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# Anti-acne, preliminary phytochemical and physico-chemical investigation of *Saraca asoca* bark

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## ABSTRACT

*Acne is a common disorder of pilosebaceous follicle which mainly affects the teenagers up to 95% boys and 83% girls due to the hormonal changes. Modern acne therapy which includes comedolytic agents, antibiotics and various anti-inflammatory agents has many side effects due to prolonged therapy. Propionibacterium acnes and Staphylococcus epidermidis have been recognized as pus-forming bacteria triggering an inflammation in acne. Excessive and prolonged use of antibiotics has lead to the development of resistance in acne causing bacteria, viz, Propionibacterium acne and Staphylococcus epidermidis. In the present study Saraca asoca were chosen based on their antibacterial activity against other Gram+ve and Gram-ve bacteria and was found to be effective against S.epidermidis. and P.acne and in present study physico-chemical parameter, phytochemical analysis also determine.*

**Keywords**: *Propionibactrium acne, Staphylococcus epidermidis*, Physico-Chemical Parameter, Phytochemical Analysis.

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## INTRODUCTION

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous follicle, characterized by comedones, papules, pustules, cysts, nodules and often scars in certain sites of predilection, namely, the face, neck, upper trunk and arms [1]. Acne is an inflammatory chronic disease, whose clinical presentation can range from a mild comedonal form to severe cystic acne of the face, chest, and back. Factors which contribute to the development of acne include hormonal imbalance, bacterial infection, stress, food, or cosmetic application [2]. *Propionibacterium acnes* release pro-inflammatory cytokines as well as antigens and mitogen(s), with cellular and non-cellular responses to these products triggering inflammation. Acne vulgaris is a common skin disease that involves individuals of all ages. It has been estimated to affect 79% to 95% of the

adolescent population, 40% to 54% of individuals older than 25 years, and 12% of women and 3% of men in their mid ages [1]. These factors provide a potential target for treatment. *Propionibacterium acnes* and *Staphylococcus epidermidis* are the target sites of antiacne drugs. Long term use of antibiotics against acne is outdated because of exacerbated antibiotic resistance [3].

*Saraca asoca* is an indigenous herbal drug belonging to the family Caesalpiniaceae. *Saraca asoca* is distributed in evergreen forests of India up to an elevation of about 750 meters. It is cultivated in many gardens because of its decorative orange red flowers and evergreen beautiful foliage. *Saraca asoca* is reported to contain glycoside, flavanoids, tannins and saponins. It is used as spasmogenic, oxytocic, uterotonic, anti-bacterial, anti-implantation, anti-tumour, anti-progestational, antiestrogenic activity against menorrhagia and anti-cancer. Bark extracts of *Saraca asoca* (Roxb.) de Wilde were previously investigated for in vitro antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus aureus and Klebsiella pneumoniae* at 4 mg/ml using agar well diffusion method. The ethanol and distilled water extracts showed significant broad spectrum antibacterial activity [4].

## MATERIALS AND METHODS

**Collection and Authentication of Plant Material:** The Ashoka plant was collected from Kamala Nehru Bal Udhyan, Barkheda, BHEL, Bhopal in month of December. The plant was authenticated by Botanist Mrs. Madhuri Modak Professor, Department of Botany, M.V.M College, Bhopal.

**Macroscopy:** The following macroscopic characters for the fresh leaves were noted: colour, odour, taste, size, shape, texture and fracture. Results are shown in table 1.

**Physical evaluation:** [4-6] Results are shown in table 2.

**Foreign Matter:** 50 gm sample was spread in a thin layer, and the pieces of foreign matter were sorted out by visual inspection. The powder of foreign matter was sifted through a 250 micron sieve. All portions of the foreign matter were pooled and weighed.

**Loss on Drying:** 10 gm of the drug was weighted in a tarred evaporating dish. It was dried at 105°C for 5 hours and weighed. The drying and weighing was continue at 1 hour interval until difference two successive weighing correspond not more than 0.25% .

**Extractive value:** 5 gm of the air dried coarsely powdered drug was macerate with 100 ml of distilled water in a closed flask for 24 hour. Shaking frequently during 6 hour and allowed to stand for 18 hour. Filter rapidly and evaporate 25 ml of the filtrate to dryness in tarred flat bottomed shallow dish and dry at 105°C to constant weight and weigh.

**Preparation of Extracts:** Plant materials were washed with water and shade dried. The powdered material was defatted with petroleum ether (60-80 ºC). The powdered materials were extracted using soxhlet apparatus with ethanol and by maceration with distilled water. Different

extracts were collected and subjected for preliminary phytochemical evaluation, and antibacterial screening.

