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Phytochemical screening of *Saraca asoca* (Ashoka tree)

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Abstract

Saraca asoca also known as Ashoka tree, belongs to the family Caesalpiniaceae, which is a wild native that has significant traditional value. A variety of ailments can be cured by such amazing herbs that have the qualities of Ayurveda medicines. *Saraca Indica* is a significant conventional Indian medicinal herb utilized in various rituals and diseases. The literature references the use of various plant parts, including leaves and resins, as medications to treat various inflammatory diseases. There are, however, few investigations on the phytochemical components and therapeutic qualities of these plants' bark skin. In this research, it's been tested and examined presence of phytochemicals in the Ashoka tree along with its therapeutic uses. Also considering with positive and negative outcomes of presence and absence of phytochemicals such as saponin, tannin, etc.

Keywords: *Saraca asoca*, phytochemical analysis, ayurvedic properties

Introduction

One of India's most revered and mythical trees is Asoka. The Asoka tree, also known as *Saraca Indica* or De. wild, is a member of the Caesalpiniaceae family, and is commonly recognized by its binomial Latin name *Saraca asoca* (Wani *et al.*, 2012) ^[22]. The tree is always green. Other names for it include Asokam (Malayalam), Ashok (Marathi), Ashoka (Oriya), Ashok (Punjabi), Asogam (Tamil), Ashoka Damara (Kannada), Ashok (Hindi), Ashoka (Assamese), Ashoka (Bengali), Ashoka (Hindi), Ashoka Damara (Kannada), Ashok (Kashmiri), and Kankeli (Sanskrit). One of the holiest plants in Hinduism, Ashoka is particularly revered by the Hindu God of Love, Kamadeva, in whose honor it is offered annually on December 27 (Pradhan *et al.*, 2009) ^[14]. In Hindu mythology, Ashoka is described as the Ashoka tree, under which the Indian philosopher and founder of Buddhism, Gautham Siddhartha (563-583 B.C.), is said to have lived (Biswas *et al.*, 1972) ^[4]. Although *Saraca Indica* leaf extract's phytochemical analysis and analgesic qualities have been assessed in earlier research (Verma *et al.*, 2010) ^[22]. The phytochemical analysis and analgesic qualities of the plant's bark, or Ashok Chhal, which is referenced in Ayurvedic literature (the Charak Samhita), have not yet been investigated (Mishra *et al.*, 2013) ^[12]. It can be found in India's evergreen woods up to an altitude of roughly 750 meters. It can be found all over India, particularly in Bengal, Kerala, the Himalayas, and the entire South. According to (Warrier *et al.* 2000) ^[23], it can be found in the Kerala region's Palakkad district, Thrisur, Kollam, and Kannur districts, as well as the Himalaya's Khasi, Garo, and Lushi hills (Wani *et al.*, 2012) ^[22]. *Saraca asoca* is a versatile herb that is mostly used in medicine to treat women's gynecological problems, vaginal dysuria, uterine inertia, uterine discomfort, and urinary calculus. Glycosides, flavonoids, tannins, and saponins are present in the *Saraca asoca* (Ashoka) plant (Pradhan *et al.*, 2009) ^[14]. It has antimicrobial, anti-implantation, anti-tumor, anti-progestational, anti-estrogenic, anti-menorrhagia, oxytocic, uterotonic, and anti-cancer properties. Dyspepsia, fever, burning feeling, colic, ulcer, menorrhagia, leucorrhoea, acne, and other conditions can benefit from the herb (Srivastava *et al.*, 1988) ^[15]. In India, menorrhagia has been treated using the dried bark of the *Saraca asoca* plant (Bhandary *et al.*, 1995) ^[10]. In India, ladies are administered dried bark and the *Saraca asoca* plant blooms as a tonic for uterine diseases. In cases of any problem related to the menstrual cycle, asoca stem bark is also employed (Middelkoop and Labadie, 1985) ^[11]. Ashoka is a blood purifier and is used to treat blood circulation and purification, cancer, diarrhea, amenorrhea, dysmenorrhea, menopause, menorrhagia, painful menstruation, and other skin conditions (Nadhkarni, 1994).

