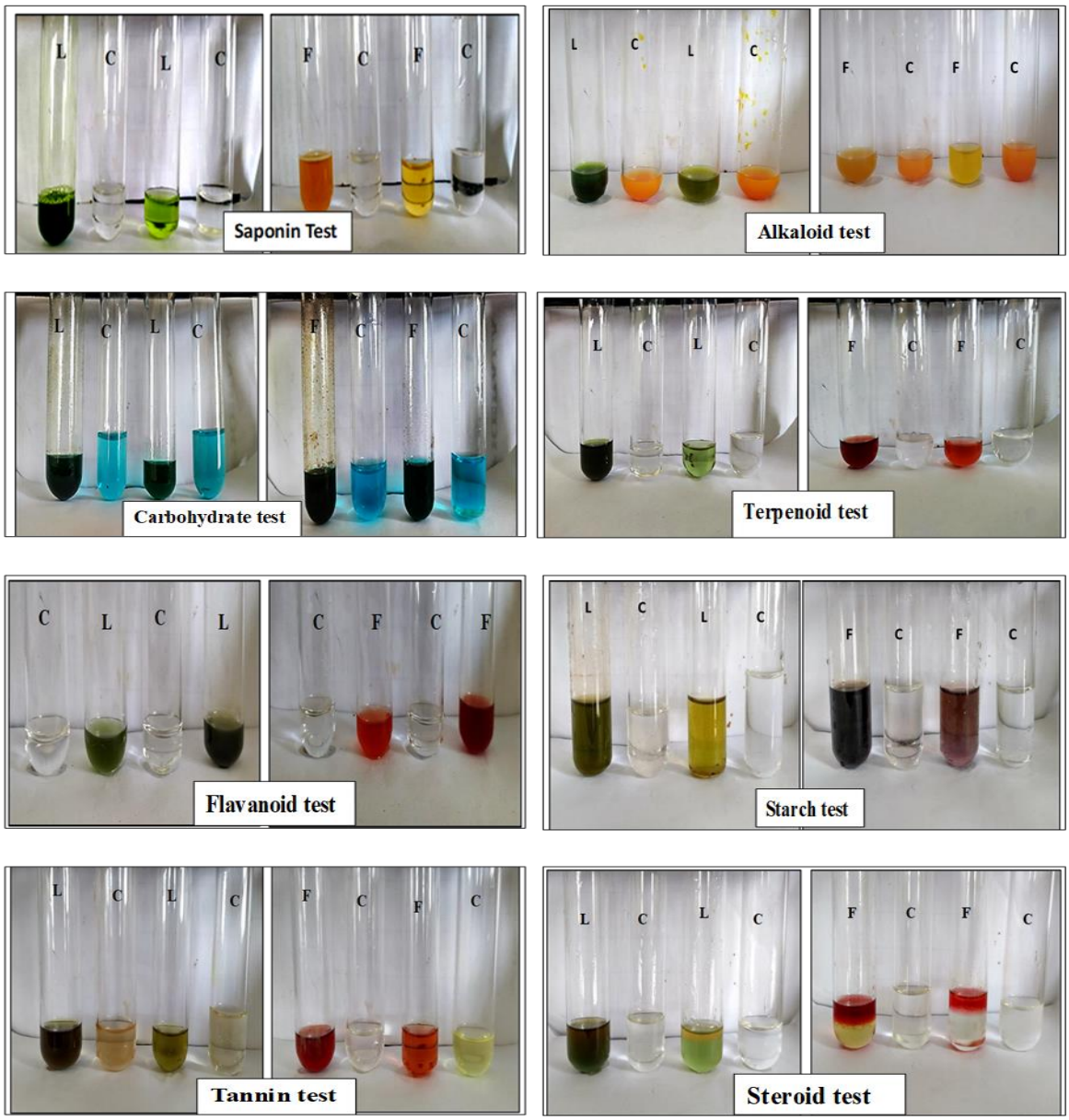

Fig. 2: Phytochemical tests in methanol and chloroform extract of *S.asoca*
L- leaf, F- flower and C- control

