HPTLC and antibacterial analysis of extracts of *Cressa cretica* Linn.

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Abstract

Plants used in traditional medicine are potential sources of new biologically active compounds many of them with antibacterial and antifungal activity. Plants can serve as a source of model compounds for synthetic or semi synthetic structure modification (Balandrin et al., 1993). Potential natural and synthetic substances with biocidal activity are considered candidates for developing new drugs for treatment of various chronic as well as infectious diseases. In the present investigation, HPTLC analysis is carried out with the various extracts of *Cressa cretica* L. to identify the number of phytoconstituents and their antibacterial activity is also tested.

Introduction

*C. cretica* is mentioned as an Ayurvedic drug, known in indigenous medicine in India as Rudanti. (Satakopan and Karandikar, 1961). It is reported as antibilious, antitubercular and an expectorant (Rizk and El-Ghazaly, 1995 and Satakopan and Karandikar, 1961). The plant is alternately used as an anthelmentic, stomachic, tonic for aphrodisiac purposes, enrichment of the blood and is useful in the treatment of constipation, leprosy, asthma and urinary discharges (Chopra et al., 1956). The plant is traditionally used in Bahrain as an expectorant and antibilious agent (Rizk and El Ghazaly, 1995). A decoction of the leaves of this plant is used as a diuretic
and crushed leaves are used to heal wounds.

Phytochemical screening of the plant *Cressa cretica* L. revealed the presence of alkaloids, coumarins and steroids (Rizk, 1995). Few compounds have been reported from previous investigations. Review of literature showed that *Cressa cretica* L. has antifungal activity. The ethanol extract of *Cressa cretica* L. exhibited higher inhibitory effect against *Pencillium citrium*, followed by *Candida albicans* and *Alternaria alternata*. Anti bacterial activity of the extracts of this plant is not reported so far. In view of the medicinal importance of *C. cretica*, we undertook a study to find out the probable number of compounds in different extracts of this plant and also their antibacterial activity.

The air-dried powdered whole plant of *C. cretica* was extracted successively with hexane, chloroform, ethyl acetate and ether by soxhlet extraction procedure. Since no work on isolation of alkaloids has been reported, the whole plant was subjected to alkaloid extraction process. The different extracts along with a sample of crude solid presumed to be an alkaloid, were sent to Anchom Analytical Pvt. Ltd., Mumbai, for HPTLC analysis. Results are given in the following pages.
**winCATS Planar Chromatography Manager**

**10 µl - Chloroform**

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area % | Assigned substance |
|------|----------|--------------|--------|------------|-------|--------|------------|--------|-------------------|
| 1    | 0.13     | 0.9          | 11.1   | 2.91       | 19.6 | 9.5    | 303.9      | 1.13   | Substance 9       |
| 2    | 0.20     | 8.6          | 22.6   | 6.48       | 28.9 | 26.8   | 155.0      | 2.94   | Substance 7       |
| 3    | 0.52     | 1.2          | 68.9   | 17.72      | 61.0 | 63.0   | 230.5      | 10.94  | Substance 11      |
| 4    | 0.61     | 6.93         | 96.6   | 23.09      | 73.5 | 9.4    | 5756.6     | 28.87  | Substance 1       |
| 5    | 0.74     | 3.6          | 164.7  | 63.5       | 16.7 | 10.6   | 10014.0    | 63.70  | Substance 3       |

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Revised:  
Report ID: 9703070103081631  
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**winCATS Planar Chromatography Manager**

**10 µl - Ether**

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area % | Assigned substance |
|------|----------|--------------|--------|------------|-------|--------|------------|--------|-------------------|
| 1    | 0.14     | 8.1          | 18.4   | 7.83       | 32.2 | 17.4   | 97.3       | 11.83  | unknown *         |
| 2    | 0.23     | 17.0         | 29.2   | 8.38       | 28.9 | 11.4   | 576.9      | 7.02   | unknown *         |
| 3    | 0.56     | 3.2          | 20.3   | 8.41       | 68.0 | 10.6   | 1108.4     | 14.47  | Substance 11      |
| 4    | 0.65     | 13.0         | 19.6   | 8.11       | 72.7 | 0.7    | 554.5      | 7.97   | Substance 4       |
| 5    | 0.75     | 6.8          | 65.9   | 27.29      | 79.7 | 63.6   | 1406.9     | 17.05  | unknown *         |
| 6    | 0.79     | 63.6         | 81.1   | 40.18      | 78.9 | 5.6    | 3419.7     | 41.84  | Substance 3       |

