

An invitro Anti-bacterial and HPTLC Study of Latexes of *Alstonia scholaris* (Linn.)R.Br and *Calotropis gigantea* (Linn.)R.Br to substantiate its ancient usage.

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Abstract

Alstonia scholaris (Linn.)R.Br known as *Saptaparna* and *Calotropis gigantea* (Linn.)R.Br. known as *Arka* in *Āyurveda* is being widely used for many medicinal preparations. But the latexes of these are not in much common use. Ancient texts of *Āyurveda* mentioned that the mixture of latexes of *Saptaparna* & *Arka* is very effective for pain in dental caries (*Krimi Danta śūla*). So an Anti-bacterial study with E.coli and Gingivitis bacteria using ampicilin as control and HPTLC profiling were done to substantiate this ancient usage which is totally neglected today. The individual samples as well as the mixture were subjected to the above studies. The results showed that in the Anti-bacterial study the mixture was as effective as the control drug ampicillin. The total area got in the HPTLC of the mixture was found to be more than the individual samples. So the ancient usage is almost substantiated.

Key words : *Alstonia scholaris*, *Saptaparna*, *Calotropis gigantea*, *Arka*, *Krimi Danta śūla*, Dental caries, Anti-bacterial study, HPTLC, E.coli.

Introduction

Alstonia scholaris (Linn.)R.Br of Apocynaceae family, commonly called Devil tree or Shaitan wood is distributed throughout India, in deciduous and evergreen forests and also in plains. It is a large evergreen tree upto 3 m in height, greyish brown bark, lenticellate abounding in bitter milky latex, leaves 4-7 in a whorl and greenish white small flowers in umbellate panicles. Known as *Saptaparna*, *Saptacchada* in Sanskrit. Bark, leaves and milky exudates are the parts used for medicinal purposes. *Saptaparna* is used in *Kapha vata* conditions, *Vrana* (ulcers and wounds), *Kusta* (skin disorders), *Krimi* (Anti-bacterial, wormicidal), *Swasa* (Dysnoea)etc.^[1] Dhanwantari Nighantu, Kaiyyadeva Nighantu, Bhavaprakasa Nighantu and Raja Nighantu mentions the wide usage of *Saptaparna* for *Krimi*^[2].

Calotropis gigantea (Linn.)R.Br. of Asclepiadaceae family, with English name Gigantic swallow wort, is distributed throughout India, especially in dry waste places. The plant is large hard much branched milky shrub, very pale in colour. Leaves are opposite, subsessile. Flowers beautiful lilac, rosy or purple in umbellate cymes. The whole plant is useful as medicine. *Calotropis gigantea* is a wasteland weed better known as milkweed, habitat of Asian countries that includes, India, Indonesia, Malaysia, Philippines, Thailand, Sri Lanka and China. Tribal people were using this plant parts to cure several illnesses such as toothache, earache, sprain, anxiety, pain, epilepsy, diarrhoea and mental disorders. *C. gigantea* is scientifically reported for its anti-*candida* activity, cytotoxic activity, antipyretic activity and wound healing activity.^{[3], [4], [5], [6], [7], [8]}

Calotropis is described as *Arka* in *Āyurveda*. It is said to be stomachic, anthelmintic, analgesic, used for *kusta*, *krimi*, *raktapitta*, *gulma*, *sotha*. Various Nighantus like Bhavaprakasā Nighantu, Dhanwantari Nighantu, Raja Nighantu mentions its *krimihara* properties.

A combination of *Saptaparnā* and *Arka Kshīra* is mentioned in *Āyurvedic* texts. In Astanga Samgraha Uttarasthana (26/16)^[9] and Astangahridaya Uttaratantra (22/20)^[10] it is mentioned for *Krimidanta*. Among the treatments described for *krimidanta* filling the cavity with the mixture of latexes of *Saptacchada* (*Alstonia scholaris* (Linn.)R.Br.) and *Arka* (*Calotropis gigantea* (Linn.)R.Br.) is said to alleviate the pain due to *krimidanta* (Dental caries).

Āyurveda has identified and mentioned *Krimidanta* (Dental caries) years back in the Samhitās. *Ācārya* describes it as a condition that occurs to the teeth due to the vitiated *dosas* with the predominance of *vāta*. He describes the symptoms as severe pain that appears and disappears without any reason, swelling, blackish discoloration, shaking of the teeth, discharge of pus mixed with blood.^[11] This is the area of interest in this research paper. This combination still now remains stagnant as a textual knowledge. So an invitro anti-bacterial study was conducted with the latexes separately and in combination, with ampicillin as standard. HPTLC profile matching was also conducted of the latexes separately and in combination. The studies were done in R&D department of Arya Vaidya Sala, Kottakkal, Kerala.

