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Phytochemical investigation and spectral studies of isolated flavonoid from ethanolic extract of whole plant *Blumea lacera* D.C.

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Abstract

Blumea lacera D.C. (Asteraceae) is one of the common Rabi weeds of India. It is an annual herb, with a strong odor of turpentine. Indigenously in the Indian system of traditional medicine- Ayurveda it is used as bitter, astringent, acrid, thermogenic, errhine, anti-inflammatory, styptic, ophthalmic, digestive, anthelmintic, liver tonic, expectorant, febrifuge, antipyretic, diuretic, deobstruant, and stimulant. Phytochemical investigation on the aerial parts of *Blumea lacera* revealed to be quite rich in essential oils contain *Nagi camphor* as its major constituent, β -caryophyllene, α -humulene and E- β -farnesene, thymoquinol dimethyl ether and precocene. It also contains other volatile fraction of cineol, d-fenchone, and citral.

In course of a systematic phytochemical studies of the *Blumea lacera* led us to isolate a *flavonoid compound-I*, (5-hydroxy-3'-methyl-3, 6, 7, 4'-tetramethoxyflavone) from the ethanolic extract of the whole plant. Isolated flavonoids from the ethanolic extract of *Blumea lacera* DC were separated and purified under column chromatography and analyzed by spectroscopy methods (UV, IR, ¹H NMR, and Mass).

Keywords: *Blumea lacera*, Asteraceae, Flavonoids, 5-hydroxy-3'-methyl-3, 6, 7, 4'-tetramethoxyflavone, *Nagi camphor*.

1. Introduction

Blumea lacera D.C. (Asteraceae) is one of the common Rabi weeds of India^[1]. It is an annual herb, with a strong odor of turpentine. Stem is erect, ash colored, densely glandular, pubescent. Leaves are often incised or lyrate. There are many yellow flower heads in single plant, arranged in axillary cymes or terminal panicle. Pappus is white. Fruits are an achene, oblong and not ribbed. Flowering time January to April^[2].

It is used indigenously in the Indian system of traditional medicine as a remedy for the various ailments, being used as *Blumea* in ayurveda as bitter, astringent, acrid, thermogenic, errhine, anti-inflammatory, styptic, ophthalmic, digestive, anthelmintic, liver tonic, expectorant, febrifuge, antipyretic, diuretic, deobstruant, and stimulant^[3]. Internally the decoction given for worms, it is very useful in various catarrhal infections, in dysentery and chronic uterine discharges^[4]. A tincture is useful in case of bleeding piles^[5]. The root kept in the mouth said to cure disease of the mouth. In the Konkan region of India, the plant used to drive away fleas and other insects. It prescribed, as an antiscorbutic in West Africa^[6]. A recent study on the hot water extract of *B. lacera* revealed its broad anti-leukemic activity at magnitudes ranging from moderate to mild^[7]. It also suppressed the replication of herpes simplex virus types 1 and 2 (HSV-1/HSV-2), indicating antiviral activities^[7].

Earlier work on the aerial parts of this plant revealed it to be quite rich in essential oils contain *Nagi camphor* as its major constituent^[8], β -caryophyllene, α -humulene and E- β -farnesene, thymoquinol dimethyl ether and precocene^[9]. It also contains other volatile fraction of cineol, d-fenchone, and citral. In a studies, Laakso I, *et al.*, isolated, two new glycosides from the whole plant extract of *B. lacera*, the triterpenoid glycoside 19 α -hydroxy-12-ene-24, 28-dioate-3-O- β -D-xylopyranoside and the phenol glycoside 2-isoprenyl-5-isopropylphenol-4-O- β -D-xylopyranoside from the whole plant of *B. lacera*^[10]. In the course of study, by Caius JF *et al.*, phytochemical investigation of *Blumea lacera* D.C. (Asteraceae) reported coniferyl alcohol diangelate, thymol-3-O- β -glucoside, β -sitosteryl-3-O- β -D-glucopyranoside, and stigmasteryl-3-O- β -D-glucopyranoside^[11].

In course of a systematic phytochemical studies of the *Blumea lacera* led us to isolate a *flavonoid compound-I*, (5-hydroxy-3'-methyl-3, 6, 7, 4'-tetramethoxyflavone) from the

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ethanolic extract of the whole plant. Isolated flavonoids from the ethanolic extract of *Blumea lacera* DC were separated and purified under column chromatography and analyzed by spectroscopy methods (UV, IR, ¹H NMR, and Mass).