Quantitative estimation of alkaloids: In the current study, the alkaloid content of flower and leaf extracts from *S.asoca* was investigated. The total alkaloid content was quantified using a method based on a calibration curve, where the equation Y = 0.0005X - 0.0371 was employed with an R^2 value of 0.9803. Here, X represents the caffeine equivalent in mg/100mg and Y denotes the absorbance, as illustrated in figure 3. The results indicated that the total alkaloid content

was higher in the leaf extracts (0.506 µg/mg) compared to the flower extracts (0.4104 µg/mg), as presented in table 2. This trend was consistent with findings from other studies on various extracts of *S.asoca*[1,13].

Quantitative estimation of total phenol: In this study, extracts from the flowers and leaves of *S.asoca* were analyzed for their phenolic content using the Folin-Ciocalteu method, expressed as gallic acid equivalents based on a standard curve. The calibration curve, which exhibited linearity for gallic acid spanning from 100 to 1000 µg/ml and a correlation coefficient (R2) of 0.9807 (Fig. 4), was utilized for the quantification of total phenolic compounds. The total phenolic content was higher in the leaves (0.555 mg/g) compared to the flowers (0.487 mg/g) (Table 3).

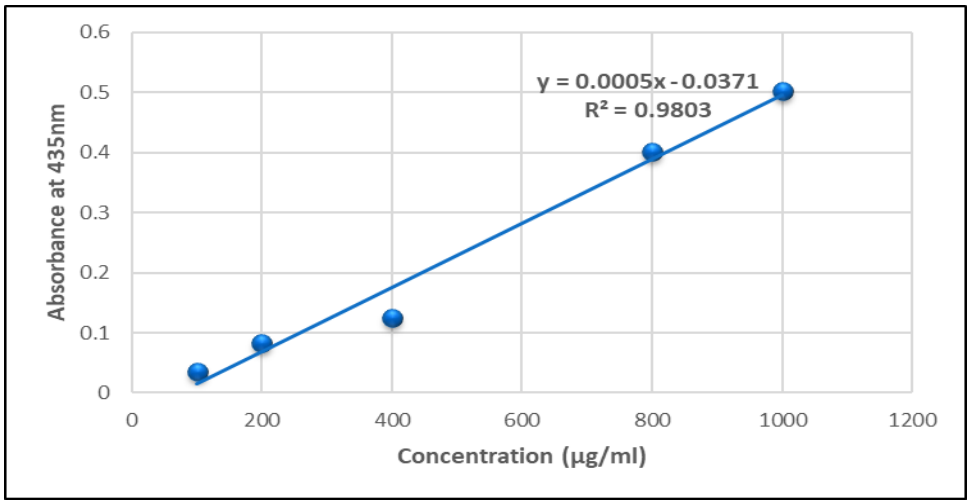

Fig. 3: Standard calibration curve of caffeine

Table 1
Preliminary phytochemical screening of various extracts of leaves and flower samples of *S.asoca*

Phytochemical Test	Methanol Extract		Chloroform Extract	
	Leaf	Flower	Leaf	Flower
Saponin	+++	++	++	+
Carbohydrate	+	++	+	++
Terpenoid	-	+++	-	+++
Flavonoid	+	++	+	++
Alkaloid	+	+++	+	+++
Phenol	+++	+++	+	++
Quinone	-	+++	-	+++
Cardiac Glycoside	+++	-	++	-
Tannin	+++	+++	+++	+++
Starch	+	-	+++	-
Steroid	+++	-	+++	-

(+++) = present in high levels; (++) =present in medium level;(+) = present in low levels; (-) =Absence

Table 2
Total alkaloid content of leaf and flower extract of *S.asoca*

Sample code	Absorbance	From the graph Amountof alkaloid in terms of caffeine units (mg)	Amount of alkaloid in per mg of sample
Leaf	0.0894	0.253	0.506
Flower	0.0655	0.2052	0.4104

