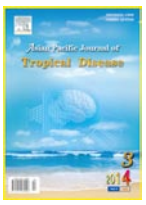
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading doi: 10.1016/S2222-1808(14)60509-0

Harnessing the medicinal properties of *Andrographis paniculata* for diseases and beyond: a review of its phytochemistry and pharmacology

Agbonlahor Okhuarobo[1], Joyce Ehizogie Falodun[2], Osayemwenre Erharuyi[3,4], Vincent Imieje[3], Abiodun Falodun[3,4,5*], Peter Langer[3]

[1]*Department of Pharmacology and Toxicology, University of Benin, Nigeria*

[2]*Crop Science, University of Benin, Nigeria*

[3]*Pharmaceutical Chemistry, University of Benin, Nigeria*

[4]*Institute of Chemistry, University of Rostock, Albert-Einstein-Str. 3A, 18059 Rostock, Germany*

[5]*Department of Pharmacognosy, School of Pharmacy, University of Mississippi, 38655 Oxford, Mississippi, USA*

PEER REVIEW

Peer reviewer

Dr. rer. nat. Iftikhar Ali, Assistant Professor, Department of Chemistry, Karakoram International University, 15100 Gilgit-Baltistan, Pakistan
Tel: 0092 333 9665739
E-mail: iftikhar.ali@kiu.edu.pk

Comments

The present review is well-written and precisely summarised. The structures of the compounds and differernt biological activities of the pure compounds and extracts as well have been represented in a good way. This type of review may lead to further research upon the plant species.
Details on Page 220

ABSTRACT

Andrographis paniculata Wall (family Acanthaceae) is one of the most popular medicinal plants used traditionally for the treatment of array of diseases such as cancer, diabetes, high blood pressure, ulcer, leprosy, bronchitis, skin diseases, flatulence, colic, influenza, dysentery, dyspepsia and malaria for centuries in Asia, America and Africa continents. It possesses several photochemical constituents with unique and interesting biological properties. This review describes the past and present state of research on *Andrographis paniculata* with respect to the medicinal usage, phytochemistry, pharmacological activities, toxicity profile and therapeutic usage, in order to bridge the gap requiring future research opportunities. This review is based on literature study on scientific journals and books from library and electronic sources. Diterpenes, flavonoids, xanthones, noriridoides and other miscellaneous compounds have been isolated from the plant. Extract and pure compounds of the plant have been reported for their anti-microbial, cytotoxicity, anti-protozoan, anti-inflammatory, anti-oxidant, immunostimulant, anti-diabetic, anti-infective, anti-angiogenic, hepato-renal protective, sex hormone/sexual function modulation, liver enzymes modulation insecticidal and toxicity activities. The results of numerous toxicity evaluations of extracts and metabolites isolated from this plant did not show any significant acute toxicity in experimental animals. Detailed and more comprehensive toxicity profile on mammalian tissues and organs is needed in future studies.

KEYWORDS

Andrographis paniculata, Phytochemistry, Medicinal uses, Pharmacology, Toxicity

1. Introduction

In many developing countries, it is estimated that about two third of the population relies heavily on traditional practitioners and medicinal plants to meet primary healthcare needs[1]. As a result of the numerous problems associated with orthodox drugs, many plant species are now been revalued by researchers based on variation in plant species and their therapeutic chemical principles. Therefore, the need to do a thorough literature search on some species with a view to update the current state of knowledge is imperative. One of such plant species is

*Corresponding author: Abiodun Falodun, Department of Pharmacognosy, School of Pharmacy, University of Mississippi, 38655 Oxford, Mississippi, USA.
Tel: 662-638-5786
E-mail: faloabi@uniben.edu
Foundation Project: This work was in part supported by a US- Senior Fulbright Award.