2. Material and Methods

2.1. Extraction and Isolation

The whole plants collected from Dist. Gorakhpur (Uttar Pradesh) during the month of November 2008, the plant material taxonomically identified and authenticated by Dr. P.B. Singh, Head in charge of Regional Research Institute Ayurveda, Gwalior Road, Jhansi, where a voucher specimen deposited. The collected materials thoroughly cleaned, air dried under shade & powdered with a mechanical grinder. The powdered plant material then passed through sieve # 40 & stored in an air-tight container for further use.

The air-dried powdered plant material (700 g) was defatted with petroleum ether (40-60 °C) in a Soxhlet apparatus. The defatted plant material completely dried under reduced pressure to obtain a dry mass. Dry defatted plant material again packed in Soxhlet and extracted with ethanol at 45 °C for complete extraction (15 cycles). The yield of ethanolic extract found to be 2.99% w/w.

2.2. Preliminary Phytochemical Investigation

The plant extract (petroleum ether and ethanolic) were subjected for the phytochemical screening for the detection of various plants constituents. The ethanol extract was used for the preliminary phytochemical investigation indicated the presence of Glycosides, Triterpenoids, Phenolics compounds, Flavonoids & Vitamin C.

TLC studies of ethanolic extract of *Blumea lacera* show nine spots of a different color, in the solvent system - Chloroform: Benzene: Formic acid (3: 1: 12 drops), shown in **fig. 1**, carried out in Lab. It indicates the presence of nine different components in the extract.

2.3. High Performance Thin Layer Chromatography (HPTLC):

HPTLC studies were carried for the better isolation and identification of the different components of ethanolic extract of *Blumea lacera*. The report of HPTLC indicates the presence of eleven spots in the solvent system - Chloroform: Benzene: Formic acid (3: 1: 12 drops), carried out at NBRI, Lucknow.

HPTLC Parameters

- Sample preparation: 10 mg/ml
- Sample application: Linomat 5 applicator (Camag).
- Volume applied: 10 µl
- Solvent system: Chloroform: Benzene: Formic acid (3: 1: 12 drops).
- TLC plate development: Presaturated Camag Twin Trough Chamber.

2.4. Isolation of Flavonoids by Fractionation Method

Flavonoids from the ethanolic extract of the *Blumea lacera* were isolated on the basis of the solubility of the different constituents by fractional method, summarized in **fig. 2**. Ethanolic extract (50 ml) was dissolved in the distilled water (100 ml). The recovered water insoluble fraction was dissolved in the chloroform (100 ml) and shake for 15 mins. The chloroform soluble fraction was discarded and insoluble fraction dissolved further in ethyl acetate (100 ml) by shaking 15 min. and filtered. Ethyl acetate soluble fraction was

discarded and the insoluble fraction left on filter paper was subjected for TLC studies and qualitative test for flavonoids. To procure the pure compound it was crystallized with methanol. There after the compound (green crystals) obtained was designated as flavonoids.

The qualitative test shown positive for the flavonoids and the TLC studies in Chloroform: Benzene: Formic Acid (7:3:12 drops) shown 3 spots. Column Chromatography was performed to isolate different flavonoids from the mixture of flavonoids obtained from the fractional separation of ethanolic extract of the *Blumea lacera*.

2.5. Column Chromatography for the Separation of the Flavonoids Mixture

Slurry of the silica gel (60-120 mesh) was prepared in n-hexane and poured into the column. Filter paper was placed when the silica gel settled down as to avoid the cracking of the column and maintained the level of solvent system about 2 cm. The isolated flavonoids were mixed uniformly with silica gel of (60-120 mesh) with the help of spatula. The prepared mixture was carefully placed over the filter paper without disturbing the column and maintained the level of solvent system up to 2 cm. After loading the sample on the column, the solvent system n-hexane, chloroform, benzene and acetone were added according to increasing order of their polarity.

The each eluent fraction was analyzed for the presence of components by TLC, using iodine vapor as locating agent. Fraction having one spot was further analyzed for the nature of the component via, ¹H NMR, FT IR and Mass spectroscopy.

3. Results and Discussions

The whole plants collected from Dist. Gorakhpur (Uttar Pradesh) during the month of November 2008. Plant material taxonomically identified and authenticated by Regional Research Institute Ayurveda, Gwalior Road, Jhansi. Dry defatted plant material packed in Soxhlet and extracted with ethanol at 45 °C for complete extraction (15 cycles). The yield of ethanolic extract found to be 2.99% w/w. The ethanol extract found (+)-tive for the presence of glycosides, triterpenoids, phenolics compounds, flavonoids and vitamin C. TLC studies of ethanolic extract of *Blumea lacera* show nine spots of a different color, in the solvent system - Chloroform: Benzene: Formic acid (3: 1: 12 drops), shown in **fig. 1**, carried out in Lab. The R_f values are mentioned in **Table 1**. It indicates the presence of nine different components in the extract.