User: Supervisor  
Revised:  
Report ID: 0703070103091631  
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10 µl - Standard

| Peak | Start RF | Start Height | Max RF | Max Height | % | End RF | End Height | Area | % | Assigned Substance |
|------|----------|--------------|--------|------------|----|--------|------------|------|----|-------------------|
| 1    | 0.02     | 0.3          | 0.04   | 17.9       | 31.95  | 0.05   | 5.6        | 196.2 | 14.36 | unknown *          |
| 2    | 0.08     | 4.9          | 0.10   | 20.3       | 38.23  | 0.11   | 0.6       | 242.2 | 17.73 | unknown *          |
| 3    | 0.56     | 1.9          | 0.81   | 15.9       | 29.82  | 0.67   | 7.1       | 927.8 | 67.91 | Substance 11      |

Track 3: ID: STD

10 µl – Ethyl acetate

User: Supervisor  
ReportID: 9T307910333031E31  
Printed: Tuesday, July 31, 2003 03:30:51 AM
10 µl - Hexane

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area % | Assigned Substance |
|------|----------|--------------|--------|------------|-------|--------|------------|--------|-------------------|
| 1    | 0.59     | 0.61         | 2.3    | 46.21      | 0.63  | 0.1    | 30.8       | 22.14  | Substance 11      |
| 2    | 0.67     | 0.72         | 2.7    | 53.79      | 0.76  | 0.1    | 108.3      | 77.86  | unknown*          |

*unknown*
**Track 2, ID: SPL2**

**10 µl - Chloroform**

| Start | Start | Max | Max | Max | End | End | Area | Assigned substance |
|-------|-------|-----|-----|-----|-----|-----|------|-------------------|
|   1  | 0.10  | 0.0 | 0.18| 19.6| 4.94| 0.23| 15.0 | Substance 9       |
|   2  | 0.20  | 15.0| 0.22| 33.0| 8.11| 0.28| 3.0  | Subs tance 7       |
|   3  | 0.49  | 0.2 | 0.51| 3.3 | 0.80| 0.51| 0.4  | unknown *         |
|   4  | 0.53  | 0.7 | 0.60| 47.6| 11.71| 0.61| 42.0 | Substance 11      |
|   5  | 0.61  | 42.0| 0.63| 56.5| 13.92| 0.72| 0.2  | Substance 3       |

**Track 3, ID: STD**

**10 µl - Standard**

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**Supervisor: [Name]**

**Revised: [Date] 07/08/03**

**Report ID: 070307010303229**

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Track 1, ID: SPL1  
10 µl - Hexane

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|------|----------|--------------|--------|------------|-------|--------|------------|------|--------|-------------------|
| 1    | 0.94     | 6.0          | 0.67   | 15.0       | 57.16 | 0.68   | 11.9       | 345.7| 51.69  | Substance 4        |
| 2    | 0.72     | 11.1         | 0.72   | 12.0       | 42.84 | 0.77   | 1.7        | 323.1| 48.31  | unknown            |

Track 2, ID: SPL2  
10 µl - Chloroform

User: Supervisor  
Reported:  
SN: 0708W003. V1.2.3  
Tuesday, July 01, 2003 03:25:07 AM  
Report ID: 07D3700310301005  
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### winCATS Planar Chromatography Manager

| Peak | Start RF | Start Height | Max RF | Max Height | Max % | End RF | End Height | Area | Area % | Assigned substance |
|------|----------|--------------|--------|------------|-------|--------|------------|------|---------|-------------------|
| 1    | 0.04     | 0.3          | 0.07   | 18.9       | 3.0   | 0.10   | 3.0        | 477.5| 1.85    | unknown *          |
| 2    | 0.16     | 8.3          | 0.18   | 19.3       | 4.13  | 0.19   | 17.7       | 382.9| 1.47    | Substance 9        |
| 3    | 0.20     | 18.0         | 0.22   | 45.9       | 0.77  | 0.23   | 24.3       | 1400.8| 5.42   | Substance 7        |
| 4    | 0.25     | 24.3         | 0.26   | 36.2       | 5.60  | 0.34   | 10.8       | 1113.9| 4.31   | unknown *          |
| 5    | 0.61     | 0.3          | 0.04   | 195.0      | 41.91 | 0.73   | 0.9        | 12967.5| 54.03  | Substance 11       |
| 6    | 0.74     | 0.0          | 0.79   | 161.5      | 54.59 | 0.84   | 28.1       | 8511.9| 32.93  | Substance 3        |

**Track 3, ID: STD**

**10 µl - Standard**

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User: Supervisor  
Revised:  
Report ID: CTD0070/100031905  
SN:0708W003, V3.2.3  
Tuesday, July 01, 2003 03:25:07 AM  
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10 µl – Ethyl acetate