Article history:
Received 27 Apr 2014
Received in revised form 2 May, 2nd revised form 6 May, 3rd revised form 11 May 2014
Accepted 15 Jun 2014
Available online 28 Jun 2014

Andrographis paniculata (*A. paniculata*) used in ancient oriental and ayurvedic medicine. The genus *Andrographis* which belongs to the Acanthaceae family comprises of about 40 species. Only a few are popular for their use in folk medicine for assorted health concerns. Of these few, *A. paniculata* is the most important. *A. paniculata*, commonly known as King of Bitters or kalmegh, is an annual, branched, erect handsome herb running half to one meter in height. It is native to peninsular India and Srilanka and is also distributed in different regions of Southeast Asia, China, America, West Indies and Christmas Island. It is cultivated because of its well known medicinal value and it grows well in most soil types thus it is widely distributed[2]. The aerial parts and roots of the plant have been widely used as traditional medicine in China, India, Thailand and other Southeast Asian countries to treat many maladies. It is known as King of Bitters (English), *Mahatikta* (Sanskrit), *Kiryato* (Gujarati), *Mahatita* (Hindi), *Kalmegh* (Bengali), or *Fah Talai Jone* (Thai)[3]. A wide array of studies has been conducted by researchers, especially in Asia, following reports about the medicinal properties possessed by this plant mostly according to traditional medical practitioners in ayurvedic medical system. Phytochemical studies have revealed that *A. paniculata* contains diverse compounds including labdane diterpenoid lactones, flavonoids and miscellaneous compounds. It has been shown to possess wide spectrum of pharmacological properties[4,5]. This review is focused on its medicinal properties, phytochemistry and the pharmacological effects of its various extracts and compounds including anti-microbial, cytotoxicity, anti-protozoan, anti-inflammatory, anti-oxidant, immunostimulant, anti-diabetic, anti-infective, anti-angiogenic, hepato-renal protective, sex hormone modulatory, liver enzymes modulatory and insecticidal activities. Furthermore, this review also discusses some toxicological aspects of this species.

1.1. Medicinal uses

The aerial parts, roots and whole plant of *A. paniculata* have been used for centuries in Asia as traditional medicine for the treatment of various ailments. It has been used by traditional medical practitioners for stomachaches, inflammation, pyrexia, and intermittent fevers[6-9]. The whole plant has been used for several applications such as anti-dote for snake-bite and poisonous stings of some insects, and to treat dyspepsia, influenza, dysentery, malaria and respiratory infections[6,7]. The leaf extract is a traditional remedy for the treatment of infectious disease, fever-causing diseases, colic pain, loss of appetite, irregular stools and diarrhea[10]. In Malaysia, a decoction of the aerial parts is used to treat common cold, hypertension, diabetes, cancer, malaria and snakebite[11]. Table 1 describes the medicinal uses of the parts of *A. paniculata*. It is an important constituent of at least 26 Ayurvedic formulas in Indian pharmacopoeia. In traditional Chinese medicine, it is seen as the cold-property herb used to rid the body of heat and fever and to dispel toxins from the body[12]. In Ayurvedic medicinal system, tribals of Tamilnadu, India use this herb for a variety of ailments like dysmenorrhoea, leucorrhoea, pre-natal and post-natal care, complicated diseases such malaria, jaundice, gonorrhea and general ailments like wounds, cuts, boils and skin diseases[13-16]. The different modes of usage of *A. paniculata* by these tribals are described in Table 2.

Table 1

Medicinal uses of *A. paniculata*.

