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COLUMN CHROMATOGRAPHY FOR ISOLATION OF ANDROGRAPHOLIDE FROM *IN VITRO* GROWN MEDICINAL PLANT-*ANDROGRAPHIS PANICULATA* WALL. EX NEES.

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ABSTRACT: *Andrographis paniculata* which is commonly known as Kalmegh is an important medicinal plant. The Methanol extract of *in vitro* grown *A. paniculata* was used for column chromatography to isolate its active ingredient- Andrographolide. The melting point and UV detection wave length of the colourless crystal recovered after column chromatography were recorded as 235°C and 220nm respectively. Comparison of recorded UV spectral data, characteristic IR spectra and melting point of isolated constituents with previous literatures indicates that the isolated crystal is the andrographolide of *A. paniculata.*

Key words: Tissue culture, Phytoconstituent, Andrographolide, Methanol.

INTRODUCTION

India is blessed with varieties of aromatic and medicinal plants. Among the medicinal plants, *Andrographis paniculata* (Kalmegh) has been used in Indian and Chinese herbal medicine. *Andrographis* contains active principle andrographolide (Fig.1).

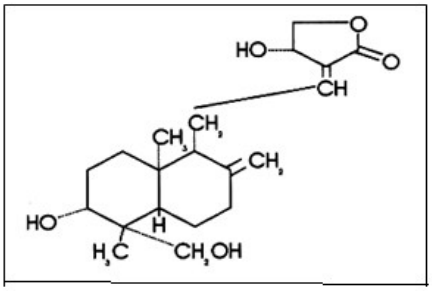

Fig.1-. Molecular structure of Andrographolide

It is a diterpenelactone with a very bitter taste and colourless crystalline in appearance. Other active components include 14- deoxy- 11,12 didehydroandrographolide (andrographolide-D), Homoandrographolide, andrographan, andrographon, andrographosterin and stigmasterol. Besides these compounds, the chemicals isolated from leaves are diterpenoids viz- deoxyandrographolide, 19 β-D-glucoside and neo-andrographolide (Chem Wiming 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-hydroxy 7,8,2',3'-tetramethoxyflavone.

It also contains a monohydroxy trimethyl flavone, andrographin and a dihydroxy-di-methoxyflavone, panicolin. In ancient time *Andrographis* was used for digestion problem, snakebite and infections ranging from malaria to dysentery (Nadkarni and Nadkarni, 1976; Bensky *et al*, 1993). The bitter constituents of Kalmegh are believed to have immune stimulating, fertility decreasing, anti-inflammatory, liver protective and bile secretion stimulating actions (Bone, 1998). Though *Andrographis* has weak direct antibacterial action, it has remarkably beneficial effect in reducing diarrhea and symptoms arising from bacterial infections. *Andrographis* can reduce symptoms of common cold (Thamlikitkul *et al,* 1991; Melchior *et al*, 1996; Hancke *et al*, 1995; Cacers *et al*, 1999). Andrographolide decreases viral load and increases CD4, lymphocytelevel in people with HIV infection (Calabrese *et al*, 2000). *Andrographis* in combination with antibiotics is beneficial in dysentery, a severe form of diarrhea (Thanagkul and Chaichantipayut, 1985). It has also shown preliminary benefit for people with chronic viral hepatitis (Chaturvedi, 1983). Besides these beneficial actions andrographolide acts as analgesic, antiperiodic, antipyretic, antithrombotic, cancerolytic, cardioprotective, choleretic, depurative, expectorant, hepatoprotective,hypoglycemic, laxative, sedative, thrombolytic and vermicidal. Considering the importance of medicinal plant, National Medicinal Plant Board (NMPB) has identified 32 medicinal plants for their overall development viz- value addition and quality production, simple agro technique, conservation, multiplication etc. *A. paniculata* is also rightly included in this list (Rawat and Uniyal, 2003). Due to it high demand, tones of *A. paniculata* is being harvested from wild source every year. Tissue culture is the only technique through which numerous plants can be developed within a short span of time to fulfill its growing demand. Large-scale plant tissue culture is found to be an attractive alternative approach to traditional methods of plantation as it offers a controlled supply of biochemical independent of plant availability (Sajc *et al*., 2000). But tissue culture is meaningless if *in vitro* regenerated plant cannot synthesize active ingredients under natural condition. Therefore an experiment has been designed to isolate andrographolide from *in vitro* grown *A. paniculata* plant.

MATERIALS AND METHODS

Collection of *in vitro* grown plant material

Aerial shoots of *in vitro* grown *A. paniculata* were collected from Medicinal Plant Garden of Life Sciences Department, Dibrugarh University.