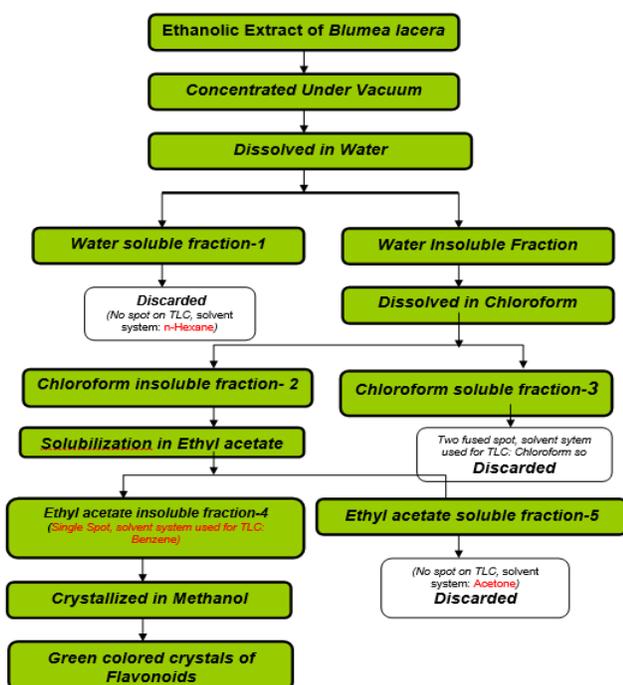
Fig 1: TLC Chromatogram of Ethanolic Extract of *Blumea lacera* in Solvent system Chloroform: Benzene: Formic acid (3:1:12 drops), performed in lab.

Table 1: Rf values and color of the TLC spots in solvent system - Chloroform: Benzene: Formic Acid (3:1:12 drops).

S. No.	Rf value	Color
1	0.03	Dark green
2	0.05	Light green
3	0.13	Light Brown
4	0.18	Green
5	0.23	Light yellow
6	0.28	Light green
7	0.33	Yellow
8	0.40	Light Brown
9	0.47	Dark green

HPTLC studies indicates the presence of eleven spots in the same solvent system - Chloroform: Benzene: Formic acid (3: 1: 12 drops), carried out at NBRI, Lucknow.

The ethanolic extract subjected for the fractional isolation of the flavonoids based on the solubility principal of the different constituents, **fig. 2**.

**Fig 2:** Scheme: Isolation of Flavonoids of *Blumea lacera* DC. by Fractional method based on the solubility of different constituents.

The qualitative test for isolated mixture obtained after the fractional separation shown positive for the flavonoids, **Table 2**, and its TLC studies in Chloroform: Benzene: Formic Acid (7:3:12 drops) shown 3 spots, **Table 3**.

Table 2: Qualitative tests for the Isolated Flavonoids from Ethanolic Extract of *Blumea lacera*.

S. No.	Tests for flavonoids	Observation	Inferences
1.	Shinoda test	Pink color	Flavonoids present
2.	Drug + lead acetate	Yellow ppt.	Flavonoids present
3.	Drug + NaOH followed by addition HCl.	Yellow color appeared and disappeared on addition of HCl	Flavonoids present
4.	Drug + FeCl ₃	Deep Blue black color	Flavonoids present

Table 3: TLC Studies of isolated flavonoids from the ethanolic extract of *Blumea lacera*.

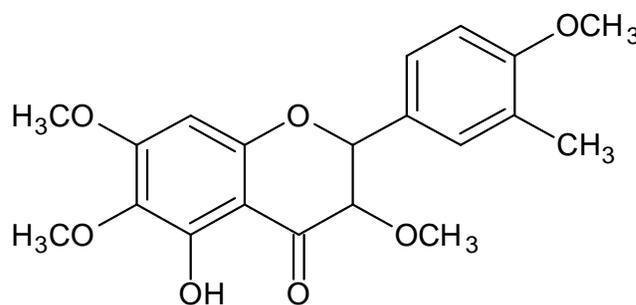
S. No.	Solvent system	No of spots	Rf values	Color of Spots
1.	Chloroform: Benzene: Formic Acid (7:3:12 drops)	3	0.03	Dark green
			0.23	Light yellow
			0.41	Yellow

