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Research Paper

A NEW ANTICANCER FLAVONOID FROM THE LEAVES OF *Andrographis paniculata*

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Abstract

Extract of *Andrographis paniculata*is traditionally used as a medicine to treat different diseases in India, China and Southeast Asia. Inthe present study, we evaluated the anticancer activity of the methanolic extract of *Andrographis paniculata* in human cancer and immune cells.The chloroform extract of the leaves of *Andrographis paniculata* yielded a labdanediterpenoid Andrographolide and a new flavonoid. These compounds were evaluated for their anticancer activity against selected human cancer cell lines. These isolateswere significantly inhibited M14 (malonoma) and U251 (CNS) human cancer cell lines. These two compounds can serve as a scaffold for design and synthesis of novel, potent, non-toxic anticancer molecules.

Key words: *Andrographis paniculata,* chloroform extract, Andrographolide, flavonoid, anticancer activity.

INTRODUCTION

Andrographis paniculata belongs to the family ofAcanthaceae. It is also commonly known as "kalmegh,"and a well-known medicinal plant of Ayurveda and has been usedfor centuries in Asian countries. Severalpolyherbal formulationsof this plant are stated in Ayurveda as a popularremedy for the treatment of various disorders. *Andrographispaniculata*is an annual shurb grows abundantly in India andcultivated extensively in China and Thailand. The aerial partsof the plant (leaves and stems) are used to extract the activephytochemicals. The plant extract is known to contain labdane, neoclerodane–type of diterpenoids,sesquiterepenes, flavonoids and stigmasterols[45].Extracts of plants and their major metabolites including labdanediterpenoids, neoclerodanediterpenoids and flavonoids have been reported to exhibit a wide range of biological activities [2-44]of therapeutic importance that include anti-inflammatory, hepatoprotective, antimalarial, antibacterial, antithrombotic, immune stimulant, antidepressive, antiallergic, central nervous system disorders [15, 17, 19, 21, 26-31], anti HIV, and anticancer.

Andrographolide is the major diterpenoid of the *Andrographispaniculata*extract has shown cytotoxic activityagainst KB (human epidermoid carcinoma) and P388 (lymphocyticleukaemia) [45] cells. Themethanol extract of aerial parts of *Andrographis paniculata*and some of the isolated compounds showed growth inhibitoryand differentiating activity on M1 (mouse myeloidleukaemia) cells [46]. However, no systematicstudy has been reported addressing the cytotoxicactivity of *Andrographis paniculata*extract in human immunecells. Here we report the isolation of new flavonoid and

labdanediterpenoidandrographolidefrom chloroform extract of leaves of *Andrographis paniculata,*and evaluated their anticancer activity against selected humancancer cell lines.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Andrographis paniculata*was collectedfrom Chandigarh, Haryana, India in in October 2014. Botanical identification was done by Prof.Jnanendra Shukla, taxonomist, Ayurvedic medicinal Plants Division, and voucher specimen HR-102/1A was deposited at the herbarium.

Extraction and isolation

The powdered plant material (750 mg) was extracted with chloroform for 24 h (5 L × 2 times). The chloroform extractwas concentrated *in vacuo,* and 1 g of crude extract was obtained. A portion of the chloroform soluble fraction (1 g)was chromatographed on a column of silica geleluted with chloroform and methanol mixture in order of increasingpolarity. Fractions were collected andcombined according to similar TLC pattern. Fractions 5-7 of chloroform–methanol (9:1) were found to be mixture (250 mg) ofcompounds andrographolide and a new flavonoid.