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The phytochemical analysis and analgesic properties of its bark skin, which are referenced in Ayurvedic literature (*Charak Samhita*), have, however, been the subject of few investigations. Given the aforementioned therapeutic significance, the current investigation was conducted to identify the phytochemical components in Ashoka and Shal. (Santapau, *et al.* 1998) ^[16].

Kingdom	Plantae
Division	<i>Magnoliophyta</i>
Class	<i>Magnoliopsida Order</i>
Family	<i>Caesalpiniaceae</i>
Genus	<i>Saraca</i>
Species	<i>asoca</i>

Materials and Methods

Collection of plant material

The leaves of Ashoka received from Rapture Biotech's institute, Mohali in July 2024 were used for the study. In the first instance, all leaves were observed under a microscope for perceivable characteristics like appearance, shape, size, and color.

Preparation of extract

Ashoka leaf samples were collected and washed with ethanol, crushed in a mortar, and placed with a little bit of water in it. A smooth slurry was made by increasing the water in it. The aftermath was boiled at 100 degrees Celsius for 15 minutes. The slurry was strained and poured into a conical flask. The conical flask was labeled and kept in the refrigerator for 24 hours.

Qualitative Phytochemical Analysis

Tests for qualitative analysis and the presence of phytochemicals were tested by taking different types of solutions poured into various test tubes to check the presence of phytochemicals. Presence is denoted by a '+' sign and absence is denoted by a – sign.

Test: Saponin test

2 ml sample extract (Ashoka plant) in 8ml H₂O
Froth formation means test positive

Test: Tanin or Braymer's test

1 ml Ashoka plant extract in FeCl₃ (10% of few drops)
Blue-green indicates positive

Test 3: Phenolic test

1ml Ashoka extract in 10% lead acetate solution
Brown precipitate formed will indicate a positive sign

Test 4: Alkaloid test or Wagner reagent test

1 ml filtrate + a few drops of Wagner's reagent
Reddish brown precipitates means test positive.

Test 5: Flavonoid test

Concentration sample in 2 ml of NaOH

Positive: Colourless

Negative: Yellow

Terpenoid test:

Description: Terpenoids represent the largest class of secondary metabolites and usually do not contain nitrogen or sulfur in their structures. Many terpenoids serve as defense compounds against microbes and herbivores and/or are signal molecules to attract pollinating insects, fruit-dispersing animals, or predators which

can destroy insect herbivores.

Procedure

2 ml Ashoka plant sample in 800 microlitres chloroform and addition of concentrated H₂SO₄ in it.

For safety purposes place the test tube in H₂O while adding H₂SO₄.

Positive: Green color solution

Phenolic or lead acetate test

Procedure: 2ml sample in 10% of lead acetate

Positive: White precipitate

Alkaloid test

Few ml of Ashoka filtrate + 1-2ml Mayer's reagent

Positive: Cream white yellow

Dragendroff's reagent

1 ml filtrate or extract + 1-2ml dragendroff's reagent

Positive: If only reddish-brown precipitates formed

Flavanoid test

A) Cyanidin test

1ml extract dissolved in 1-2ml methanol by heating addition of metal magnesium followed by 5-6 drops of concentrated HCL

Positive: If red/orange color occurs.

H₂SO₄ test

Ashoka plant extract in concentration H₂SO₄

Positive: If an orange color occurs

Carbohydrate test

2ml Ashoka filtrate in 2 drops alcoholic followed by concentrated H₂SO₄

Positive: Violet ring positive occurs

Reducing sugar:

A) Benedict's test

Take 0.5ml Ashoka filtrate and add 0.5ml Benedict's reagent
Boil for 2min

Positive: If green/Yellow/ Red occurs

B) Fehling test

1ml each of Fehling solution A & B followed by 1ml of Ashoka extract

Boil in hot water

Positive: If red precipitate occurs

Phenolics test

A) Hot water test

Warm water in a beaker along with the addition of the motor plant part dipped

Positive: Black/Brown color ring.

B) Potassium dichromate

Plant extract adding a few drops of potassium dichromate solution

Positive: If dark purple color.