Table 3
Total phenolic content of leaf and flower extract of *S.asoca*

Sample code	Absorbance	Amount of phenol in terms of gallic acid units (mg)	Amount of phenol in 1mg
Leaf	0.169	0.1110	0.555
Flower	0.154	0.0973	0.487

This study further confirmed the presence of phenolic compounds in both leaves and flowers of *S.asoca*. Previous research has also noted similar findings regarding the prevalence and concentration of phenolic compounds in *S.asoca* leaf extracts[1,13]. Phenolic compounds are significant secondary metabolites in plant tissues, known to vary in concentration across different stages of plant development [3].

Detection of reducing power activity: In this study, the research investigated the reducing power activity of extracts from both flowers and leaves of *S.asoca*. Quercetin (10mg/mL DMSO) served as the standard and absorbance was measured at 700nm. The leaf extracts of *S.asoca* exhibited the highest absorbance (0.9475) at a concentration of 2000 µg/ml while the flower extracts showed an absorbance of 0.1910 (Fig. 5 and Fig. 6). This study confirms that both flower and leaf extracts of *S.asoca* possess significant reducing power activity. Previous research on this medicinal plant has also supported these findings [6,19]. Antioxidants are known to neutralize free radicals, which are implicated in the development of various diseases and the aging process.

Determination of anticancer activity: To assess the anticancer potential of methanolic extracts from *S.asoca* flowers and leaves against the SKOV-3 human ovarian cancer cell line, different concentrations (6.25 µg, 12.5 µg, 25 µg, 50 µg and 100 µg) of each extract were used for 24-hour incubations. Cell viability was measured using the MTT assay, revealing a dose-dependent cytotoxic effect for both extracts. Microscopic examination (Figures 7, 8 and 9) showed a significant decrease in cell viability with increasing extract concentration. The flower extract's anticancer activity was further illustrated in a graphical representation (Fig. 10) while the IC50 values were calculated (Fig. 11) as 86.925 µg/ml for the flower extract and 66.775 µg/ml for the leaf extract.

Cell viability was assessed using the MTT assay. Ovarian cancer ranks as the seventh most common malignancy affecting women, posing a significant threat to reproductive health[18]. The use of natural phytochemicals for cancer chemoprevention is emerging as a promising strategy to prevent, delay, or treat malignant diseases[4,10,19].