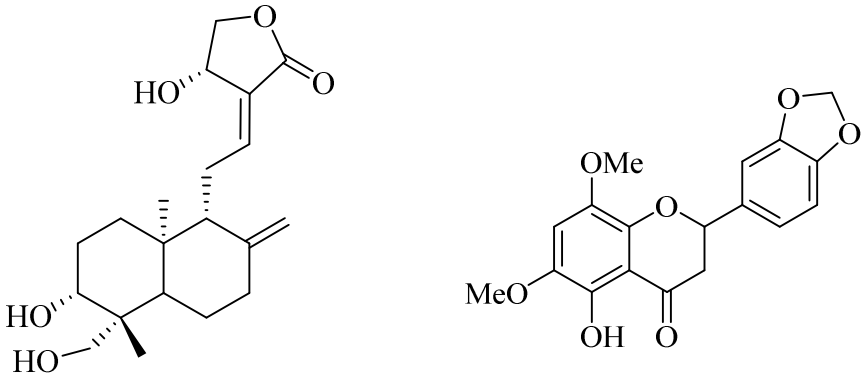
Cell growth assay

Cells undergoing exponential growth were seeded ona 96-well cell culture plates at a concentration of 10 000cells per well and incubated at 37◦C in a CO_2 incubator.Twenty-four hours later cells were treated with differentconcentrations of extracts or pure compounds dissolvedin DMSO to a final concentration of 0.05% in the culturemedium and incubated for 48 h. Cells were fixed by addingice-cold 50% trichloroacetic acid (TCA) and incubatingfor 1 h at 4◦C. The plates were washed with distilled water,air-dried and stained with SRB solution (0.4%, w/v, in1% acetic acid) for 10 min at room temperature. UnboundSRB was removed by washing thoroughly with 1% aceticacid and the plates were air-dried. The bound SRB stainwas solubilized with 10mM Tris buffer, and the opticaldensity was read on a spectrophotometric plate reader ata single wavelength of 515 nm. At the time of drug addition,a separate reference plate for cell growth at time 0 h(the time at which drugs were added) was also terminatedas described above. The percentage growths were calculatedand the GI_{50} values were calculated from the growthcurves.

RESULTS AND DISCUSSION

The portion of the chloroform soluble fraction (1 g)was chromatographed on a column of silica geleluted with chloroform and methanol mixture in order of increasingpolarity. Fractions were collected andcombined according to similar TLC pattern. Fractions 5-7 of chloroform–methanol (9:1) were found to be mixture (250 mg) ofcompounds andrographolide and a new flavonoid (Fig. 1).

Compound **1**structure was confirmed as andrographolide by its spectral data (NMR and MS data).



Compound 1
Andrographolide

Compound 2
New Flavonoid

Fig 1: Structures of isolated compounds

Compound **2** was obtained as white amorphous solid. Its molecular formuala $C_{18}H_{14}O_7$ $[M+H]^+$ at m/z 343.1, and gave a positive flavonoid test with magnesium-hydrochloric acid and exhibited UV absorption atλmax (MeOH) nm (logϵ) 283 (4.27) and 329 (3.76).Its IR spectrum showed absorption bands at μmax(KBr) 3380 (bonded hydroxyl), 1635, 1610,1510 cm^{-1} (chelated α, β-unsaturated carbonyl). The^{1}HNMR spectrum (400 MHz, $CDCl_3$) of **2** displayedresonances at d 3.9 (6H, s, two Ar-OCH_3 groups),6.1 (2H, s, -O-CH2-O-), 6.4 (1H, s, H-7), 6.72(1H, s C_3-H), 6.9 (1H, d, *J* =8 Hz, H-5'), 7.3 (1H,d, *J* = 2 Hz, H-2'), 7.5 (1H, dd, *J* =2, 8 Hz, H-6')and δ 13 (phenolic OH). The EIMS exhibited significant mass peaks at m/z (% rel. int.) 343.1 (M+H,60.2), 327 (M+H-CH3, 100), 314 (M+H - CO, 7.9),313 (M+H – CO- H, 18.2), 299 (M+ H--CH_3CO,20.7), 196 and 146 (R.D.A. of 1; 21.6, 14.7), 181and 146 (R.D.A. of mass fragment 327; 15.2, 14.7),149 (4.3) and 132 (146 - CH_2, 50.6). This characteristic MS fragmentation suggests the presence oftwo methoxyls and one hydroxyl in ring-A, while the^{1}HNMR spectral analysis indicated one –O-CH2-O- chain at the 3',4'-position in ring B.

