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Research Article

ISOLATION AND BIOLOGICAL EVALUATION OF SOME NOVEL PHYTOCONSTITUENTS FROM *BLUMEA LACERA* (BURN F.) D.C.

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ABSTRACT

Blumea lacera(burn. F) D.C. known as *Kukraunda* is an important drugdescribed in Ayurveda as bitter, thermo-genic, anti-inflammatory, styptic ophthalmic, digestive, anthelmintic, liver tonic, expectorant, febrifuge, antipyretic, diuretic and stimulant. The whole plant was extracted with hexane followed by ethanol, the extract so obtained was fractionated using chloroform to obtain chloroform soluble fraction. Four compounds were isolated from chloroform soluble fraction using column chromatography and characterized by techniques likeInfrared spectroscopy (FTIR) and Nuclear magnetic resonance ^{1}HNMR. Isolated compounds were screened for antibacterial activity. The compounds showed significant antibacterial activity against *B. subtilis* and *E. coli*bacteria.

Keywords: *Blumea lacera,* Antibacterial, Isolation, Column chromatography, *Kukraunda.*

INTRODUCTION

Nature has been a source of medicinal treatment for thousands of years. Now days, the search for new chemotherapeutic agents has been expended to the whole biodiversity. An increasing number of chemotherapeutic agents are discovered as a result of chemical studies directed towards the isolation of the active substances from plants used in traditional medicine. Despite the great success achieved in natural products chemistry and drug development, we have barely begun to tap the potential of our molecular diversity.

Blumea lacera (burn. F) D.C. in an important Ayurvedic, Homeopathic, Unani drug entering into several formulations. The drug is known by the name of *Kukraunda* in India. Blumea in described in Ayurveda as bitter, thermo-genic, anti-inflammatory, styptic ophthalmic, digestive, anthelmintic, liver tonic, expectorant, febrifuge, antipyretic, diuretic and stimulant.[1]The plant is used to drive away fleas and others insects in several parts of India. It is described as an anti-scorbutic in West Africa,[2] essential oils from Blumeahas been shown analgesic, hypothermic and tranquilizing activities.[3] The phytoconstituents separated in leaves are 5-hydroxy-3,6,7,3',4'-pentamethoxy flavone, 5,3',4'-trihydroxy flavone and an unidentified flavone and in aerial parts compestrol has been isolated.[4]The leaves are stomachic, anti-spasmodic and diaphoretic.[5,6,7]

MATERIALS AND METHODS

Plant material

Whole plants were collected from the fields around the campus of Indira Gandhi Krishi Vishwavidyalaya (Agriculture University) Raipur, Chhattisgarh, Indiaduring the months of November and December 2007,identified and authenticated by Dr.Pushpa Patel, Govt. PG College, Khargon, MP.

Chemical and reagent

All the chemicals and reagents used in the study were of analytical grade obtained from MERCK and their boiling points were compared with the available literature values.

Extraction and fractionation

The collected plant material was segregated from the extraneous material and dried in shade to prevent the loss of active ingredients. About 1 kg of shade dried coarsely powdered drug was extracted with n-hexane followed by 95% ethanol in a soxhlet apparatus by continuous hot extraction. Ethanol extract were filtered and concentrated to dryness under reduced pressure to obtain semisolid mass.A portion of ethanol extract was fractioned with chloroform. The solvent was removed by distillation to obtain the fraction. Ethanol and chloroform fraction were subjected to preliminary phytochemical analysis[8,9,10]to detect the presence of various types of phytoconstituents.

Chromatographic studies

Chromatographic studies were carried out following Harborne (1998)[9], Stahl (2005)[11]and Wagner et al (1996)[12]. Thin layer chromatography fingerprinting was performed to detected presence of various phytoconstituents. The TLC plates were prepared by using silica gel G and activated at 105 ^{0}C for 1 hr prior to experiment. Extract was applied on the silica gel plates as a single spot using a capillary tube. Chloroform fraction chromatogram was developed in the TLC chamber containing hexane: ethyl acetate: methanol (10:3:1.5) as solvent system and ethanol fraction was developed in ethyl acetate: methanol: toluene: water (5:4:7:0.5) as solvent system.

Plates were developed for a migration distance of about 80% of the total height of the plate and then after drying, plates were observed under 254 and 365 nm. And finally after exposing to iodine vapours. The Rf value was calculated for each observed spot.