#Ninhydrin test

2 ml filtrate adding 2 drops of ninhydrin solution (10 mg ninhydrin + 200 ml acetone)

Positive: Purple

B) Xanthopretic test

Plant extract + few drops of concentration nitric acid

Positive: Yellow-colored solution

Thin Layer Chromatography

A thin-layer chromatogram of the extracts was done in TLC plates. 2-5 µg of 1% solution of the sample was spotted using a micropipette. Various solvents like acetone, butanol, and acetone-butanol (1:1). Plate is placed under UV light, and dark spots are observed. The Formula calculated the Rf value of the sample, $R_f = \frac{\text{Distance moved by solute from the origin}}{\text{Distance moved by solvent from the origin}}$. The five sequential extracts are used for TLC profiling (Debprasad *et al.*, 2012) [8]. Before spotting the extracts are filtered and concentrated, to remove the solvents.

$R_f = \frac{\text{Distance moved by solute from the origin}}{\text{Distance moved by solvent from the origin}}$

Distance moved by solvent from the origin

Result and Discussions

The results of a phytochemical investigation carried out on the extracts of *Saraca asoca* leaves and flowers indicated the presence of components known to have both physiological and therapeutic properties (Datta, 2011) [6]. Phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, phenolic compounds, and alkaloids were found in the leaf extracts after analysis. All of the *Saraca asoca* flower extracts contained carbohydrates; the leaf extracts solely contained acetone without any. Phenolic substances found in distilled water, acetone, and extracts of flowers and leaves in methanol solvents. Regarding leaf and flower extracts, flavonoids were found in three different solvents: methanol, distilled water, and acetone. Methanol extracted from leaves and distilled water both include phenols, tannins, and steroids. The flower extracts in methanol, distilled water, and acetone revealed the presence of steroids, tannins, and phenol (Rosenthaler., 1930) [21]. Acetone is volatile, miscible with water, dissolves a wide variety of hydrophilic and lipophilic plant components, and is not harmful to the bioassay employed. It is a highly helpful extractant, particularly in antimicrobial research where more phenolic chemicals are needed.

According to a study published by (Das *et al.*, 2010) [7] aqueous acetone extracts tannins and other phenolics more effectively than aqueous methanol. Furthermore, tests conducted with ethanol discovered that ethanol was more easily able to cross the cell membrane and collect the intracellular components from the plant material. In the current investigation, maximal levels of ethanol were found in the flower to include substances including carbohydrates, phenols, flavonoids, saponins, glycosides, and steroids. We found saponins in acetone, ethanol, and water. There were flavonoids in each of the five extracts. The least amount of flavonoids, phenols, glycosides, and steroids were found in petroleum benzene. (Bimakr, 2010) [3]. Phytochemicals such as saponin, and tannin were not present in the leaf extract of Ashoka whereas phenolic compounds and alkaloids were found to be present when tests and research were done (Bendigeri *et al.* 2019) [2]. Moreover, the article discusses *Saraca asoca*'s medical importance which is unquestionably denoted by the fact that is used to treat a variety of health-related issues such as dysentery, stomach pain, menues-related diseases, and many more (Singh, *et al.*, 2015) [17]. It's been researched about phytochemicals present in Ashoka tree leaf extract by analyzing and testing the phytochemicals of the extract. For instance, in the case of saponin, tannin, flavonoid, dragendroff's reagent, and carbohydrate test when analyzed and researched they were negative which is considered that such phytochemicals are not present in the Ashoka tree as per the requirement of result. (Satyavati GV *et al.*, 1970) [18]. Positive tests for phytochemicals such as phenolic test, alkaloid test, terpenoid test, lead acetate test, H₂SO₄, and Benedict's test for reducing sugar resulted as positive showing positive and with required endpoint results. (Aggarwal BB., 2011) [1]. It was discovered through phytochemical research of *Saraca asoca* that certain compounds had physiological and therapeutic benefits. Leaf extract was discovered to include flavonoids, phenols, glycosides, steroids, saponins, tannins, and alkaloids through phytochemical examination. All of the *Saraca asoca* flower extracts included carbohydrates, except the acetone extract of the *Saraca asoca* leaves. The phytochemicals that this plant contains are both necessary for its own growth and provide its therapeutic benefits. (Somani, *et al.*, 2015) [19].