The bathochromic shift of UV band I by 20(283-303) nm in the presence of $AlCl_3$, whichremained unchanged on addition of hydrochloricacid suggested the presence of a hydroxyl functionat C_5 and one of the methoxyl groups at the C_6position. Thus, the other methoxyl must beeither at C_7 or C_8 in ring-A. The presence of an^{1}HNMR signal at δ 6.4 (1H, s, H-7) and failure of thecompound **2** to respond to the gossypetone test confirrmed the presence of the second methoxylgroup at C_8. The above data led us to formulatethe new flavone as 5-hydroxy-6,8-dimethoxy-3',4'-methylenedioxyflavone**2** and the structure wasconfirmed by ^{13}CNMR spectral analysis.

^{13}CNMR data (100 MHz, $CDCl_3$):δ 183.7, 163.9, 152.8, 149.7, 147.2, 132.1, 129.2, 122.9, 119.5, 118.6, 110.2 104.2, 104.1, 101.1, 61.9, 60.8.

Table: Effect of isolated compounds on growth of human cell lines: Growth Inhibition (GI_{50}):

Cell line	Andrographolide	flavonoid
M14 (malonoma)	9	11
U251 (CNS)	8	7

Human cancer cell lines representing two types of cancers were incubated in complete medium with and without test compounds. Percentage growth of the treated cells was calculated compared to the control untreated cells and the concentration required inhibiting the 50% growth (GI50 concentration). The numbers represent the GI50 values in micromolar concentration.

From these two results it is evident that the major constituentandrographolide shows anticancer
activity. The in vivo results from hollow fiberassay conducted in immunocompetent Swiss albino mice,demonstrated that andrographolide and new flavonoid significantly inhibitsthe cancer cell proliferation without showing any signsof toxicity in mice even at high doses.

We conclude that, owing to its potent anticancer activity, thediterpenoidandrographolide and flavonoid can serve as a scaffold fordesign and synthesis of novel, potent, non-toxic anticancermolecules.