The column chromatography of isolated flavonoids mixture were carried out with four solvent system n-hexane, chloroform, benzene and acetone and stationary phase silica gel. Four fractions were collected F-1, F-2, F-3 and F-4, on performing the TLC of the fractions; F-2 and F-3 were showing one and two spots respectively, **Table 4**. Since fraction F2 showing only one spots, so, it was further investigated via ¹H NMR, FTIR and Mass spectroscopy. Infra red (IR) spectra carried out on a Perkin Elmer spectrophotometer in KBr pellets. Ultraviolet (UV) spectra recorded with a Shimadzu UV-2501PC spectrophotometer. ¹H NMR spectra recorded in DMSO-d₆ using a JEOL A-500 spectrometer with TMS as an internal stand. The FAB mass spectra recorded with a JEOL HX-110 spectrometer. Chemical shifts values are given in δ-value (ppm) with tetramethylsilane (TMS) as internal standard.

Table 4: TLC Studies of different Elutes of Column Chromatography.

S. No.	Fractions	Solvent system (Elutes)	No. of spots
1.	F-1	n-Hexane	Nil
2.	F-2	Benzene	One
3.	F-3	Chloroform	Two
4.	F-4	Acetone	Nil

On the basis of the available spectral data and on comparing the reported literature of Mabry *et al.*, 1970; Markham *et al.*, 1978, the compound –I were identified as 5-hydroxy-3'-methyl-3, 6, 7, 4'-tetramethoxyflavone (Flavonoid), **fig 3**.

**Fig 3:** Isolated Flavonoid: 5-hydroxy-3'-methyl-3, 6, 7, 4'-tetramethoxyflavone.**Compound I**

Compound I obtained as pale yellow amorphous solid with m.p.:150-152 °C, eluted with benzene, showed a positive ferric chloride and Shinoda test for flavonoids, indicating that compound may be a flavonoid. The positive ferric chloride test indicates that the compound have free hydroxyl group at C-5 [14]. The **UV spectrum study reveals** that the Bands in the 1605-1527 cm⁻¹ range are typical characteristic of a flavanone skeleton. IR spectrum indicated the presence of three hydroxyl groups (3412.5 cm⁻¹). The band at 1368.2 cm⁻¹ corresponds the keto-enol tautomerism between C=O and 5-OH groups. The band at 2928.1 cm⁻¹ indicates the presence of symmetric –

OCH₃ groups. Peak reported at 571 cm⁻¹ corresponds to oxo (-O-) group stretching.

The ¹H NMR spectrum, measured in MeOD, revealed a down field signal at δ 5.78 ppm, indicating a hydroxyl group at C-5, which was deshielded by the hydrogen bonding with the carbonyl carbon at C-4. Two meta-coupling protons of ring-A also observed at δ 6.18 ppm (1H, d, J_{6,8}=2.5Hz) and δ 6.22 ppm (1H, d, J_{8,6}=2.5 Hz). The ¹H NMR showed methoxy singlets resonating at δ 3.80 ppm (-OCH₃, C-7 and C-6). Furthermore, a methylene proton, H-2, occurred as double doublet at 3.20 ppm, which indicated an axial-axial and an axial-equatorial coupling to H-3a and H-3b, respectively. The H-2' and H-6' pair occur in an identical environment and these are centered at δ 6.99 ppm and δ 6.89 for C-5'.

The mass fragmentation pattern of isolated flavonoid was shown in fig 4. The mass spectrum of the compound showed important mass peaks at m/z 361 [M+H]⁺, 320 [M+H-3 x CH₃]⁺, 302 [M+H-3 x CH₃]⁺, 168 [R. D. A. cleavage at ^{1,3}A⁺], 181 [^{0,2}A⁺, fragmented ion peak], 136 [^{0,2}B⁺, fragmented ion peak]⁺, 135 [^{1,3}B⁺, fragmented ion peak]⁺. The MS fragmentation pattern clearly indicated that three methoxy group, attached C-7 and C-6 of the ring A and one at C-3' of ring B. It also reveals the presence of three hydroxyl group at C-5, C-3 and C-4'.