Test	Method used	Result
Saponin test	2 ml extract + 8ml h ₂ o	Negative
Tannin test	1 ml extract +10% lead acetate	Negative
Phenolic test	1 ml extract+ 10% lead acetate	Positive
Alkaloid test	1 ml filtrate + Wagner's reagent	Positive
Flavonoid test	Conc. Sample + 2ml NaOH	Negative
Terpenoid test	2ml sample+800ul chloroform+ 1ml conc. H ₂ SO ₄	Positive
Lead acetate test	2ml+10% lead acetate	Positive
Dragendroff's reagent test	1ml filtrate extract+1-2ml dragendroff's reagent	Negative
Cynadin test	1ml extract+dissolved in 1ml CH ₃ OH +metal magnesium +5-6 drops of HCL	Slightly positive
H ₂ SO ₄ test	Plant extract + conc. H ₂ SO ₄	Positive
Carbohydrate test	2ml filtrate+2 drops alcohol+1ml conc. H ₂ SO ₄	negative
Reducing sugar		
A)Benedict's test	0.5ml filtrate+ Benedict's reagent	positive
B)Fehling test	1ml fehling solution+1ml extract	negative



Ashoka tree (*Saraca asoca*)



Tanin test



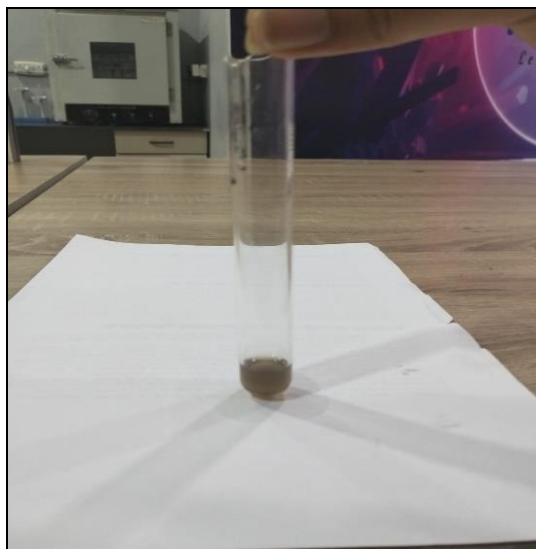
Phenolic test



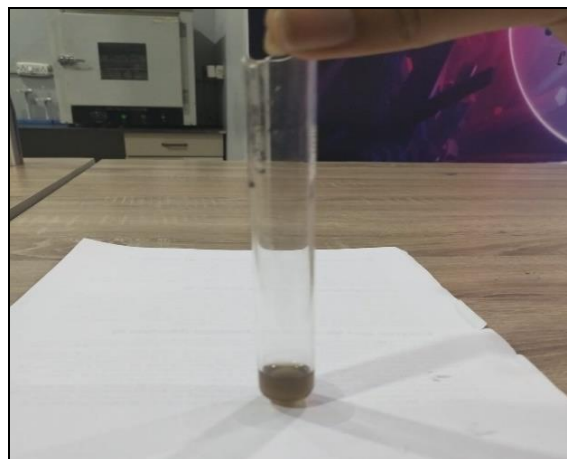
Saponin test



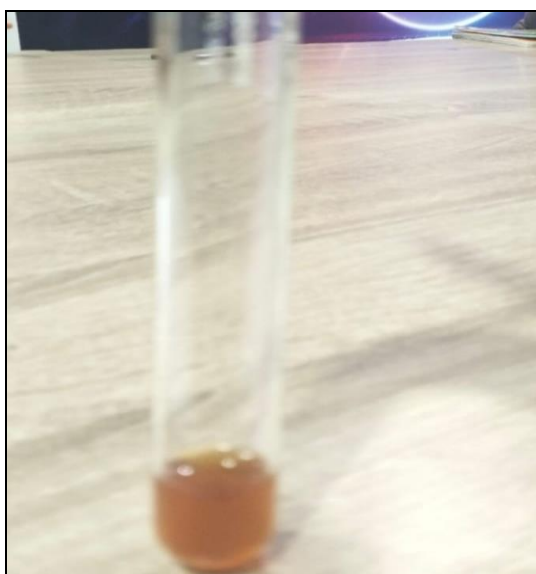
Alkaloid test



Flavanoid test



H₂SO₄ TEST



Alkaloid test or mater's reagent test

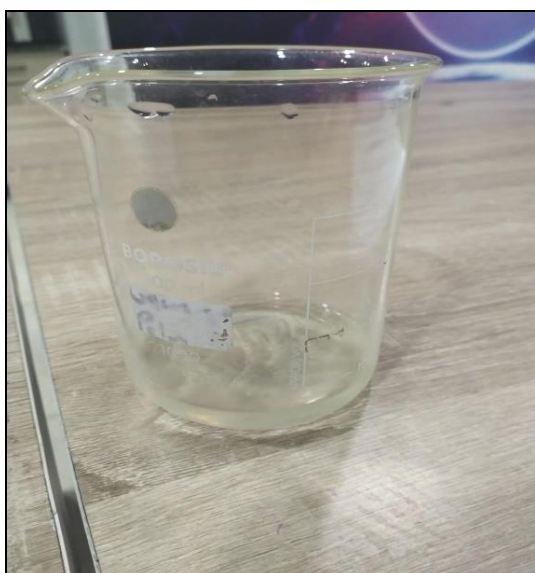


Carbohydrate test



Dragendroff's reagent test



Reducing sugar test**A) Benedict's test****B) Fehling test****Protein test****Ninhydrin test****Conclusion**

Herbal medications are used in many medical therapies to treat patients due to their remarkable influence; nevertheless, the mechanism of action of these medicines is not well understood. Since many of the ingredients in Ayurvedic medicine have several mechanisms of action, using herbal extracts rather than purified chemicals has been practiced for ages to provide the necessary holistic therapeutic impact. Among the most revered and mythical trees is the Ashoka tree. In Ayurvedic medicine, *sarraca asoca* is highly esteemed as a universal remedy. It is a ubiquitous plant with therapeutic properties and a rich source of many kinds of chemicals. The development of contemporary drug discovery has a barrier to applying Asoka's phytochemicals