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EXTRACTION AND IDENTIFICATION OF DITERPENOID LACTONE FROM ANDROGRAPHIS PANICULATA

Kavita Kirar[1],
Deepak Kaurav[1], Jyoti Chourasiya[1], R.N. Shukla[1]

[1]P. G. Department of Applied Chemistry, Samrat Ashok Technological Institute, Vidisha (M.P.) India

ABSTRACT

Andrographispaniculatawhich is commonly known as Kalmegh is an important medicinal plant. Andrographolide is the main diterpenoid lactone contained in the leaves of Andrographispaniculata. This bioactive component has multifunctional medicinal properties such asactivity against fever, dysentery, diarrhoea, inflammation, and sore throat as well as immunedisorder. To date, extraction of andrographolide from Andrographispaniculatais usuallycarried out using liquid organic solvent.The extraction was carried out by employingmethanol as solvent using standard soxhlet method.The Methanol extract ofA.paniculatawas used for column chromatography to isolate its active ingredient- Andrographolide. The melting point of the colourless crystal recovered after column chromatography were recorded as 235^{0}C. Comparison of recorded characteristic IR spectra and melting point of isolated constituents with previous literatures indicates that the isolated crystal is the andrographolide of A. paniculata.

Correspondence to Author

Kavita Kirar

P. G. Department of Applied Chemistry, Samrat Ashok Technological Institute, Vidisha (M.P.) India

Email:

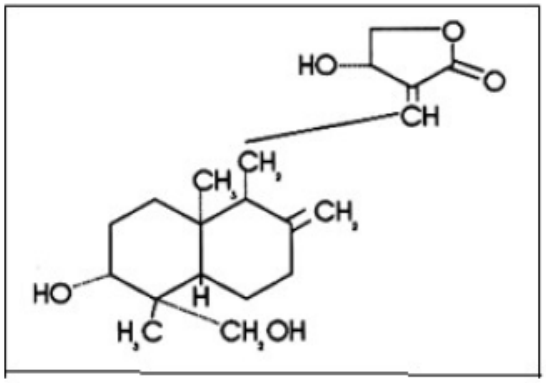
KEYWORDS *:* Andrographolide, Methanol.extraction, andrographolide, Andrographispaniculata, soxhlet.

INTRODUCTION

India is blessed with varieties of aromatic and medicinal plants. Among the medicinal plants, Andrographispaniculata (Kalmegh) has been used in Indian and Chinese herbal medicine..*Andrographispaniculata*NEES, locally known as HempeduBumi and commonly called as "King of Bitter" grows widely in the tropical area of South East Asia, India and China with annual growth of 0.30 - 0.70 m height. In Malaysia, this plant has been extensively used for traditional medicine and help against fever, dysentery, diarrhoea, inflammation, and sore throat. Furthermore, it is a promising new way for the treatment of many diseases, including HIV, AIDS, and numerous symptoms associated with immune disorders.[(1)]Andrographis contains active principle andrographolide (Fig.1).is a diterpenelactone with a very bitter taste and colourless crystalline in appearance.The molecular formula of andrographolide,
which is an unsaturated trihydroxy lactone, is $C_{20}H_{30}O_5$.Other active components include 14-deoxy- 11,12 didehydroandrographolide

(andrographolide-D),Homoandrographolide, andrographan, andrographon, andrographosterin and stigmasterol. Besides thesecompounds, the chemicals isolated from leaves are diterpenoidsviz-deoxyandrographolide, 19 b-Dglucoside and neo-andrographolide (ChemWiming and Liang Xiaotion, 1982). The leaves contain the highest amount of andrographolide (2.39%) while the seeds contain the lowest (Sharma et al., 1992). The roots contain Apigenin-7, 4'-di-O-methyl ether, andrographolide and a natural flavone, 5-hydroxy7,8,2',3'-tetramethoxyflavon.[(2)]

Fig.1-. Molecular structure of Andrographolide



MATERIALS AND METHODS

the plant andrographispaniculata were collected from vidisha and bhopal (m.p.) region.the leaves were then washed with water to remove mud and dust particles. the leaves were first dried in room temperature and then in the oven. the dried leaves were grinded to powder by a cyclotec grinder the powder was separated by sieving into five size classes (0.10–0.30, 0.30–0.45, 0.45–0.60 and 0.60–0.80 mm).Collect approx 100mg of andrographispaniculata air dried powder fill this air dried powder in a cotton cloth bag (thimble). at the bottom of the apparatus fill the solvent with 500 ml of a 90% in volume of methanol 500ml. continue the extraction process for 48 hours. after complete extraction cool the apparatus. the extract was filtered through fresh cotton bed and finally whatman no.1 filters paper. the filtrate was concentrated with a rotary evaporator at low temperature (400-5000c) and reduced pressure. the weight of the crude extract was 100mg. the concentrated methanol extract was fractionated by the column chromatography.