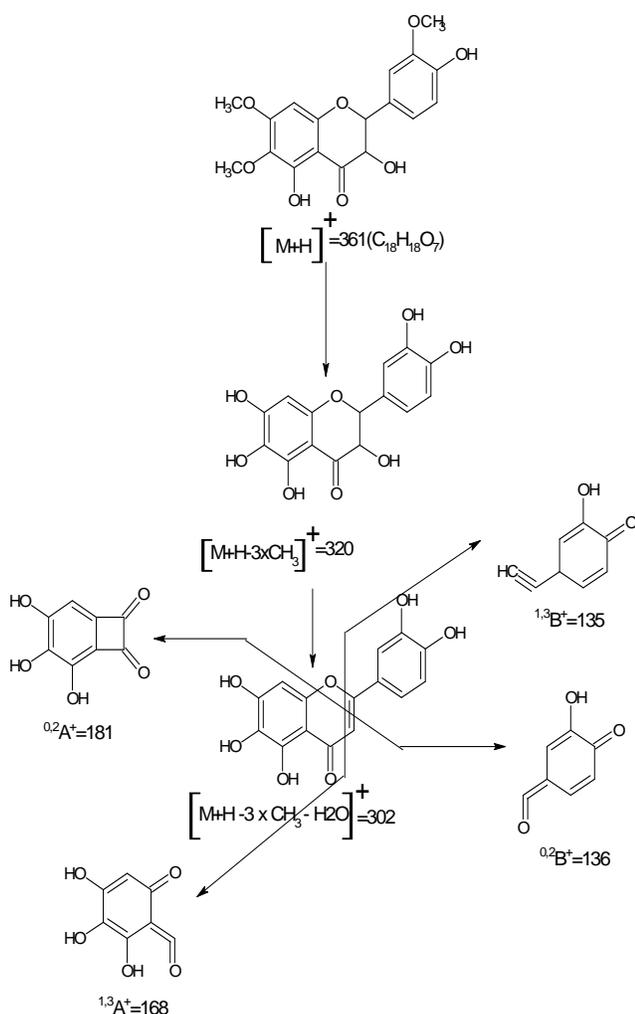


Fig 4: Proposed Mass fragmentation pattern of Compound -I

4. Acknowledgement

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5. References

- Oudhia P, Tripathi RS. A useful weed *Blumea lacera* L: A review. Indian J Weed Sci 1999b; 31(1-2):108-109.
- Agharkar SP. Medicinal plants of Bombay presidency. Scientific Publishers, Jodhpur, India, 1991.
- Chopra IC, Handa KL, Kapur LD, RN. Chopra's. Indigenous drugs of India. Edn 2, Calcutta: Academic Publishers, 1982.
- Kapoor LD. Hand book of Ayurvedic medicine Plant. Herbal Reference Library, 78.
- Ghosh NC. Comparative Materia Medica. Calcutta, India: Hannemann Publ. Co. Pvt. Ltd, 1988, 855.
- Caius JF. The Medicinal and Poisonous Plants of India. Scientific Publ. Jodhpur, India 1986, 323-325.
- Chiang LC, Cheng HY, Chen CC, Lin CC. *In vitro* antileukemic and antiviral activities of traditionally used medicinal plants in Taiwan. Am J Chin Med 2004; 32:695-704.
- Manjunath L. Wealth of India: Raw Materials. Council of Scientific and Industrial Research, New Delhi 1948; 1:197.
- Anon C, Sureeporn H, Kanjana N, Thanidtha T, Nuchnipa N, Apichart S. Chemical constituents of *Blumea lacera* (burm.f.) DC. 31st Congress on Science and Technology of Thailand at Suranaree University of Technology, 18 – 20 October, 2005.
- Laakso T, Seppänen L, Hiltunen R, Ekundayo O. Composition of the essential oil of *Blumea lacera* DC. (Asteraceae) leaves from Nigeria. Flavour and Fragrance Journal 2006; 4:73-75.
- Agarwal R, Singh R, Siddiqui IR, Singh J. Triterpenoid and prenylated phenol glycosides from *Blumea lacera*. Phytochemistry March 1995, 38:935-938.
- Rao CB, Rao TN, Muralikrishna B. Flavonoids from *Blumea lacera*. Planta Medica 1997; 31:235-237.
- Pal R, Moitra SK, Chakravarti NN, Adhya RN. Campesterol from *Blumea lacera*. Phytochemistry 1972; 11:1855-1857.
- Geissman A. The Chemistry of Flavonoid Compounds. New York: The MacMillan Company, 1962.
- Harborne JB, Mabry TJ, Mabry H. The Flavonoids, London: Chapman and Hall, 1978, 45-77.
- Mabry TJ, Markham KR, Thomas MB. The Systematic Identification of Flavonoids, New York: Springer, 1970, 24-26.