Part	Medicinal uses	References
Whole Plant	Snakebite and insect sting treatment, dyspepsia, influenza, dysentery, malaria and respiratory infections.	[6,7]
Leaf	Fever, colic pain, loss of appetite, irregular stools and diarrhea, common cold, cough, fever, hepatitis, tuberculosis, mouth ulcers, bronchitis gastro-intestinal disorder and sores .	[10,14,16]
Aerial part	Common cold, hypertension, diabetes, cancer, malaria and snakebite, urinary tract infection.	[10,11,16]
Root	Febrifuge, tonic, stomachic and anthelmintic.	[6]

Table 2

A. paniculata in folk medicine of Tamilnadu, India[14].

Medicinal uses	Mode of uses
Malaria	About 20 g of the whole plant is pounded, mixed in water, filtered and given internally. Alternatively, the plant is cut into small pieces and kept overnight in 100 mL of Water. About 40 mL of the cold infusion obtained is given internally, twice a day (Shevaroy Hills, Malayali).
Post-natal care	About 25 g of powdered herb is boiled in 400 mL of water, reduced to 50 mL. cooled filtered and given internally to arrest unusual thirst. This decoction is also given to alleviate burning sensation in the palm and foot of the subject (Shevaroy Hills, Malayali).
Dysmenorrhoea	About 10 g of leaf together with 3 black peppers is grounded well and given once a day for 7 d (Kolli Hills, Malayali).
Intestinal worm infestation	A total of 2 g each of root and stem along with 7 mustard are made into paste, mixed in mother's milk and given internally. Alternatively, paste made of 5 fresh leaves or juice extracted from 5 g of root is mixed in hot water and given internally (Kolli Hills, Malayali).
Eczema	Powdered herb is mixed in oil and applied on the lesions. About 2 g of powder is also given internally once a day for 40 d (Pachamalais, Malayali).
Leucoderma	A total of 2 g of powdered herb is given, once a day for 40 d (Panchamalais, Malayali).
Jaundice	Water extract of 10 g of the herb together with equal quantities of stem bark extracts of *Azadirachta indica* and *Holarrhena antidysenterica*, which is heat treated by dropping a hot sonte, is given 3 times a day for 6 d, in dose of 30 mL (KolliHills, Malayali).
Abscess	About 10 g of leaf paste is given internally. Some paste is also applied externally (Shevaroy Hills, Malayali).
Gonorrhea	Powdered herb mixed in oil is applied externally. Alternatively, plant juice is applied on the wounds. In addition 2 g of the powder is also given internally (Shevaroy Hills, Malayali).
Infected wounds	The herb is grounded into paste together with turmeric and applied externally. Alternatively, the leaf paste is smeared on the affected parts and kept for two days (Kolli Hills, Malayali). Juice extracted from 100 g of herb is given internally (Shevaroy Hills, Malayali).

Table 3
Terpenes of *A. paniculata*.

Compound	Type	Plant part	Reference
Andrographolide	Diterpenoid lactone	Leaves/aerial	[20,35-37, 55-57]
Neoandrographolide	Diterpenoid lactone	Leaves/aerial	[25,58-60]
14-deoxyandrographolide	Diterpenoid lactone	Aerial parts	[25,36,37,61]
Andrographoside	Diterpene	Leaves/aerial parts	[25,26]
14-deoxy-11, 12-didehydroandrographolide	Diterpenoid lactone	Aerial parts	[59-62]
19-O-β-D-glucopyranosyl-ent-labda-8(17), 13-dien-15, 16, 19-triol	Ent-labdane diterpenoid lactone	Aerial parts	[63]
8α-methoxy-14-deoxy-17β- hydroxyandrographolide	Ent-labdane diterpenoid lactone	Aerial parts	[64]
Andrographolactone	Diterpenoid lactone	Aerial parts	[65]
3, 13, 14, 19-tetrahydroxy- ent-labda-8(17), 11-dien-16, 15 olide and 3, 19 isopropylidene- 14-deoxy- ent-labda-8(17), 13-diene-16, 15-olide	Diterpenoid lactone	Aerial parts	[65]
14-deoxy-15-isopropylidene-11,12-didehydroandrographolide	Unusual Terpenoid	Aerial parts/roots	[24]
3,7,19-trihydroxyl-8,11, 13- ent-labdatriene-15, 16-olide and 8α,17β-epoxy-3, 19-dihydroxy-11,13-ent-labdatrien-15, 16-olide	Diterpene lactone	Aerial parts	[64]
Andrograpanin	Diterpene	Leaves	[31]