Isolation of phytoconstituents by column chromatography

The chloroform soluble fraction obtained from ethanol extract of *Blumea lacera*was packed in a column. The elution in the silica gel column was started with hexane and thereafter ethyl acetate was added up to 100%and then methanol was gradually added from 10-100%. The fractions (100 ml) were collected from the column and those having the same TLC profile were mixed together. The mixed crude fractions from the column were subjected to re-chromatography in silica gel column,

Fraction A (hexane-ethyl acetate, 9:1) yielded a colourless liquid on chromatographing this fraction with hexane, which was designated as compound no. 80. On addition of hexane to fraction B (hexane-ethyl acetate, 2:8) compound no. 81 crystalize out and collected as white amorphous solid mass. After separating 82 the remaining liquid was dried and again chromatographed with mixture of hexane and chloroform (6:4), which yielded compound no. 83.

Spectral analysis and structural elucidation

The identification of a molecule was done through the interpretation of the data obtained from spectroscopic analysis. The functional groups were identified by Infrared spectroscopy (FTIR). ^{1}HNMR analysis was performed to determine the chemical shift values of the protons as well as the number of neighboring protons from the splitting of the multiplets. The determination of carbon, hydrogen,

and nitrogen (CHN analysis) was performed to check sample purity, and in conjugation with mass spectrometry and NMR data, to characterize the compound.

Antibacterial activity

All isolated phytoconstituents were screened for *In vitro* antimicrobial activity by agar diffusion cup plate method following standard protocol.[13,14] All the compounds were screened for antibacterial activity at 50 and 100 µg/ml concentrations against the strains *Escherichia coli* and *Bacillus subtilis*. The melted nutrient agar was cooled to 45^0C, mixed with culture media by gentle shaking and then poured onto a sterilized petri dish and allowed to solidify. Cups were made by punching the agar surface with the help of a sterile cork borer (6 mm). Suspension of isolated compounds in the strength of 50 and 100µg/ml was preparedusing distilled water as solvent and added to each bore. The drug was allowed to diffuse for about 4 h into the agar medium before the addition of bacterial suspension. Norfloxacin was used as standard drug. The inoculated plates were then incubated at 37^0C for 48 h. The zone of inhibition for each isolated compound was recorded and measured in millimeters (mm).

RESULTS AND DISCUSSION

Phytochemical and chromatographic studies

Preliminary phytochemical studies of *Blumea lacera* revealed the presence of alkaloids, carbohydrates, glycosides, phytosterols, phenolic compounds and flavonoids.

Thin layer chromatographic studies of the chloroform fraction revealed 4 phytoconstituents of Rf 0.75, 0.56, 0.37 and 0.31 under sunlight and 7 phytoconstituents as Rf 0.82, 0.70, 0.58, 0.30, 0.27, 0.23 and 0.17 when exposed to iodine vapours.

Isolation of phytoconstituents

Four compounds were isolated by silica gel column chromatography from chloroform soluble fraction of ethanol extract of *Blumea lacera*.

Compound 80 was isolated as a colourless oily liquid. Its IR peaks (cm^{-1}) at 3425 (free –OH of an aliphatic moiety), 2923 (aromatic C-H), 1734 (conjugated C-O) 1653, 1377 (aromatic C-C), 1460 (ά- CH_2), 1260 and 1094 (aryl ether C-O-) and ^{1}HNMR values δ 4.12m br (-OCH_2CH_3), 4.17s, 6.14s (-OH) Confirmed that it is a derivative of protocatechuic acid with an aliphatic moiety having a free hydroxyl group.

Compound 81 gave positive test with dragendroff's reagent and exhibited IR peaks (cm^{-1}) at 3429 (primary amide linkage), 2921(aliphatic –CH_3), 2852(-CH_2), 1737 (-COO-NH_2), 1664 (-$CONH_2$), 1459, 1373 (CH_3-CH_2 bending)1176, 1163 (C-N stretching) and 1051 (-C-O-C). Its ^{1}HNMR peaks were atΔH (ppm) 0.82dd (J=1.5, 1.01Hz, -CH_3), 1.29t (CH_2-CH_3), 2.15s (-$COCH_3$), 3.47m br (-$CONH_2$), 7.26s (Ar-H).

Compound 82 exhibited IR Peaks (cm-1) at 3413, 2922, 2852, 1664,1379, 1077, 658 and 1 H NMR peaks (ppm) at δ_H 0.10 s, 0. 86s br, 1.25s br, 1.61s, 1.95s, 2.13s, 2.29s br, 2.51d (J=1.6 Hz), 2.77d br (J=14.0Hz), 3.7t (J=4.0) were showed some similarity to 82 The IR and 1HNMR values of these two compounds matched with published literature for compounds with primary and secondary amide moieties respectively.