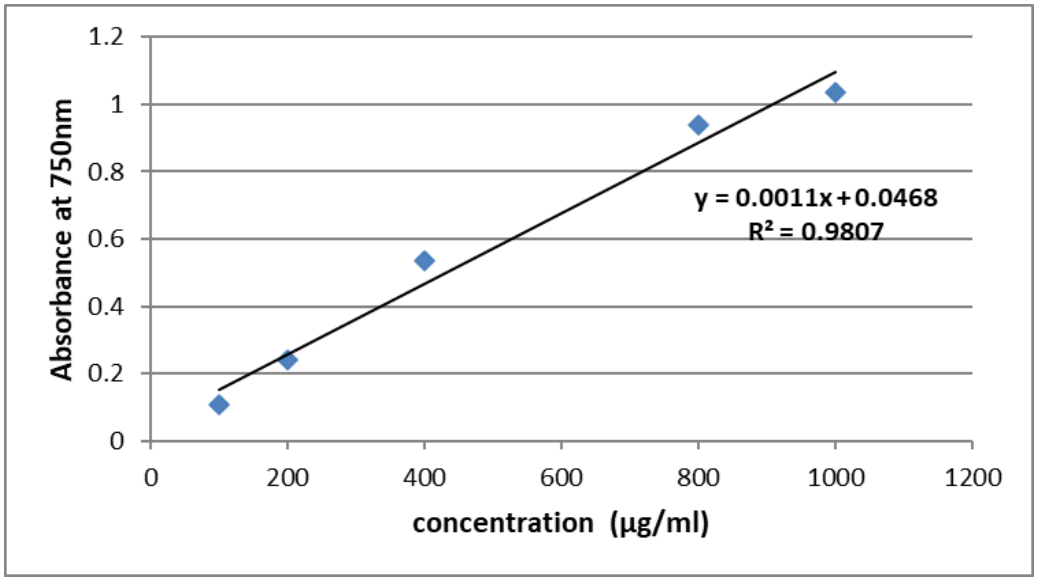

Fig. 4: Standard calibration curve of gallic acid

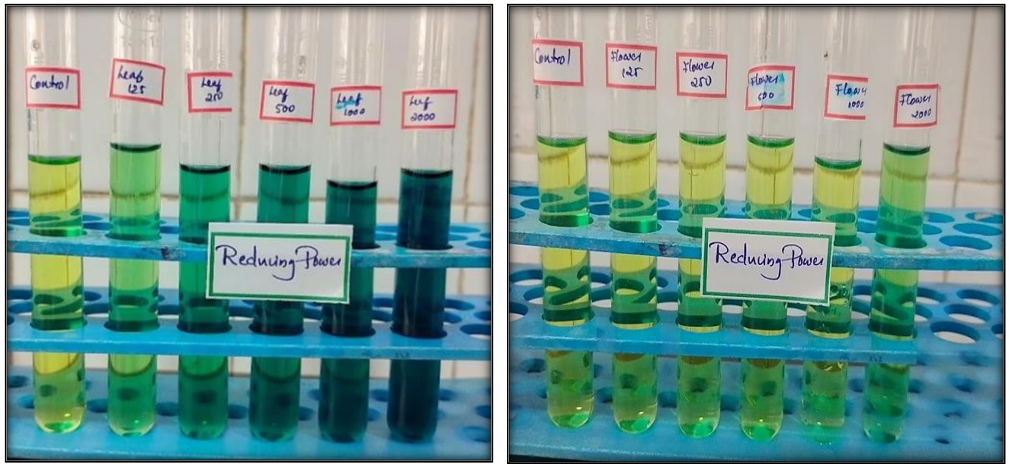

Fig. 5: Colour change of reagent by leaf and flower sample at various concentrations

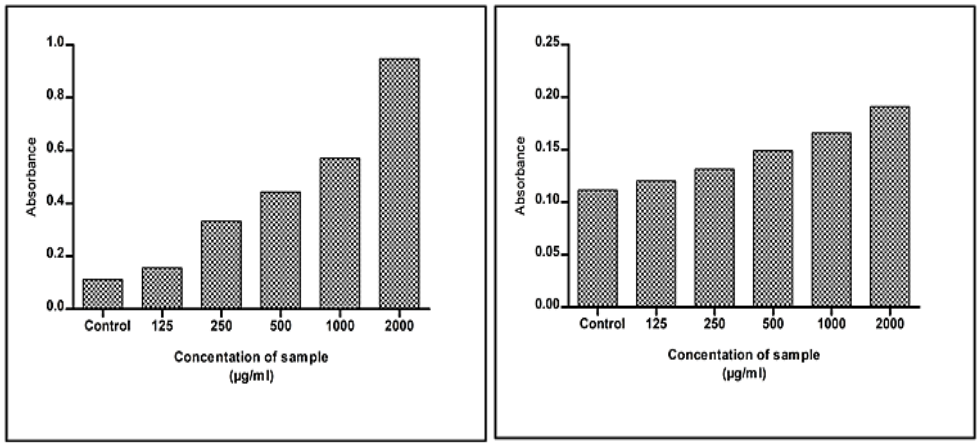

Fig. 6: Graph showing the absorbance of leaf and flower sample at different concentrations

