

Two bioactive compounds from leaf extracts of *Colebrookea oppositifolia* Smith

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ABSTRACT

Colebrookea oppositifolia Smith, is a small bushy plant with enormous ethnomedicinal importance. The plant is used to cure the diseases like epilepsy and urinary problems. It was also used for its antiseptic and hepatoprotective activity. In the present study, antimicrobial and antioxidant activity of *C. oppositifolia* leaf extracts was determined. The extracts were further screened for bioactive compounds. After IR, NMR and MS analysis of extracts, eugenol and luteoline-7-glucoside were identified as major active compounds in the extracts.

Key words: Antimicrobial activity, antioxidant activity, bioactive compounds, *Colebrookea oppositifolia*.

Medicinal plants are the source of many bioactive compounds of therapeutic use⁶. Indian subcontinent is a great reservoir of medicinal plants and many of them are also mentioned in our traditional health care systems like Ayurveda, Siddha, and Unani medicine^{8,9&13}. Melghat reserve forest area is one of major floristic zone in Maharashtra (India). This region also supports the aboriginal tribal inhabitants like Gond, Rajgond, Korku, Bhil etc. These tribals solely depend on forest products for their livelihood and health care.

For the present study, author had selected *Colebrookea oppositifolia* for its bioactivity and bioactive compounds. *C. oppositifolia* is a bushy shrub commonly found in elevated forest

ranges. It belongs to Lamiaceae and locally known as Bhaman. The tribal healers are found to use this plant/ plant parts to cure various ailments like epilepsy and urinary problems. It also claimed to have antiseptic and hepatoprotective properties^{10&15}.

Present paper is focused on the biological activities like antimicrobial and antioxidant activities and identification of major bioactive compounds in leaf extracts of *C. oppositifolia*.

The fresh plant material was collected from Melghat forest ranges, Chikhaldara region, District Amravati (MS) India, during November-December 2010. The plant material was identified taxonomically, using floras^{7&12}.

A specimen voucher was submitted in the herbarium of Department of Botany, Shri Shivaji College, Akola (MS).

The collected plant material was shade dried and powdered. This powder was used for further analysis. The powder was then extracted using Soxhlet extracts to obtain methanol and chloroform fractions. These methanolic and chloroform extracts were then used to analyze their antimicrobial activity⁴ against *E. coli*, *S. aureus*, *S. subtilis* and *C. albicans*; and antioxidant activity¹⁴. The antimicrobial activity was determined as zone of inhibition and the antioxidant activity was analyzed as percentage scavenging of free radicals. The IR, NMR and MS data of these extracts were obtained from SAIF, CDRI, Lucknow to detect the major bioactive compounds in them.

Plants being rich in chemicals, showed varied effects when used as individual or in combination. These various properties are efficiently being utilized by mankind for their benefits. A large number of plant based

medicines are the examples of such utilizations⁸.

Biological activities:

The methanolic and chloroform extracts of *Colebrookea oppositifolia* were tested for antimicrobial activity against *E. coli*, *S. aureus*, *S. subtilis* and *C. albicans* using disc diffusion method. Tetracycline was used as standard positive control for antimicrobial activity. It was found that, methanolic extract showed significant high antimicrobial activity than chloroform extract. This activity was also comparable to standard positive control (Table-1).

Antioxidant activity of methanolic and chloroform extracts was analyzed as % scavenging activity of DPPH radicals. BTH was used as standard control. It was found that both extracts showed significant antioxidant activity, however methanolic extract showed slightly more activity than chloroform extract. It was also observed that as the concentration of extract increases, the activity also increases (Table- 2).