against illnesses. This adaptable plant yields a wide range of chemicals. Nowadays, a wide variety of plants are utilized to cure a wide range of illnesses. However, Ashoka is utilized in many pharmaceutical processes since it is a dependable and old source of medicine. It can cure a variety of conditions, including skin infections, neurological disorders, and genitor-urinary disorders. Given the current shift in world healthcare towards the use of nontoxic plant products, emphasis should be placed on developing contemporary drugs derived from *Saraca asoca* to manage a range of illnesses. According to Cowan (1999), ether is frequently used sparingly to extract fatty acids and coumarins. In the current investigation of phenols, carbohydrates, and leaf extract, there have been reports of steroids and glycosides from acetone.

In petroleum benzene, flavonoids, carbohydrates, and reports of phenolic compounds have been made. In ethanol, sugars, glycosides, and saponins; phenols, glucosides, flavonoids, saponins, phenolic substances, and steroids have been reported in water. The excerpts were preliminary phytochemical analyses conducted utilizing conventional chemical techniques, which mostly showed the presence of tannins, saponins, flavonoids, and carbs. It was discovered that the percentage extractability of *Shorea robusta*'s aqueous and alcoholic extracts was 24.57% and 18.33%, respectively, and the percentage extractability of *Saraca Indica*'s aqueous and alcoholic extracts was 20.26% and 18.33%, respectively. It was tracked down, that the percentage of alcoholic extracts of *Saraca asoca* was 25% and 17.93% respectively. It was discovered that Ashoka has phytochemicals including tannin, terpenoid essential oil, and resin. Based on the phytochemical study, the many therapeutic qualities of Ashoka listed in Ayurvedic literature can be related to the presence of these bioactive chemicals. Nevertheless, more clinical research is required to investigate the medicinal effectiveness of Shal and Ashoka bark skin.

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