**Preliminary Phytochemical Analysis:** All the extracts obtained by the powdered bark of *Saraca asoca* were subjected to various qualitative tests for the identification of phytoconstituents present [4-6]. The results are shown in table 3.

**Tests for Alkaloids:**
**Dragendorff's test**: To the 1 ml of extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

**Mayer's test:** To the 1 ml of extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

**Hager's test:** To 1 ml of extract add 3ml of Hager's reagent (saturated aqueous solution of picric acid) yellow colored precipitate indicates the presence of alkaloids.

**Wagner's test:** To the 1 ml of extract add 2 ml of Wagner's reagent (iodine in potassium iodide) formation of reddish brown precipitate indicates the presence of alkaloids.

**Tests for Glycosides:**
**Legal's test:** Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. No formation of pink to red colour shows absence of glycosides.

**Baljet's test:** To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange color reveals the presence of glycosides.

**Keller-Killani test:** 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer was separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

**Borntrager's test:** Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer is treated with 1ml of ammonia. The formation of red color of the ammonical layer shows the presence of anthraquinone glycosides.

**Modified Borntrager's test:** To 5 ml extract, add 5 ml 5% $FeCl_3$ and 5 ml dil. HCl. Heat for 5 min in boiling water bath. Cool and add benzene. Shake well. Separate organic layer, add equal volume dilute ammonia. No formation of pinkish red color of the ammonical layer shows the absence of glycosides.

**Tests for Carbohydrates:**
**Molisch's test:** To 2ml of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence.

**Fehling's test:** To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

**Benedict's test:** To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

**Barfoed's test:** Mix equal volume of Barfoed's reagent and test solution. Heat for 1-2 minutes in boiling water bath and cool. Red ppt is obtained.

**Tests for Tannins:**
i) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.
ii) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins.
iii) To the test extract, add strong potassium dichromate solution, a yellow color precipitate indicates the presence of tannins and phenolic compounds.
iv) To the test extract, add dilute nitric acid solution, reddish to yellow colour indicates the presence of tannins.

**Tests for Flavonoids:**
**Shinoda's Test**: i) The alcoholic extract is treated with magnesium foil and concentrated HCl give intense cherry red color indicates the presence of flavonones or orange red color indicates the presence of flavonols.

ii) The extract is treated with sodium hydroxide; formation of yellow color indicates the presence of flavones.
iii) The extract is treated with concentrated $H_2SO_4$, formation of yellow or orange color indicates flavones.

**Tests for Steroids:**
**Salkowski test:** Dissolve the extract in chloroform and add equal volume of conc. $H_2SO_4$. Formation of bluish red to cherry color in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

**Tests for Proteins:**
**Biuret test:** Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color is produced, and then add to the 1ml of the extract. Formation of pinkish or purple violet color indicates the presence of proteins.

**Ninhydrin test:** Add two drops of freshly prepared 0.2% Ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. Development of blue color reveals the presence of proteins, peptides or amino acids.

**Millon's test:** 1ml of test solution is made acidify with sulphuric acid and add reagent and boil this solution. A yellow precipitate is formed indicates the presence of protein Millon's.

**Microorganisms and media**

The test organisms used in this study were as followed: *Propionibacterium acnes* (MTCC 1951) and *Staphylococcus epidermidis* (MTCC 3382). These bacteria were obtained from the Microcare laboratory Surat India.

**Determination of Minimal Inhibition Concentration (MIC)[**7-8]

The main advantage of the **'Broth Dilution Method'** for MIC determination lies in the fact that it can readily be converted to determine the MIC as well. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic is immediately sub cultured [before inoculation] by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 $^0$C overnight. The tubes are then incubated overnight. The MIC of the control organism is read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism is recorded as the MIC. The amount of growth from the control tube before incubation [which represents the original inoculum] is compared.

## RESULTS AND DISCUSSION

**Table 1**: **Morphological evaluation of *Saraca asoca* bark**

| S.No. | Features | Observations |
|---|---|---|
| 1. | Colour | Brown (outer surface), pale yellow (inner surface) |
| 2. | Odour | Characteristic |
| 3. | Taste | Characteristic |
| 4. | Fracture | Irregular |
| 5. | Touch | Rough |
| 6. | Extra feature | The bark shows minute longitudinal wrinkles and fibrous fracture, internal surface smooth to touch |

**Table 2: Physical evaluation of *Saraca asoca* bark**

| S.No. | Parameter | Determined value |
|---|---|---|
| 1) | Loss on drying | 4.4% |
| 2) | Water soluble extractive | 14.4% |
| 3) | Ethanol soluble extractive | 15.2% |
| 4) | Benzene soluble extractive | 1.6% |
| 5) | Acetone soluble extractive | 5.6% |
| 6) | Ethyl acetate soluble extractive | 4% |
| 7) | Chloroform soluble extractive | 2.4% |
| 8) | Petroleum ether soluble extractive | 1.6% |
| 9) | Foreign matter | Not seen |