Table-1. Antimicrobial activity (zone of inhibition in mm) of *Colebrookea oppositifolia* leaf extracts (n=3)

Samples	<i>E. coli</i>	<i>S. aureus</i>	<i>S. subtilis</i>	<i>C. albicans</i>
	Zone of inhibition in mm			
Methanolic extract	17.60± 1.30	20.50± 0.95	15.10± 1.05	17.80± 1.60
Chloroform extract	12.50± 0.85	15.30± 1.10	11.50± 2.20	13.35± 0.90
Tetracycline	28.15± 0.85	32.50± 1.25	25.80± 1.20	30.50± 1.25

Table- 2 Antioxidant activity (% scavenging of DPPH radical) of *C. oppositifolia* methanolic and chloroform extract (n=3)

Samples	Conc. In assay (mg/ml)	% scavenging of DPPH radical
<i>C. oppositifolia</i> methanolic extract	500	83.42± 0.18
	250	72.16± 1.20
	125	55.20± 0.17
	50	32.50 ± 0.10
<i>C. oppositifolia</i> chloroform extract	500	72.60± 0.24
	250	66.50± 0.60
	125	51.11± 0.78
	50	27.46± 0.23
BTH (standard antioxidant)	50	85.20 ± 0.20
	25	68.45± 0.12
	10	40.58± 0.04

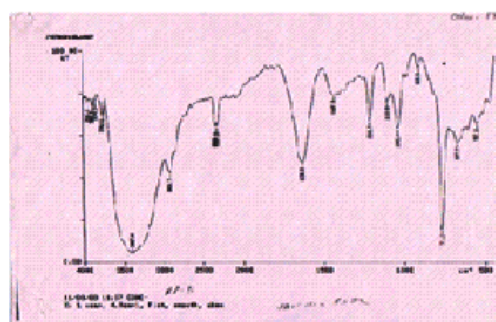
IR, NMR, MS analysis of Extracts:

The IR, NMR, MS chromatograms of methanolic extracts are presented in fig. 1. The data obtained was interpreted. The IR analysis showed 3480-3300 (OH), 2923-2853 (C-H), 1710 (COOH), 1655 (C= O in flavon), 1607-1498 (aromatic rings). NMR analysis showed δ ppm: 7.43 (d, J=2 Hz, H-6'); 7.41 (d, J=2 Hz, H-2'); 6.8 (d, J=8.4 Hz, H-5'); 6.77 (d, J=2 Hz, H-8'); 6.42 (d, J=2 Hz, H-6'); 3.68 (d, J=9.5 Hz, H-5'') leading to structural skeleton. The MS analysis gives about the actual structure with molecular mass of the compound. It was found that the molecular mass of detected compound was 287 and the chemical formula as C₁₂H₂₀O₁₁. From it was concluded that the compound identified as leuteolin-7- glucoside (Fig. 2).

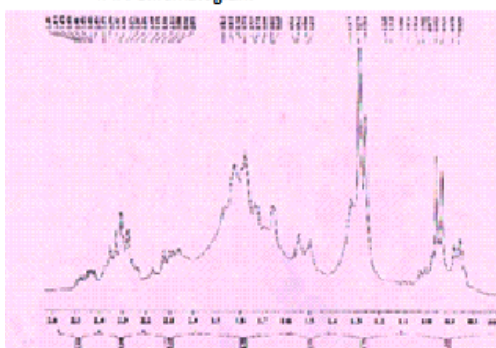
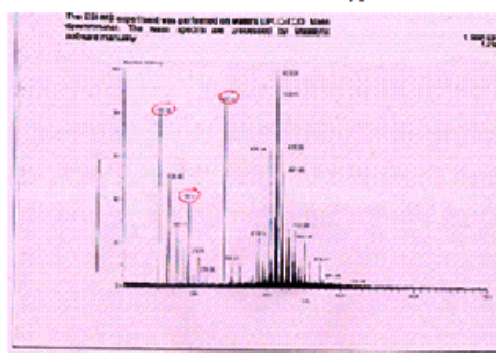
The IR, NMR, MS chromatograms of

methanolic extracts are presented in fig. 3. The data obtained was interpreted. It was found that, the detected compound has at least one aromatic ring attached to methanolic and hydroxyl functional groups. The MS analysis gives about the actual structure with molecular mass of the compound. It was found that the molecular mass of detected compound was 165 and the chemical formula as C₁₀H₁₂O₂. From it was concluded that the compound identified as leuteolin-7- glucoside (Fig. 4).