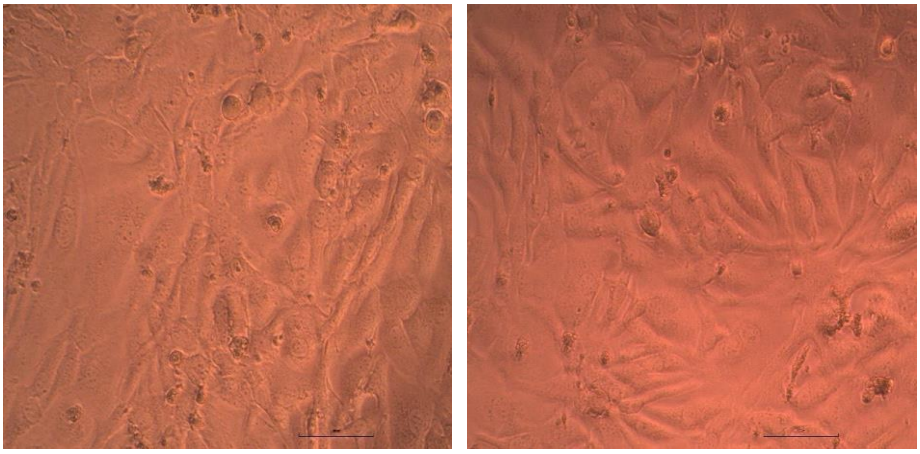

Fig. 7: Microscopic image of cell line (SKOV-3) in control 1 and control 2 after incubation.

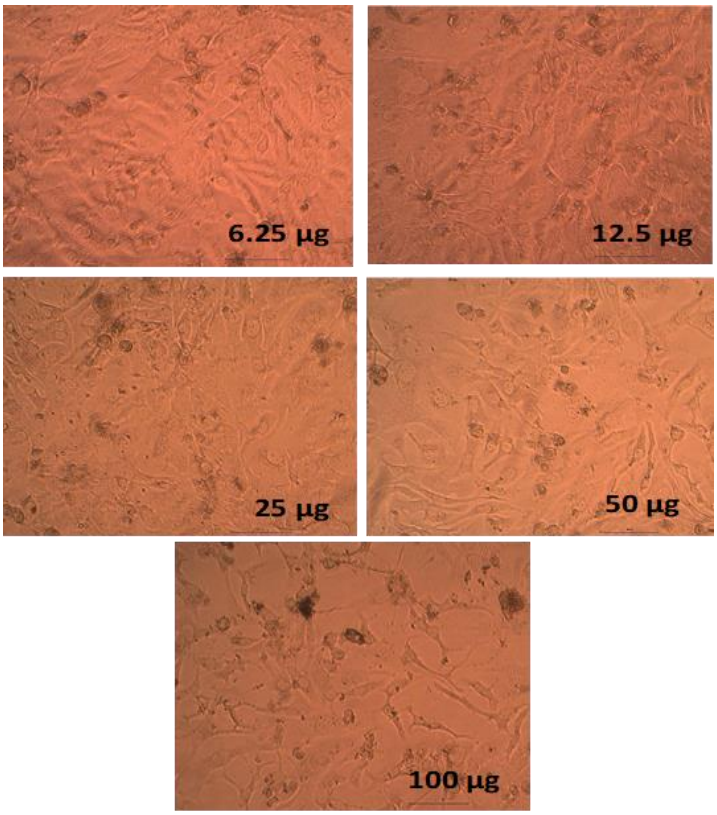

Fig. 8: Microscopic image of cell line treated with different concentration of *S.asoca* leaf extract