Materials and Methods

Collection of Latex: The Latex of *Alstonia scholaris* (Linn.)R.Br was collected from the herbal garden and campus of V.P.S.V Ayurveda college, Kottakkal. *Calotropis gigantea* (Linn.)R.Br latex was collected from surrounding areas of Ayurveda college. Both were collected on the same day in the month of February 2016.

Extraction of Latex :

10ml *Alstonia scholaris* sample and 10ml *Calotropis gigantea* sample was evaporated to dryness, extracted with 10ml Methanol each. 10ml equal volume of *Alstonia scholaris* & *Calotropis gigantea* was mixed well, evaporated to dryness, extracted with 10ml Methanol.

Test microorganism: The antimicrobial activity was individually tested against *Escherichia coli* and Gingivitis bacteria. Both test strains were maintained on nutrient agar and were sub-cultured every two weeks.

Bioassay for Anti-bacterial activity: The disc diffusion method was adopted to test the antibacterial activity where ampicillin was used as a standard drug to compare the results of experimental plant.

Disc diffusion method

The disc diffusion method was used to determine the growth inhibition of bacteria by the plant latex extracts of *Alstonia scholaris* (Linn.)R.Br and *Calotropis gigantea* (Linn.)R.Br separately and in combination. Discs containing different concentration (200, 100, 50 and 25 mg/ml) of dissolved plant latex extracts and prepared by using sterile Whatman filter paper No. 1 (6 mm in diameter). The discs were dried at 50°C. Overnight cultures of each of bacterial isolates was diluted with sterile normal saline to give inoculum size of 10⁶ cfu/ml. Nutrient agar medium was prepared, sterilized, cooled and poured in to sterile petri dishes to a depth of 4 mm about 25 ml/plate to solidify. Pure cultures of the test organism were used to inoculate the petri dishes. This was done by spreading the inoculum on the surface of the prepared nutrient agar plate using sterile cotton swabs which have been dipped in the diluted suspension of the organism. The discs were then aseptically placed evenly on the surface of the inoculation and gently pressed down to ensure contact using a pair of forceps. The plates were finally incubated at 37°C for 18-24hrs. The plates were examined after 24 hrs for clear zone of inhibition. All measurements were taken in mm.^[12]

HPTLC Study

HPTLC studies were done for 3 samples, *Alstonia scholaris* and *Calotropis gigantea* latexes separately and in combination.

TEST SOLUTIONS

01. 10ml equal volume of *Alstonia scholaris* & *Calotropis gigantea* is mixed well, evaporated to dryness, extracted with 10ml Methanol, and spotted as 10 microlitre.
02. 10ml *Alstonia scholaris* sample is evaporated to dryness, extracted with 10ml Methanol, and spotted as 10 microlitre.
03. 10ml *Calotropis gigantea* sample is evaporated to dryness, extracted with 10ml Methanol, and spotted as 10 microlitre.

STATIONARY PHASE

Merk, 1.05554.0007, TLC Silica gel 60 F₂₅₄, 20x10 cm Aluminium sheet.

MOBILE PHASE

Toluene: Ethyl acetate: Formic acid: Methanol (14:10:2:1)

DEVELOPMENT

CAMAG 20 x 10 cm Twin trough chamber.

HPTLC INSTRUMENTATION

CAMAG Linomat 5, CAMAG TLC Scanner 3, CAMAG Reprostar 3.

DERIVATIZATION

10% sulphuric acid reagent.

RESULTS AND DISCUSSION

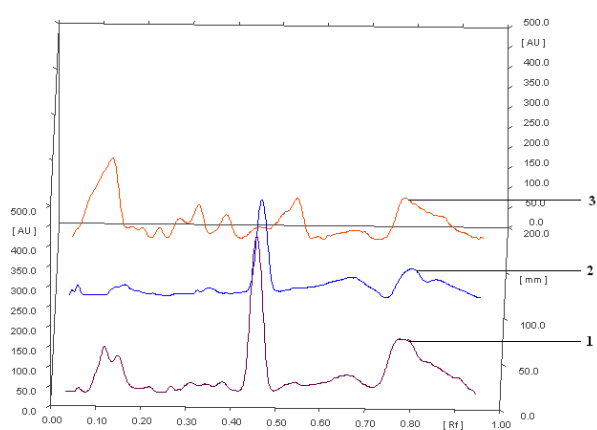
The zone of inhibition of *Alstonia scholaris* Latex as well as *Calotropis gigantia* Latex individually were lesser compared to the control ampicillin. But the combination showed the same inhibition as the control.