**Table 3: Phytochemical screening of aqueous and ethanolic extracts of *Saraca asoca* bark**

| S.No | Compound | Test | Extracts | |
|---|---|---|---|---|
| | | | Water | Ethanol |
| **1.** | Carbohydrates | Molisch's test | - | - |
| | | Fehling test | - | - |
| | | Benedict's test | + | + |
| | | Barfoed test | - | - |
| **2.** | Proteins & amino acids | Biuret test | - | - |
| | | Millons test | - | - |
| | | Ninhydrin test | - | - |
| **3.** | Steroids | Salkowaski test | - | - |
| | | Libermann- Burchards test | - | - |
| | | Libermann's test | - | - |
| **4.** | Glycosides | Legal test | + | + |
| | | Keller killani test | + | + |
| | | Borntragers test | - | - |
| | | Modified Borntragers test | - | - |
| **5.** | Flavonoids | Shinoda test | - | - |
| | | Lead acetate test | - | + |
| | | NaOH test | - | + |
| | | $H_2SO_4$ test | - | - |
| **6.** | Alkaloids | Wagner test | + | - |
| | | Hager test | + | + |
| | | Mayer test | - | - |
| | | Dragendorff test | + | + |
| **7.** | Tannins | 5% $FeCl_3$ Solution | + | + |
| | | Acetic acid test | - | - |
| | | Potassium dichromate test | + | + |
| | | Dil. Iodine solution test | + | + |
| | | Dil. Potassium permanganate | - | - |
| | | Dil. Nitric acid test | + | + |

*- = Negative (absent)*
*+ = Positive (present)*

**Antibacterial activity:** The aqueous and ethanolic extract of *Saraca asoca* bark was treated for antibacterial activity against *Propionibacterium acnes* and *Staphylococcus epidermidis,* the antibacterial activity will processed by micro dilution method by using plastic trays. The results are shown in table 4 and 5.

**Table 4: Antibacterial activity of *Saraca asoca* bark**

| Minimum Inhibition Concentration (microgramme/ml) | | | |
|---|---|---|---|
| **SR. NO.** | **Solvent** | ***Propionibacterium acnes*** **MTCC 1951** | ***Staphylococcus epidermidis*** **MTCC 3382** |
| 1 | Aqueous | 125 | 100 |
| 2 | Ethanol | 100 | 100 |

**Table 5: Minimal Inhibition Concentration of Standard Drugs**

| **Standard Drug** (microgramme/ml) | ***Propionibacterium acnes*** **MTCC 1951** | ***Staphylococcus epidermidis*** **MTCC 3382** |
|---|---|---|
| GENTAMYCIN | 0.5 | 0.25 |
| AMPICILLIN | 100 | 250 |

Acne vulgaris is an extremely common skin disorder that affects virtually all individuals at least once during life. The incidence of acne peaks at teenage, but substantial numbers of men and women between 20-40 years of age are also affected by the disorder. Acne can have important negative psychosocial consequences for the affected individual, including diminished self-esteem, social withdrawal due to embarrassment and depression. Herbal medication are

considered safer than allopathic medicines as allopathic medicines are associated with side effects such as like contact allergy, local irritation, scaling, photosensitivity, itching, pruritus, redness, skin peeling of the skin etc. The present research work deals with evaluation of anti-acne activity of *Saraca asoca* bark. The mechanism of action of flavonoids and tannins is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds. These results suggested that the preparations incorporating flavonoids and tannins of the *Saraca asoca* bark could be used as an alternative treatment for acne [9]. Phytochemical screening of *Saraca asoca* revealed the presence of alkaloid and tannins which could be responsible for activity against *Propionibacterium acnes* and *Staphylococcus epidermidis* [10]. The preliminary screening of aqueous and ethanolic extract of *Saraca asoca* was found to effective against *Propionibacterium acnes* and *Staphylococcus epidermidis*. The extract were screened for their activity against acne causing bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis* using broth dilution method and MIC techniques.

## CONCLUSION

The extract of *Saraca asoca* barks having antimicrobial activity against *Propionibacterium acnes* and *Staphylococcus epidermidis* with different MIC (minimum inhibitory concentration). The ethanolic extract (MIC 100 µg/ml) show better as compare to aqueous extract (125 µg/ml) for *Propionibacterium acnes* and ethanolic and aqueous extract show similar effect as MIC 100 µg/ml for *Staphylococcus epidermidis.*

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Therefore, the active component alkaloids and tannins of the *Saraca asoca* bark could be of interest for further development as anti-acne products, however further clinical research will be necessary.

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