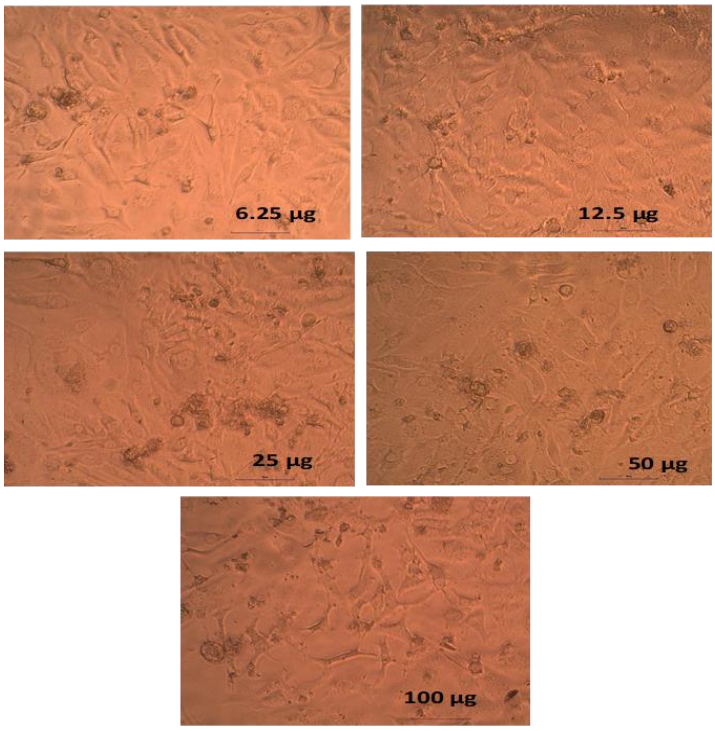

Fig. 9: Microscopic image of cell line treated with different concentration of *S.asoca* flower extract

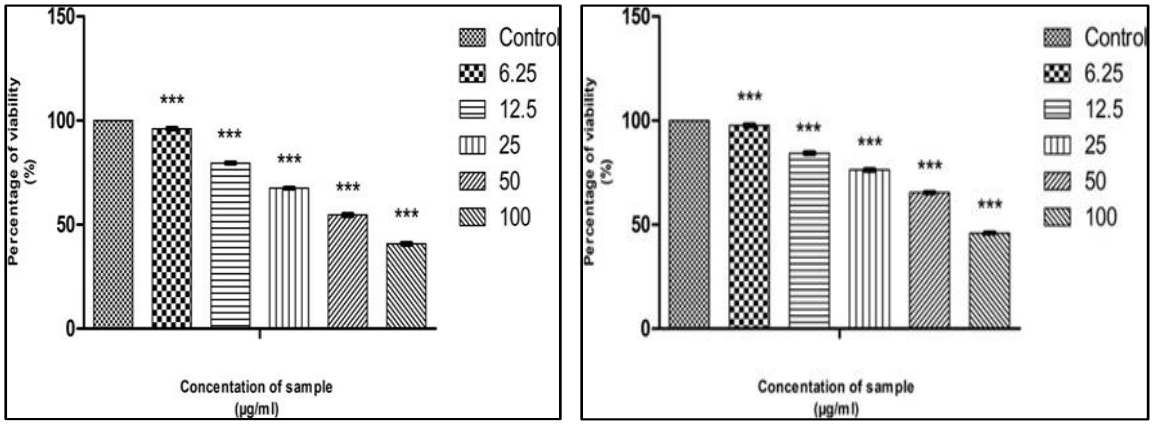

Fig. 10: Graphical representation depicting the anticancer effect of leaf and flower by MTT assay- along Y axis percentage viability, along X axis varied concentration of leaf and flower.

All experiments were done in triplicate and results represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyse data. ***p< 0.001 compared to control group.