Preparation of crude extract

Collected plant materials (500 gm) were shade dried at room temperature, ground and treated successively with different polarity solvents – Petroleum ether, Chloroform and Methanol. Plant materials were treated in each solvent for 72hrs.

Column chromatography

Column was packed with slurry of silica gel (mesh size, 60-120) with chloroform. Then dried Methanol extract (4 gm) of *A. paniculata* was first dissolved in Methanol and carefully applied by pipette at the top of 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 column was 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 crystals of two fractions were first separately treated with Petroleum ether and then filtered. The crystalline residues were then retreated with Chloroform and were recovered after filtration. The identity of crystals was confirmed by spectroscopic analysis.

Identification of isolated crystals

The isolated constituent of *A. paniculata* (colourless crystal), were identified through IR spectrophotometer (Perkin Elmer-833 Infrared Spectrophotometer), UV spectrophotometer (Shimadza, UV-240, Graphicord) and melting point. IR and UV spectroscopy of crystals of *A. paniculata* was taken in KBr and methanol (Blank) respectively. Finally the recorded UV spectral data, characteristic IR spectra and melting points of isolated constituents of *A. paniculata,* were compared with previous literatures to assign their identity.

RESULTS AND DISCUSSION

The melting point and other recorded properties of the isolated constituents (Fig-2) were presented in Table-1. The UV detection wavelength of isolated constituent was recorded at 220nm (Fig-3). Same finding has been reported by Pholphana *et al.* (2004) for UV spectrum of andrographolide of *A. paniculata*. While UV detection wavelength at 214 nm of andrographolide was reported by Zhao *et al*. (2002). Rajani *et al*. (2000) reported melting point of andrographolide as 235.3 ºC. The IR spectrum (Fig-4) showed characteristic peak positions (Table.2) 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 of C=O, C=C, C-O-C of lactone ring and O-H group of alcohol respectively, present in the molecular structure of andrographolide.

Table-1. Properties of isolated colourless crystals of *A. paniculata.*

Sl. No.	Properties	
1	Appearance	Crystalline
2	Colour	Colourless
3	Taste	Bitter
4	Odour	Odourless
5	Melting point	235 °C
6	Solubility	Soluble in Methanol

Table-2. 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 α, β- 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

Fig-2. Colourless crystalline Andrographolide.

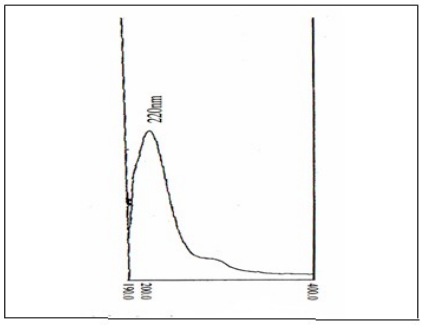

Fig-3. UV spectrum of Andrographoliode

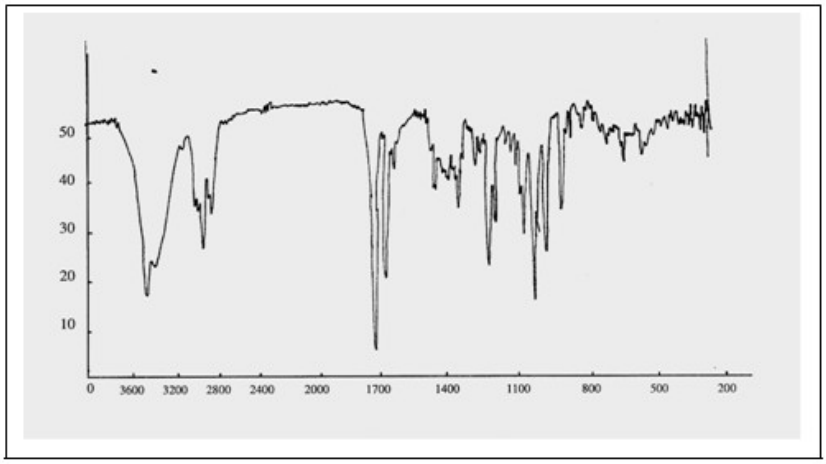

Fig-4. IR spectrum of Andrographolide.

CONCLUSION

Finally it can be concluded that the isolated colourless crystals of *in vitro* grown *A. paniculata* is the Andrographolide. From the present study it can be confirmed that *in vitro* grown *Andrographis paniculata* can produces active ingredient- Andrographolide. Thus tissue culture method can be safely used for its large scale propagation.

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