1.2. Phytochemistry

A. paniculata has various compounds in its aerial parts and roots and these are often used in extracting its active principles. Diverse factors such as geographical region, harvest time and processing method account for the variability in its chemical content[17,18]. Phytochemical studies of *A. paniculata* has led to the isolation of various plant metabolites. Notable among these metabolites are the terpenoids (entalabdane diterpene lactones) which account for a large proportion of its components and therapeutic activity. Other categories of compounds that have also been isolated include flavonoids (flavones), noriridoides, xanthones, polyphenols and trace and macro elements.

1.2.1. Terpenoids

Diterpenoid lactones are the commonest terpenoid compounds isolated from *A. paniculata* (Table 3). Diterpenoids are distributed in and have been isolated from the aerial parts and roots of this plant. Of the diterpenoids that have been identified and isolated from *A. paniculata*, andrographolide is the most prominent in occurrence and quantity. Andrographolide has a very bitter taste, and it is colourless and crystalline in appearance[19] and was first isolated in pure form by Gorter in 1911. Dominant diterpenoids other than andrographolide which have been isolated mostly from the aerial parts of *A. paniculata* include deoxyandrographolide and neoandrographolide. These diterpenoids (Table 3) have been isolated by several workers. Other diterpenes (Table 3) besides the dominant ones have also been isolated by various workers over the years, among these is an unusual 23 carbon terpenoid isolated from the roots and aerial parts of the plant[20].

1.2.2. Flavonoids

Flavones are the major flavonoids that have been isolated from the aerial parts, roots and whole plant of *A. paniculata* (Table 4).

Table 4
Flavonoids of *A. paniculata*.

Compound	Type	Plant part	Reference
5, 7, 2′, 3′-tetramethoxyflavone	Flavonone	Whole plant	[25]
5-hydroxy-7, 2′, 3′-trimethoxy flavones	Flavone	Whole plant	[25]
5-hydroxy-7, 2′, 6′trimethoxyflavone	Flavone	Root	[25]
7-O-methyldihydrowogonin	Flavone	Root/aerial part	[20,25]
7-O-methylwogonin	Flavone	Root/aerial part/whole plant	[20,25,66,67]
Flavone-1, 2′methylether	Flavone	Root/aerial part/whole plant	[20,25,68]
7-O-methylwogonin-5-glucoside	Flavones	Root/aerial parts	[20,25,67]
Flavone-1, 2′-O-glucoside	Flavonoids	Root /aerial part/whole plant	[20,25,67]
5-hydroxy-7, 8, 2′, 5′-tetramethoxyflavone	Flavonoids	Whole plant	[69]
Dihydroskullcapflavone	Flavone	Whole plant	[70]
5-hydroxy-7, 8, 2, 3′ tetramethoxyflavone	Flavone	Whole plant	[20,25,67]

1.2.3. Miscellaneous compounds

Several miscellaneous compounds (Table 5) have been isolated, especially, from the roots of *A. paniculata*. Four xanthones were isolated from the roots using a combination of thin layer chromatography and column chromatography, and were characterized by infrared radiation, mass and nuclear magnetic resonance spectroscopic methods as 1, 8-dihydroxy-3,7-dimethoxy-xanthone, 4,8-dihydroxy-2,7-dimethoxy-xanthone, 1,2-dihydroxy-6,8-dimethoxy-xanthone and 3,7,8-trimethoxy-1-hydroxy-xanthone[21]. Five rare noriridoids designated as andrographolide A-E, along with curvifloruside were isolated from the roots of *A. paniculata*[22]. Arabinogalactan proteins were isolated from the dried herbs by Prajjal and his colleagues in 2007[23]. Trace elements (Cr, Mn, Co, Ni, Zn, Cu, Se, Rb, Sr, and Pb) and macro-element (potassium and calcium) were identified and quantified in the roots[24]. Cinnamic acid, caffeic acid, ferulic acid and chlorogenic acid were also isolated from the whole plant[25,26].