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Assigned substance |
|------|----------|--------------|--------|------------|-------|--------|------------|------|-------------------|
| 1    | 0.15     | 5.9          | 0.19   | 16.7       | 7.48  | 0.20   | 16.5       | 496.7| unknown*           |
| 2    | 0.22     | 17.0         | 0.25   | 22.1       | 9.86  | 0.26   | 15.7       | 825.8| unknown*           |
| 3    | 0.37     | 8.7          | 0.04   | 65.1       | 23.11 | 0.72   | 0.5        | 3417.3| Substance 11       |
| 4    | 0.75     | 0.8          | 0.80   | 119.9      | 53.56 | 0.84   | 27.6       | 9075.3| Substance 3        |

10 µl - Ether

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Assigned substance |
|------|----------|--------------|--------|------------|-------|--------|------------|------|-------------------|
| 1    | 0.02     | 17.5         | 0.03   | 28.9       | 25.99 | 0.06   | 34.3       | 1290.4| 24.43             |
| 2    | 0.18     | 26.5         | 0.18   | 34.1       | 22.75 | 0.24   | 19.0       | 1812.0| 34.31             |
| 3    | 0.34     | 19.0         | 0.25   | 21.0       | 14.54 | 0.29   | 11.7       | 798.2 | 14.89             |
| 4    | 0.35     | 11.3         | 0.38   | 14.5       | 9.70  | 0.42   | 3.0        | 547.1 | 10.36             |
| 5    | 0.63     | 15.4         | 0.85   | 23.5       | 15.66 | 0.65   | 4.5        | 639.7 | 12.11             |
| 6    | 0.79     | 0.2          | 0.81   | 17.7       | 11.84 | 0.82   | 0.0        | 206.0 | 3.90              |

Substance 5

Substance 9

unknown* 10

Substance 4

Substance 3
Finger print data for all extracts and scan data at 200 nm, 254 nm and 366 nm is given as images. Analysis showed that extracts are responding mainly to vanillin sulphuric acid reagent, which offers proof for the presence of steroid type of compounds especially in the chloroform extract. Scan results are consolidated as follows:

Scan results show proof for presence of 11 compounds with Rf values given in the table below:

| Substane | Rf | Substane | Rf |
|----------|----|----------|----|
| 1        | 0.62 | 6        | 0.06 |
| 2        | 0.74 | 7        | 0.23 |
| 3        | 0.81 | 8        | 0.27 |
| 4        | 0.67 | 9        | 0.18 |
| 5        | 0.03 | 10       | 0.37 |
|          |      | 11       | 0.61 |

Scan result at 200 nm for all the extracts along with standard.

| Assigned Sub | Unknown |
|--------------|---------|
| 3, 7, 9, 11  | 1       |
| 3, 4, 7      | 2       |
| 3, 11        | 2       |
| 3, 4, 5      | 2       |
| 9, 10        | 1       |

Scan result at 254 nm

| Assigned Sub | Unknown |
|--------------|---------|
| -            | -       |
| 1, 3, 7, 9, 11 | - |
| 11           | 2       |
| 3, 4, 11     | 3       |
| -            | -       |
Scan result at 366 nm

| Track – 1 – Hexane – 10 µl | Assigned Sub | Unknown |
|----------------------------|--------------|---------|
| Track – 2 – CHCl₃ – 10 µl  | 3, 6, 9, 11  | 2       |
| Track – 3 – Standard – 10 µl | 7, 11       | 4       |
| Track – 4 – Ethyl acetate – 10 µl | 3, 4, 11 | 2       |
| Track – 5 – Ether – 10 µl   | 11           | 2       |

Finger print data showed that the intensity of colour developed with the spray reagent is maximum in CHCl₃ extract.

The crude alkaloid extract was fractionated by column chromatography on SiO₂ using chloroform as solvent, followed by chloroform methanol mixtures to isolate the pure alkaloids.

Anti Bacterial Activity

Anti bacterial activity of various extracts of Cressa cretica L. and the crude alkaloid solution was tested against four micro organisms and the zone of inhibition values are tabulated below:

| Extracts          | I    | II   | III  | IV   |
|-------------------|------|------|------|------|
| Hexane            | Nil  | Nil  | Nil  | Nil  |
| Ethyl acetate     | 4    | 4    | 2    | 3    |
| Methanol          | 5    | 5    | 7    | 4    |
| Chloroform        | 4    | 6    | 4    | 5    |
| Ether             | 6    | 3    | 3    | 2    |
| Crude alkaloid    | 6    | 4    | 6    | 4    |

I - E. coli ; II - Staphylococcus aureus ; III - Proteus spp. ; IV - Pseudomonas spp.

Antibacterial analysis revealed considerable antibacterial activity in all the extracts except hexane extract and in the case of Proteus spp. the extracts show greater activity compared to the control. All extracts show maximum activity against E. coli.

Acknowledgement

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