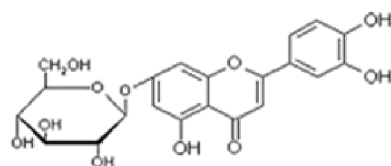
The data obtained from the antimicrobial and antioxidant activities of methanolic and chloroform extracts of leaves of *C. oppositifolia* showed that both extracts has significant antimicrobial and antioxidant activity. These activities can be correlated with the detected compounds in these extracts. There are some



i. IR Chromatogram

ii. NMR Chromatogram
of methanolic extract of *Colebrookea oppositifolia*

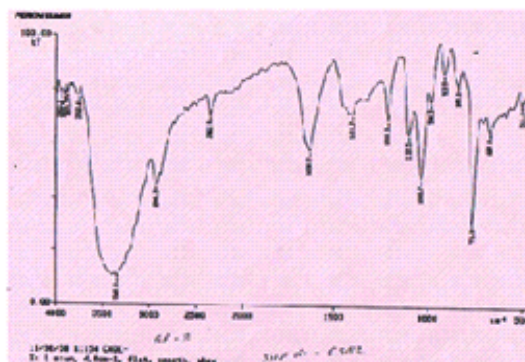
iii. MS Chromatogram

Fig. 1. IR, NMR, MS chromatogram of methanolic extract of *C. oppositifolia*

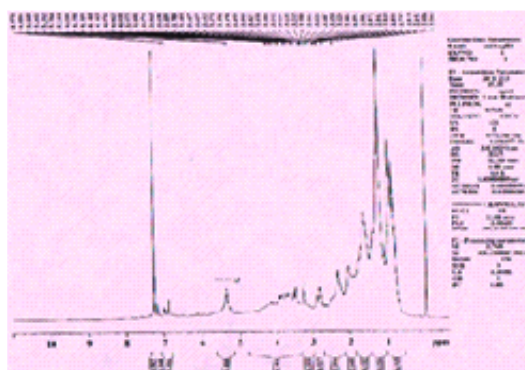
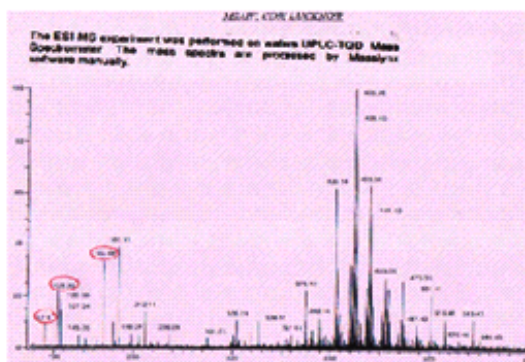
Compound predicted as Luteolin-7- glucoside

Molecular mass= 287

Fig. 2. Structure of identified compound with molecular mass



i. IR Chromatogram

ii. NMR Chromatogram
of Chloroform extract of *Colebrookea oppositifolia*

iii. MS chromatogram

Fig.3. IR, NMR, MS chromatogram of Chloroform
extract of *C. oppositifolia*

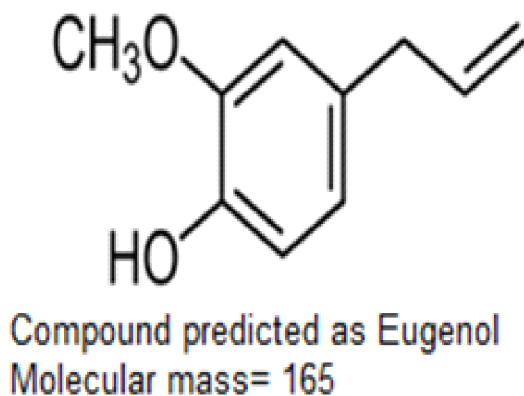


Fig. 4. Structure of identified compound with molecular mass

previous reports indicating that compounds like leuteoline-7 – glucoside and eugenol having aromatic ring with hydroxyl as one of the functional groups showed such bioactivities^{1-3, 10-11 & 15}. However, this is the first report on detection of such bioactive compounds from the leaf extracts of *C. oppositifolia*. These phytocompounds must have to be further screened for their individual bioactivities which might lead to the development of drug material in future.

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