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REFERENCES

[1] Chakravarti, R. N.,Chakravarti, D.1951, Andrographolide, The Active Constituent of *Andrographis paniculata*Nees; A Preliminary Communication. *Ind. Med. Gaz.*86: 96.
[2] Shen, Y. C., Chen, C. F.,Chiou, W. F.2002, Andrographolide Prevents Oxygen RadicalProduction by Human Neutrophils: Possible Mechanism(s) Involved in its Anti-Inflammatory Effect.*Br. J. Pharmacol.* 135: 399-406.
[3] Najib, N. A. R. N., Furuta, T., Kojima, S., Takane, K., Ali, M. M.1999, Antimalarial Activity of Extracts of Malaysian Medicinal Plants. *J. Ethanopharmacol.*64: 249-254.
[4] Gupta, S., Choudary, M. A., Yadava, J. N. S., Srivastava, V., Tandon, J. S. 1990,AntidiarrhoealActivity of Diterpenes of *Andrographis paniculata* (Kal-Megh) against *Escherichia coli* Enterotoxin in *in vivo* Models.*Pharm. Biol.* 28:273-283.
[5] Gupta, P. P., Tandon, J. S.,Patnaik, G. K.1998,Antiallergic Activity of AndrographolidesIsolated from *Andrographis paniculata*(Burm. F) Wall. *Pharm. Biol.* 36,72-74.
[6] Madav, S., Tanda, S. K., Lal, J.,Tripatthi, H. C.1996, Anti-inflammatory Activity ofAndrographolide.*Fitoterapia,* 67:452-458.
[7] Cheung, S. C., and Li, N. H.1978, Chinese Medicinal Herbs of Hong Kong. 1 (12): 8.
[8] Reddy, P. P., Tiwari, A. K., Rao, R. R., Madhusudhana, K., Rao, V. R. S., Ali, A. Z., Babu, K. S., and Rao, J. M.2009, New LabdaneDiterpenes as Intestinal α-glucosidase Inhibitor from Antihyperglycemic Extract of *Hedychiumspicatum* (Ham. Ex Smith) Rhizomes. Bioorg. Med. Chem. Lett. 19(9): 2562-2565.
[9] Husen, R.,Pihie, A. H. L., Nallappan, M. 2004, Screening for Antihyperglycaemic Activity in Several Local Herbs of Malaysia.*J. Ethanopharmacol.* 95: 205-208.
[10] Reddy, P. P., Rao, R. R., Rekha, K. S., Babu, K. S., Shashidhar, J., Shashikiran, G., Vijaya Lakshmi, V., and Rao, J.M.2009, Two New Cytotoxic Diterpenes from the Rhizomes of *Hedychiumspicatum*. Bioorg. Med. Chem. Lett. 19(1):192-195.
[11] Handa, S. S., Sharma, A.1990,Hepatoprotective Activity of Andrographolide from *Andrographis paniculata* Against Carbon tetrachloride. *Indian J. Med. Res.*92: 284-292.
[12] Reddy, P. P., Rao, R. R., Shashidhar, J., Sastry, B. S., Rao, J. M., and Babu, K. S. 2009, Phytochemical Investigation of LabdaneDiterpenes from the Rhizomes of *Hedychiumspicatum* and Their Cytotoxic Activity. *Bioorg. Med. Chem. Lett.*19(21): 6078-6081.
[13] Reddy, P. P., Lavekar, A. G., Babu, K. S., Rao, R. R., Shashidhar, J., Shashikiran, G., and Rao, J. M., 2010, Synthesis, Cytotoxic Activity and Structure-Activity Relationships of Hedychenone Analogues. *Bioorg. Med. Chem. Lett.*20(8): 2525-2528.
[14] Fajemiroye, J.O., Galdino, P. M., Florentino, I. F., Da Rocha, F. F., Ghedini, P. C., et al.2014, Plurality of Anxiety and Depression Alteration Mechanism by Oleanolic Acid. *J. Psychopharmacol.*98:923-934.
[15] Polepally, P. R., White, K., Vardy, E., Roth, B. L., Ferreira, D., and Zjawiony, J. K.2013, Kappa-Opioid Receptor-Selective Dicarboxylic Ester-Derived Salvinorin A Ligands. *Bioorg. Med. Chem. Lett.*23:2860-2862.
[16] Shen, Y. C., Chen, C. F.,Chiou, W. F.2000, Suppression of Rat Neutrophil Reactive OxygenSpecies Production and Adhesion by the Diterpenoid Lactone Andrographolide. *Planta Medica.*66: 314-317.
[17] Polepally, P.R., Setola, V., Vardy, E., Roth, B.L., and Zjawiony, J. K.2013, New Michael Acceptor-Type of Salvinorin A Ligands to Kappa-Opioid Receptor. *Planta Medica,* 79(05)*:*P41.
[18] Li, Z., Huang, W., Zhang, H., Wang, X., Zhou, H.2007, Synthesis of Andrographolide Derivatives and their TNF-α and IL-6 Expression Inhibitory Activities. *Bioorg. Med.Chem. Lett.* 17:6891-6894.
[19] Polepally, P. R., White, K., Roth, B. L., and Zjawiony, J. K.2013, Convenient Synthesis and *InVitro* Pharmacological Activity of Thioesters of Salvinorin B. *Planta Medica,* 79(05):P43.
[20] Nanduri, S.,Nyavanandi, V. K., Thunuguntla, S. S. R., Kasu, S.,Pallerla, M.K.,*et al.*2004, Synthesis and Structure-Activity Relationships of Andrographolide Analogues as NovelCytotoxic Agents.*Bioorg. Med. Chem. Lett.* 14:4711-4717.

[21] Polepally, P.R., Roth, B.L., White, K., and Zjawiony, J.K.2013, Synthesis and Biological Evaluation of New Salvinorin B-Sulfonate Ester Ligands to Opioid Receptors. *Planta Medica,* 79(05): P44.