Table 5
Miscellaneous compounds of *A. paniculata*.

compound	Type	Plant part	Reference
Arabinogalactan	Protein	Herbs	[71]
1, 8-dihydroxy-3,7-dimethoxy-xanthone	Xanthone	Root	[21]
4,8-dihydroxy-2,7-dimethoxy-xanthone	Xanthone	Root	[21]
1,2-dihydroxy-6,8-dimethoxy-xanthone	Xanthone	Root	[21]
3,7,8-trimethoxy-1-hydroxy-xanthone	Xanthone	Root	[21]
Andrographidoid A	Noriridoid	Root	[22]
Andrographidoid B	Noriridoid	Root	[22]
Andrographidoid C	Noriridoid	Root	[22]
Andrographidoid D	Noriridoid	Root	[22]
Andrographidoid E	Noriridoid	Root	[22]

1.3. Pharmacology

The robust use of the different parts of *A. paniculata* plant in folk medicine, especially, in Asia led scientists to study its pharmacological properties to validate its use as a therapeutic agent in the remedy of various ailments. Several studies showed that this plant exhibited various biological activities such as anti-microbial, cytotoxicity, anti-protozoan, anti-inflammatory, anti-oxidant, immunostimulant, anti-diabetic, anti-infective, anti-angiogenic, hepato-renal protective, sex hormone modulatory, liver enzymes modulatory and insecticidal and toxicity activities[27,28].

1.4. Anti-microbial activity

Aqueous extract, andrographolides and arabinogalactan proteins isolated from the dried herb of *A. paniculata* were screened for anti-microbial activity. The result showed that the aqueous extract and arabinogalactan proteins have antibacterial activity against *Bacillus subtilis (B. subtilis)*, *Escherichia coli (E. coli)*, *Pseudomonas aeruginosa* while andrographolide was only active against *B. subtilis*. All three were also reported to possess anti-fungal activity against *Candida albicans*[27].

Five rare noriridoides, andrographidoides A-E were screened for anti-bacterial activity against *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *B. subtilis*. None of the compounds showed any inhibitory activity (MIC>100 μg/mL). Gentamycin, chloramphenicol and Ciprofloxacin were used as positive controls[22].

1.5. Anti-inflammatory/anti-allergic activity

The aqueous extract combined with the methanol extract of the leaves showed significant alleviation of lipopolysaccharide induced release of pro-inflammatory mediators (NO, IL-1β and IL-6), inflammatory mediators (PGE2 and TXB2) and allergic mediators (LTB4) but no inhibition was observed against histamine release[28]. Seven photochemicals, namely, andrographolide, neoandrographolide, isoandrographolide, andrograpanin, 7-O-methylwogonin, 14-deoxy-11,12-didehydroandrographolide and skullcapflavone isolated from *A. paniculata* leaves were screened for *in vitro* anti-inflammatory and anti-allergic potential. The results showed that andrographolide, isoandrographolide, 7-O-methylwogonin and skullcapflavone-1 significantly inhibited inflammatory mediators NO and PGE2 release from lipopolysacharide (LPS) stimulated cultured macrophages. Whereas, IL-1β production in LPS stimulated macrophages was inhibited by andrographolide, isoandrographolide and 7-O-methylwogonin. Also, IL-6 production from LPS induced macrophages was significantly (P<0.01) inhibited by andrographolide, isoandrographolide and skullcapflavone-1 in a concentration dependent manner. The results also showed that andrographolide, isoandrographolide and skullcapflavone-1 significantly suppressed TXB4 released in A23187 activated HL-60 promyelocytic leukemia cells. Furthermore, the anti-allergic properties of the phytoconstituents was investigated on A23187 induced LTB4 production. The result showed 30.5% and 19.6% inhibition of LTB4 production in A23187 induced HL-60 promyeolocytic leukemia cells at concentrations of 63 μmol/L and 33.5 μmol/L for skullcapflavone and 7-O-methylwogonin respectively. The IC_{50} value for the reference standard captopril was 48 μmol/L. 7-O-methylwogonin was the only phytoconstituent that potently inhibited A23187 induced histamine release in RBL-2H3 rat basophil leukemic cells in a dose dependent manner[29]. Andrographolide, dehydroandrographolide and neoandrographolide isolated from the aerial parts of *A. paniculata* exhibited anti-inflammatory effects by interfering with COX enzyme activity. Andrographolide (30.1 μM) and dehydroandrographolide (28.5 μmol/L) markedly inhibited COX-1 in ionophore A23187-induced human platelets. Dehydroandrographolide (28.5 μmol/L) and neoandrographolide (20.8 μmol/L) strongly suppressed the LPS-stimulated COX-2 activity in human blood. In addition, dehydroandrographolide modulated the level of LPS-induced TNF-α, IL-6, IL-1β, and IL-10 secretion in human blood in a concentration dependent manner, showing that dehydroandrographolide has the highest efficacy. The result further showed that the mechanism of dehydroandrographolide may be related to down-expression of genes involved in the inflammatory cascade[30].