ISOLATION OF ANDROGRAPHOLIDE BY COLUMN CHROMATOGRAPHY:-Column chromatography Column was packed with slurry of silica gel (400gm -mesh size, 60-120) in ethyl acetate. The column 50cm in lenth and 5cm in diameter was used. Then dried Methanolextract (4 gm) of *A. paniculata*was first dissolved in Methanol and carefully applied by pipette at the topof prepared column. Immediately after application of sample, a gradient of Chloroform and Methanol(mobile phase) was used as eluant to collect fractions of Methanol extract of *A. paniculata*. The columnwas run with a gradient of Chloroform : Methanol (98:2, 95:5, 90:10, 80:20, 70:30, 50:50, 30:70, 20:80,10:90, 5:95, 2:98) finally 100% Methanol and 12 fractions (F1-F12) were collected.Thereafter, from all the collected fractions solvent was removed by evaporation at room temperature.After evaporation of solvent from the fractions F4 and F5, colourless crystals were isolated. The crystalsof two fractions were first separately treated with Petroleum ether and then filtered. The crystallineresidues were then retreated with Chloroform and were recovered after filtration. The identity of crystalswas confirmed by spectroscopic analysis.[(3)]

IDENTIFICATION OFISOLATED CRYSTALS :-The isolated constituent of A. paniculata (colourless crystal), were identified through IRspectrophotometer and melting point. IR spectroscopy of crystals of A. paniculata was takenin KBr. Finally the recorded characteristic IR spectraand melting points of isolated constituents of A. paniculata, were compared with previous literatures toassign their identity.[(3

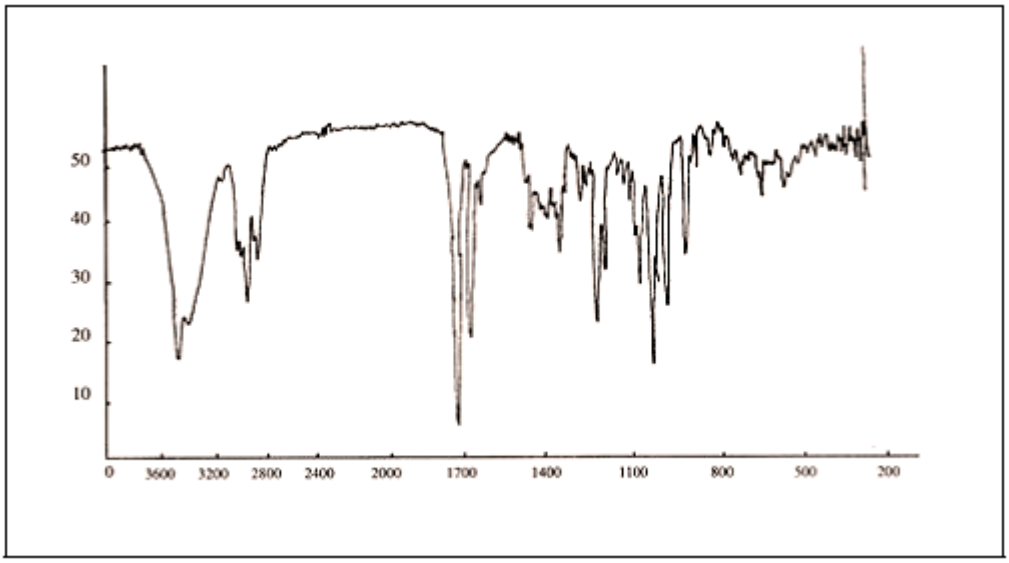


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Fig-2. IR spectrum of Andrographolide

Table-1.Peak positions and probable interatomic bonds of IR spectrum of crystal of A.paniculata.

Peak Position	*Interatomic Bond*
3100-3500 cm $^{-1}$	*O-H Stretching*
2800 – 3000 cm $^{-1}$	*C-H Stretching*
1725 cm $^{-1}$	*C=O Stretching*
1680 cm $^{-1}$	*C=O Stretching due to $_{\alpha,\beta}$ – unsaturation*
1640 cm^{-1}	*C=C Stretching*
1480 cm^{-1}	*C=C Stretching*
1380, 1420 cm $^{-1}$	*C = H deformation*
1220 , 1240 cm $^{-1}$	*C-O-C Stretching of Lactone ring*
980 , 1040 ,1090 cm^{-1}	*O-H deformation of alcohol*

RESULTS AND DISCUSSION :-

The melting point and other recorded properties of the isolated constituents (Fig-2) were presented in Table-2.melting point of andrographolide as 235^{0}C. The IR spectrum (Fig-2) showed characteristic peak positions (Table.1) of active ingredient-Andrographolide. The peak at 1725, 1680 cm-1; 1640, 1480 cm-1; 1220, 1240 cm-1; 980, 1040, 1090 cm-1 may be due to presence ofC=O, C=C, C-O-C of lactone ring and O-H group of alcohol respectively, present in the molecular structure of andrographolide.

Table-2. Properties of isolated colourless crystals of *A.* paniculata.

S no.	**Properties**	
1	**Appearance**	**crystalline**
2	**colour**	**colourless**
3	**Taste**	**Bitter**
4	**Odour**	**Odourless**
5	**Solubility**	**Soluble in methanol**
6	**Melting point**	**235 ^{0} C**

CONCLUSION

Finally it can be concluded that the isolated colourless crystals of *A. paniculata*is the Andrographolide. From the present study it can be confirmed that *Andrographispaniculata*can produces active ingredient-Andrographolide.Although andrographolide keeps the potency of a suitable lead candidate against

malaria, further optimizations are needed for its inclusion in the future prophylaxis as atherapeutic agent. Relatively rapid development of resistance to most of the drugs arises in response to drug pressure It can also happen in the recent future tothe ACT,which is now being promoted as a gold standard for malaria therapy by World Health Organization.

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