Table 1 Showing Inhibition zone

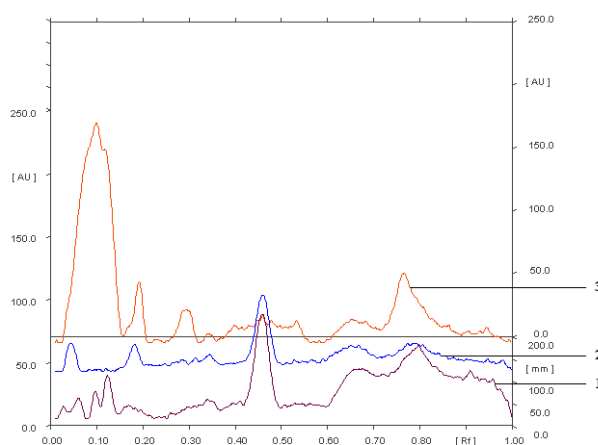
		Inhibition zone	
		E.coli	Gingivitis Bac.
Control	Ampicilin	2mm	2mm
Test Drug:	Alstonia scholaris	1.3mm	1.5mm
	Calotropis gigantia	1.4mm	1.6mm
	Mixture	2mm	2mm

HPTLC

Graph No.I -OVERVIEW GRAPH OF ALSTONIA SCHOLARIS & CALOTROPIS GIGANTIA SAMPLES



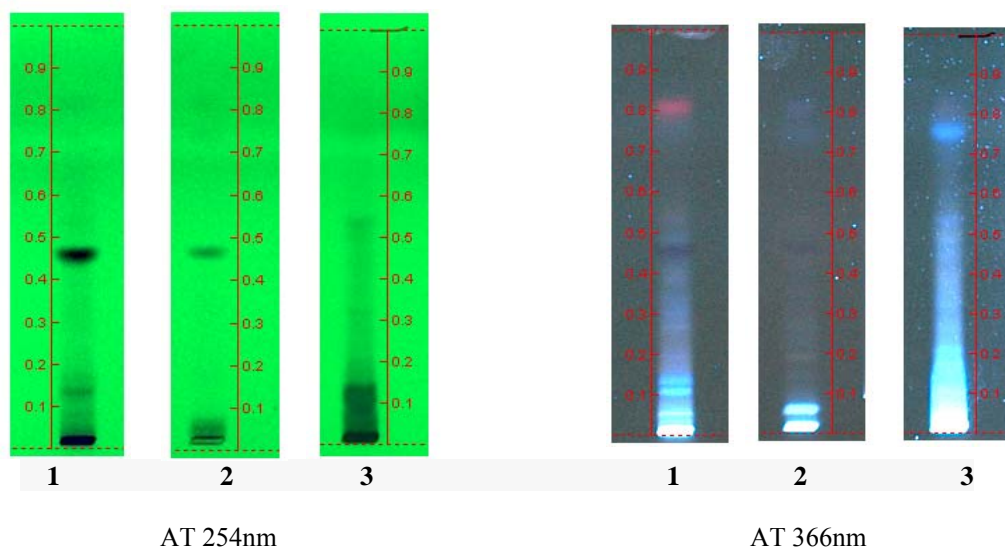
AT 254nm



AT 366nm

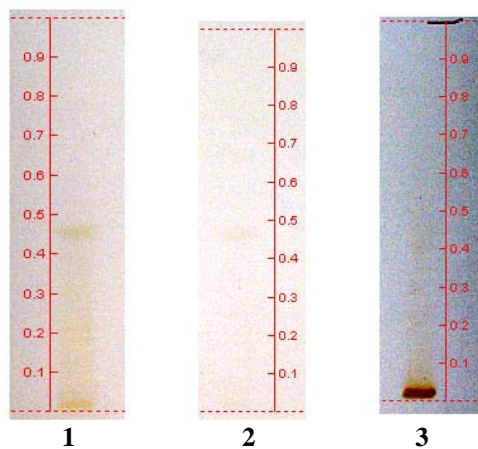
- 1 - Mixture of *Alstonia scholaris* & *Calotropis gigantia*
- 2 - *Alstonia scholaris*
- 3 - *Calotropis gigantia*

Plate No.I TLC PLATE VIEWS OF ALSTONIA SCHOLARIS & CALOTROPIS GIGANTIA SAMPLES



- 1 - Mixture of *Alstonia scholaris* & *Calotropis gigantea*
- 2 - *Alstonia scholaris*
- 3 - *Calotropis gigantea*

Plate No.II TLC PLATE VIEWS OF ALSTONIA SCHOLARIS & CALOTROPIS GIGANTIA SAMPLES AT WHITE LIGHT



Conflict of Interest

The authors declare that there are no conflict of interest regarding this manuscript.

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