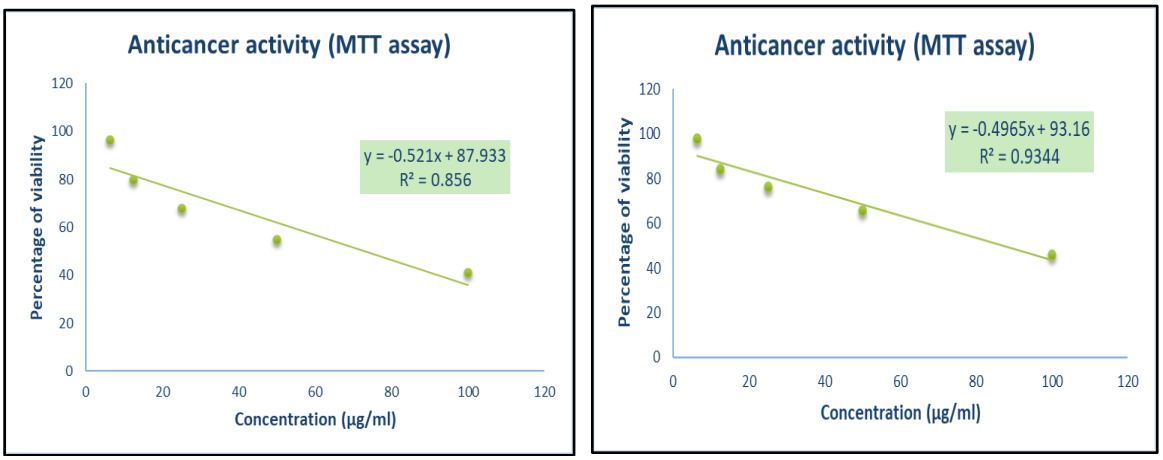

Fig. 11: Determination of IC50 value of *S.asoca* leaf and flower

The current research emphasized the presence of key phytochemicals in methanol extracts from the leaves and flowers of *S.asoca*. These extracts contain significant quantities of secondary metabolites such as phenols, steroids and oils. The study also demonstrated that both leaf and flower extracts exhibit strong antioxidant and anticancer properties, likely due to the presence of these secondary metabolites.

Conclusion

The *S.asoca* tree holds significant reverence in ancient Indian texts as a sacred plant and has been traditionally utilized in Ayurveda for managing gynecological disorders. It possesses various beneficial properties including antibacterial, uterotonic, anticancer, anthelmintic, antioxidant, hypolipidemic and antiulcer effects. The study conducted here analyzed leaf and flower extracts of *S.asoca* in chloroform and methanol, revealing rich phytochemical content. Preliminary analysis identified compounds such as saponins, flavonoids, terpenoids, alkaloids, tannins, phenols and reducing sugars, with higher concentrations of alkaloids and total phenols found in the leaves.

Spectrophotometric assessment demonstrated superior reducing power activity in the leaf extract compared to the flower extract. Anticancer evaluations against SKOV-3 (human ovarian cancer) cells showed dose-dependent reductions in cell viability with both extracts, indicating potential therapeutic applications. Further comprehensive clinical research is necessary to fully explore and validate the therapeutic benefits of *S.asoca* for potential standardization in medicinal use.

Acknowledgement

The authors express gratitude to the Department of Biotechnology at Sree Narayana College, located in Kollam-691 001, Kerala, India, for generously providing the laboratory facilities essential for conducting this research. Additionally, we appreciate the Research Division at BRMAS, Center for Research on Molecular Biology and Applied Science, for their support in facilitating the anticancer research.

References

1. Arindam G., Sidhesh N. and Ambrish V., Evaluation of antioxidant activity, total phenolic content, total flavonoids and LC-MS characterization of *Saraca asoca* (Roxb.) De.Wilde, *International Journal of Advanced Research* Volume, **3(5)**, 318-327 **(2015)**

2. Athiralakshmy T.R., Divyamol A.S. and Nisha P., Phytochemical screening of *Saraca asoca* and antimicrobial activity against bacterial species, *Asian Journal of Plant Science and Research*, **6(2)**, 30-36 **(2016)**

3. Gayathri P. and Jeyanthi G.P., Radical scavenging activity of *Saraca indica* bark extracts and its inhibitory effect on the enzymes of carbohydrate metabolism, *Int. J. Chemical. Pharm. Sci*., **4**, 87-96 **(2013)**

4. Hosseini A. and Ghorbani A., Cancer therapy with phytochemicals: evidence from clinical studies, *Avicenna J Phytomed*, **5(2)**, 84-97 **(2015)**