[22] He, X. J., Li, J. K., Gao, H., Qiu, F., Hu, K., Cui, X. M., Yao, X. S.2003, Four New Andrographolide Metabolites in Rats. *Tetrahedron Lett.*59:6603-6607.

[23] Polepally, P. R., Roth, B.L., White, K., Ferriera, D., and Zjawiony, J. K., 2013, Synthesis and *In Vitro* Biological Evaluation of New Dicarboxylic Ester-Type Salvinorin A Analogs. *Planta Medica,* 79(05):P42.

[24] Kumar, R. A.,Sridevi, K., Kumar, N., V.; Nanduri, S., Srinivas, N.,Rajagopal, S. J., 2004,Anticancer and Immunostimulatory Compounds from *Andrographis paniculata*. *J. Ethanopharmacol,*92: 291-295.

[25] Li, Z.; Huang, W.; Zhang, H.; Wang, X.; Zhou, H. 2006, Synthesis of Andrographolide Derivatives and their TNF-α and IL-6 Expression Inhibitory Activities. *Bioorg. Med. Chem. Lett.* 16:6891.

[26] Polepally, P.R., Huben, K., Vardy, E., Setola, V., Roth, B.L, Mosier, P.D., and Zjawiony, J.K.2014, Michael Acceptor Approach to the Design of New Salvinorin A-Based High Affinity Ligands for the Kappa-Opioid Receptor. *European Journal of Medicinal Chemistry*, 85, 818-829.

[27] Polepally, P.R., White, K., Roth, B. L., and Zjawiony, J. K.2013, Synthesis and *In Vitro* Pharmacological Activity of C-2 Modified New Salvinorin A Analogues. *Planta Medica,* 79(05):P45.

[28] Polepally, P.R., Setola, V., Vardy, E., Roth, B.L, Mosier, P.D., and Zjawiony, J.K.2012, New Salvinorin A-Derived Ligands to Opioid Receptors. *Planta Medica.*78:PI238.

[29] Polepally, P. R., Setola, V., Vardy, E., Roth, B. L, and Zjawiony, J.K.2013, Michael Acceptor Approach to the Design of New Salvinorin A-Based High Affinity Ligands for the Kappa-Opioid Receptor. *Planta Medica*, 79(05): P45.

[30] Polepally, P. R., White, K., Vardy, E., Roth, B. L., Ferreira, D., and Zjawiony, J. K., 2013, Kappa-Opioid Receptor-Selective Dicarboxylic Ester-Derived Salvinorin A Ligands. Bioorg. Med. Chem. Lett. 23:2860-2862.

[31] Polepally, P.R., White, K.L., Roth, B.L and Zjawiony, J.K., 2014, Design, synthesis and pharmacological activity of new C(2)-modified salvinorin A analogues. *PlantaMedica*, 80(10): PF8.

[32] Rao, R. R., Tiwari, A. K., Reddy, P. P., Babu, K. S., Ali, A. Z., Madhusudana, K., and Rao J. M., 2009, New Furanoflavanoids, Intestinal α-glucosidase Inhibitory and Free-Radical (DPPH) Scavenging, Activity from Antihyperglycemic Root Extract of *Derris indica*. *Bioorg. Med. Chem.* 17(14): 5170-5175.

[33] Rao, R. R., Tiwari, A. K., Reddy, P. P., Babu, K. S., Suresh, G., Ali, A. Z., Madhusudana, K., Agawane, S. B., Badrinaraya, P., Narahari, G.S., and Rao, J.M.2012,Synthesis of Antihyperglycemic, α-glucosidase Inhibitory, and DPPH Free Radical Scavenging Furanochalcones. *Med. Chem. Res.*21(6): 760-774.

[34] Rao, R. R., Chaturvedi, V., Babu, K.S., Reddy, P. P., Rao, V. R. S., Sreekanth, P., Sreedhar, S., and Rao, J. M.2012, Synthesis and Anticancer Effects of Pongamol Derivatives on Mitogen Signaling and Cell Cycle Kinases. *Med. Chem. Res.*21: 634-641.

