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

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Abstract

*Alstonia scholaris* (Linn.)R.Br known as *Saptaparna* and *Calotropis gigantia* (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 (*Krīmi Danta śūla*). So an Anti-bacterial study with E.coli and Gingivitis bacteria using ampicillin 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 gigantia*, *Arka*, *Krīmi 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), *Krīmi* (Anti-bacterial, wormicidal), *Swasa* (Dysnoea)etc.[1] Dhanwantari Nighantu, Kaiyyadeva Nighantu, Bhavaprakasa Nighantu and Raja Nighantu mentions the wide usage of *Saptaparna* for *Krīmi*.[2]

*Calotropis gigantia* (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, krīmi, raktapitta, gulma, sotha*. Various Nighantus like Bhavaparakasā 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 Astangahridayaya Uttaratantra (22/20) [10] it is mentioned for *Krīmīdantā*. Among the treatments described for *krīmīdantā* filling the cavity with the mixture of latexes of *Saptacchada* (*Alstonia scholaris* (Linn.)R.Br.) and *Arka* (*Calotropis gigantia* (Linn.)R.Br.) is said to alleviate the pain due to *krīmīdantā* (Dental caries).
Ayurveda 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 discolouration, shaking of the teeth, discharge of pus mixed with blood. 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 gigantia (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 gigantia sample was evaporated to dryness, extracted with 10ml Methanol each. 10ml equal volume of Alstonia scholaris & Calotropis gigantia 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 gigantia (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.

HPTLC Study

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

TEST SOLUTIONS

01. 10ml equal volume of Alstonia scholaris & Calotropis gigantia 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 gigantia 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

<table>
<thead>
<tr>
<th>Inhibition zone</th>
<th>E.coli</th>
<th>Gingivitis Bac.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Ampicillin</td>
<td>2mm</td>
</tr>
<tr>
<td>Test Drug:</td>
<td><em>Alstonia scholaris</em></td>
<td>1.3mm</td>
</tr>
<tr>
<td></td>
<td><em>Calotropis gigantia</em></td>
<td>1.4mm</td>
</tr>
<tr>
<td>Mixture</td>
<td></td>
<td>2mm</td>
</tr>
</tbody>
</table>

HPTLC

**Graph No.1** - OVERVIEW GRAPH OF ALSTONIA SCHOLARIS & CALOTROPIS GIGANTIA SAMPLES

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

AT 254nm

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

AT 366nm

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

AT 366nm

AT WHITE LIGHT

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

COMPARISON OF AREA & PEAKS OF ALSTONIA SCHOLARIS & CALOTROPIS GIGANTIA SAMPLES AT 254nm

Sample –1 (Mixture of Alstonia scholaris & Calotropis gigantia)
TOTAL PEAK NO – 12
TOTAL AREA = 25417.8AU

Sample –2 (Alstonia scholaris)
TOTAL PEAK NO – 06
TOTAL AREA = 4636.2AU

Sample –3 (Calotropis gigantia)
TOTAL PEAK NO – 13
TOTAL AREA = 18869.6AU

The Anti-bacterial study shows that the mixture of Alstonia scholaris & Calotropis gigantia showed an inhibition zone of 2mm which was the same as that of control Ampicilin. The latexes individually showed lesser inhibitory zones. During the HPTLC Studies it was found that Alstonia latex was less soluble in methanol. But the mixture was more soluble. So the mixture showed greater area in HPTLC compared to individual samples.

Conclusion

The mixture of both latexes showed the same anti-bacterial effect as the control ampicillin. The high anti-bacterial effect of the mixture substantiates its action against pain in dental caries (Krimidanta sula). Also as the mixture was more soluble in methanol and more area was observed in HPTLC graph showing that more chemical constituents were available in the mixture. So some content in Calotropis latex helps for more availability of chemical constituents.

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Conflict of Interest

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

REFERENCES