5. Jerard C., Michael B.P., Chenicheri S., Vijayakumar N. and Ramachandran R., Rosmarinic Acid-Rich Fraction from Menthaarvensis Synchronizes Bcl/Bax Expression and Induces Go/G1 Arrest in Hepatocarcinoma Cells, *Proc Natl Acad Sci, India, Sect B Biol Sci.*, **90(3)**, 515–22 **(2020)**

6. Kumar S., Narwal D., Kumar G., Singh S. and Narwal R., Evaluation of Antihyperglycemic and Antioxidant Activities of *S.asoca* (Roxb.) De Wild Leaves in Streptozotocin Induced Diabetic Mice, *Asian Pacific Journal of Tropical Disease*, **2(3)**, 170-76 **(2012)**

7. Maruthappan V. and Shree K.S., Antiulcer activity of aqueous suspension of *Saraca indica* flower against gastric ulcers in albino rats, *J. Pharmaceutical Res.*, **3(1)**, 17–20 **(2010)**

8. Muthusamy R., Karthiga V., Lavanya K., Deboral P. and Bernala W., Phytochemical screening and antibacterial activity of methanol extract of *Tridax procumbens*, *International Journal of Pharmacy and Biological Sciences*, **1(3)**, 521-524 **(2017)**

9. Myrna D.C., Rosa V.M. and Maria E.G., Editorial: Pharmacological interaction between drugs and medicinal plants, *Front. Pharmacol*., **13**, 1081090 **(2022)**

10. Newman D.J. and Cragg G.M., Natural products as sources of new drugs over the last 25 years, *J Nat Prod*, **70(3)**, 461-477 **(2007)**

11. Rasekar V. and Shahi S., Medical application of Ashok tree (*Saraca asoca*): A review, *International Journal of Health Sciences*, **6(S2)**, 8752–8759 **(2022)**

12. Sabita R. and Baidyanath K., Qualitative and Quantitative screening of Phytochemicals in polar and non polar solvent extracts of stem bark and leaves of *Saraca indica* (L.), IOSR *Journal of Biotechnology and Biochemistry*, **4(5)**, 18-29 **(2018)**

13. Satyavati G.V., Prasad D.N., Sen S.P. and Das P.K., Oxytocic activity of a pure phenolic glycoside (P2) from *Saraca indica* Linn (Ashoka): a short communication, *Indian J. Med. Res.*, **58**, 660-663 **(1970)**

14. Sasmal S., Majumdar M., Gupta A. and Mukherjee P., Pharmacognostical, phytochemical and pharmacological evaluation for the antipyretic effect of the seeds of *Saraca asoca* Roxb, *Asian Pac J Trop Biomed*, **2(10)**, 782-786 **(2012)**

15. Shaikh J.R. and Patil M.K., Qualitative tests for preliminary phytochemical screening, An overview, *Int J Chem Stud*, **8**, 603-608 **(2020)**

16. Somani G. and Sathaye S., Bioactive fraction of *Saraca indica* prevents diabetes induced cataractogenesis: An aldose reductase inhibitory activity, *Pharmacogn Mag.*, **11**, 102-10 **(2015)**

17. Sreevidya N. and Mehrotra S., Spectrophotometric Method for Estimation of Alkaloids Precipitable with Dragendorff's Reagent in Plant Materials, *Journal of AOAC International*, **86(6)**, 1124-7 **(2003)**

18. Webb P.M. and Jordan S.J., Epidemiology of epithelial ovarian cancer, *Best Pract. Res. Clin. Obstet. Gynaecol.*, **41**, 3-14 **(2017)**

19. Yadav N.K., Saini K.S., Hossain Z., Omer A., Sharma C. and Gayen J.R., *Saraca indica* bark extract shows in vitro antioxidant, anti breast cancer activity and does not exhibit toxicological effects, *Oxid Med Cell Longev*., **20(5)**, 360 **(2015)**

20. Yen G.C., Duh P.D. and Chuang D.Y., Antioxidant activity of anthraquinones and anthrone, *Food Chemistry*, **5**, 437-441 **(2000)**.

(Received 03rd May 2024, accepted 09th July 2024)