[35] Raju, B. C., Pradeep, D. V. S., Reddy, P. P., Rao, J. M., 2008, CBr_4 Catalyzed Synthesis of Aryl-14H-dibenzo [a,j] Xanthenes Under Solvent-Free Conditions. *Lett. in Org. Chem.*5(6): 450-454.

[36] Polepally, P. R., Keasling, A., White, K., Vardy, E., Roth, B. L., andZjawiony, J. K.2015, New C (2)-sulfonyl ester-type Salvinorin A ligands to the kappa-opioid receptor. *Planta Medica*, 81(05): PC4.

[37] Reddy, P. P., Raju, B. C., Rao, J. M. 2008, A Facile One-Pot Friedlander Synthesis of Quinoline Derivatives. *J. Chem. Res.*12(12):679-682.

[38] Sałaga, M., Polepally, P.R., Zakrzewski, P.K., Cygankiewicz, A., Sobczak, M., Kordek, R., Zjawiony, J.K., Krajewska, W. M.,Fichna, J., 2014, Novel orally available salvinorin A analog PR-38 protects against experimental colitis and reduces abdominal pain in mice by

interaction with opioid and cannabinoid receptors. *Biochemical pharmacology*, 92(4): 618-626.

[39] Suresh, G., Reddy, P. P., Babu, K. S., Shaik, T. B., and Kalivendi, S. V., 2010, Two New Cytotoxic LabdaneDiterpenes from the Rhizomes of *Hedychiumcoronarium*. *Bioorg. Med. Chem. Lett.*20(24): 7544-7548.

[40] Salaga, M.,Polepally, P. R.,Sobczak, M., Grzywacz, D.,Sibaev, A.,storr, M., Dorego, J. C.,Zjawiony,J. K., and Fichna J. 2014, NovelorallyavailablesalvinorinAAnalogPR-38inhibitsgastrointestinalmotilityandreduces abdominalpaininmouse Modelsmimicking irritable bowel syndrome. *J. Pharmaceutical. Exper. Theraupetics*, 350(1): 69-78.

[41] White, K. L., Scopton, A. P., Rives, M. L., Bikulatov, R. V., et al. 2014, Identification of Novel Functionally Selective κ-Opioid Receptor Scaffolds. Mol. Pharmacol. 85: 83-90.

[42] Polepally, P. R., Keasling, A., White, K., Vardy, E., Mosier, P. D., Roth, B. L., andZjawiony, J. K., 2015, High affinity C (2)-thiocarbonate and thioacetate-type salvinorin A ligands to the kappa-opioid receptor. *Planta Medica*. *81*(05): PC5.

[43] White, K. L, Robinson J. E., Zhu, H., DiBerto, J. F., et al.2015, The G-Protein–Biased κ-Opioid Receptor Agonist RB-64 Is Analgesic with a Unique Spectrum of Activities In Vivo. *Journal of Pharmacology and Experimental Therapeutics.*352(1): 98-109.

[44] Zjawiony, J. K., Polepally, P. R., Roth, B. L., Setola, V., and Vardy, E.2011, Design and Synthesis of Natural-Product Based Ligands with High Affinity to the Kappa-Opioid Receptor. *Planta Medica,*77(12):SL4.

[45] Siripong, P., Kongkathip, B., Preechanukool, K., Picha, P., Tunsuwan, K.,Taylor, W.C., 1992, Cytotoxic diterpenoid constituents from *Andrographispaniculata*Nees leaves.*J. Sci. Soc. Thailand* 18: 187–194.

[46] Matsuda, T., Kuroyanagi, M., Sugiyama, S., Umehara, K., Ueno, A., Nishi,K., 1994, Cell differentiation-inducingditerpenes from *Andrographispaniculata*Nees. *Chemical and Pharmaceutical Bulletin*, Tokyo 42,1216–1225.