Compound 83 showed IR peaks (cm^{-1}) 3362 (-OH), 1602-1003(Characteristic for flavonoids skeleton) characterized the compound shaving a flavonoids moiety39, and 1HNMR values δ 4.12m br (-OCH_2CH_3), 4.71S, 6.14S (-OH) Confirmed that it is a derivative of phytosterols with an aliphatic moiety having a free hydroxyl group.

Antimicrobial activity

The determination indicates whether the organism is 'sensitive' or 'resistant' to the agent. The organism being 'sensitive' means that the antimicrobial agent at clinically attainable concentration inhibits growth of the organism; 'resistant' means that the growth of the organism is not inhibited.The test results of this study are depicted in Table 1.

Table 1: Table shows antibacterial activity (zone of inhibition) of isolated compounds.

S. No.	Compound	Diameter of zone of inhibition in (mm)			
		B. subtilis (Gram +ve)		*E. coli (Gram -ve)*	
		50 µg/ml	*100 µg/ml*	*50 µg/ml*	*100 µg/ml*
1	80	7	11	5	9
2	81	10	17	13	21
3	82	8	14	6	11
4	83	6	13	9	13
5	Norfloxacin	20	22	25	28
6	Control	NI	NI	NI	NI

NI: No inhibition

CONCLUSION

The plant *B.lacera* (burm.f) D.C. compositae has been studied to compare and give report on phytochemicals investigations.The preliminary phytochemicals investigation showed the presence of alkaloid, carbohydrate, steroids phenol, flavonoids, and glycosides in different extracts further TLC was performed for extract for identification of constituents and compound were isolated from chloroform soluble fraction by using column chromatography.The spectral analysis (NMR, IR) indicate that the isolated fraction having constituents may be phytosterols, primary and secondary amide, derivatives of protocatechuic acid and flavonoids.

The phytochemicals study of *B.lacera* reveals that the chloroform soluble fractions of its ethanol extract constituted major classes of bioactive constituents. The chloroform soluble fraction showed significant antimicrobial activity.

Hence we may conclude that *B.lacera* may prove to be a valuable medicinal plant with some bioactive constituents and through furtherstudies we may able to isolate more phytoconstituents, which can be medicinally important.

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REFERENCES

1. Warrier PK, Nambiar VRK and RamakuttyC, Indian Medicinal Plants. Vol. I.Chennai: Orient Longman (India);1996. P.278-280.
2. Caius. JF. The Medicinal and Poisonous Plants of India. Jodhpur:Scientific Publ;1986. p. 323-325.
3. Anonymous. Phytochemistry.1972, 11. P. 1855.
4. Rastogi RP and Mehrotra BN. Compendium of Indian Medicinal Plants. Vol. II. Lucknow:CDRI and New Delhi:P & I Directorate; 1991.

5. Lawrence HM. Taxonomy of vascular plants.New Delhi: Oxoford and IBH Publishing Co;1969. P.726-731.
6. Gangrade SK, Tripathi NK, Harinkhede DK, Mishra PK. Ethnomedicinal diversity used by tribal of Central India.Indore:Sam Com Digital Graphics; 2003. P. 43-44.
7. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants, New Delhi:National Institute of Science Communication, CSIR; 1999. p. 38.
8. Kokate CK. Practical Pharmacognosy. New Delhi: Vallabh Prakashan;1999. p.107-121.
9. Harborne JB. Phytochemical Methods. 3rded. London: Chapman and Hall; 1998. p. 4-7.
10. Raaman N. Phytochemical Technique. New Delhi: New Indiapublishing agency; 2006. p.18-24.
11. Stahl E. Thin layer chromatography. A Laboratory Hand Book. 2nded. Berlin: Springer; 2005. p. 60-67, 241-7.
12. Wagner H, Bladt S. Plant Drug Analysis. 2nded. Berlin: Springer; 1996. p. 54, 254.
13. NationalCommittee for Clinical Laboratory Standards (NCCLs). Standard methods for dilution antimicrobial susceptibility tests for bacteria which grows aerobically. *Nat. Comm. Lab. Stands*. Villanova; 1982. P. 242.
14. Kulkarni SK. Handbook of Experimental Pharmacology. 3rd ed.New Delhi: Vallabh Prakashan; 1999. P.125.