Andrograpanin (15-90 μmol/L) isolated from the ethanol extract of the leaves inhibited NO and pro-inflammatory cytokines (TNFα, IL-6, IL-12p70) in a dose dependent manner from lipopolysaccharide activated macrophages. Significant (P<0.05) inhibition of NO was evident at a concentration of 30 μmol/L and at a concentration of 75 μmol/L. Andrograpanin almost completely inhibited NO production. Significant inhibition of pro-inflammatory cytokines was evident at a concentration of 1.5 μmol/L and there was an almost complete inhibition at a concentration of 90 μmol/L. The RT-PCR and western blotting assays showed that andrograpanin inhibited productions of NO and pro-inflammatory cytokines through down-regulating iNOS and pro-inflammatory cytokines gene expression levels as well as p38 mitogen activator kinase signaling pathways. Further study showed that andrograpanin has more ability of downregulating IL-12 p35 and p40 proteins than their mRNA levels. This suggests that andrograrpanin might be involved in downregulating the post-translation of IL-12 p35 and 40 proteins[31].

1.6. Anti-oxidant activity

Andrographolide and aqueous extract of *A. paniculata* herbs were screened for anti-oxidant activity on nicotine induced oxidative stress in the liver, kidney, heart, lungs and spleen of male wistar rats and the result showed that intraperitoneal administration of andro (25 mg/kg) and *Aphanamixis polystachya* (25 mg/kg) for a period of 7 d

significantly (P<0.05) reduced levels of lipid peroxidation and increased the anti-oxidant enzymes status in the five organs screened compared to nicotine and vehicle only treated group[32]. The methanol and aqueous extracts of the leaves of *A. paniculata* from different locations as well as andrographolide and 14-deoxy-11, 12-didehydroandrographolide isolated exhibited lipid peroxidation inhibition in Sragues Dawley rats and free radical scavenging activities against DPPH. The lipid peroxidation inhibition activity varied from 55.6% to 63.9% and 33.78% to 33.77% for methanol and water extracts, respectively, showing that the activity of the methanol extracts were higher and significantly different (P<0.05) from that of the water extract. The methanol extract exhibited free radical scavenging activity ranging from 45.67% to 53.82%. The activity of andrographolide was 40.2% and 12-didehydroandrographolide was 46.43%. The water extract exhibited poor free radical scavenging activity ranging from 25.29% to 28.77%. The methanol and water extracts as well as the isolated compounds exhibited a lower free radical scavenging activity compared to quercetin (89%) and butylated hydroxylanisole (71%) used as positive controls[33]. A fourteen-day oral treatment of Sprague Dawley rats with methanol extract (1 g/kg body weight) of the dried leaves followed by carbon tetrachloride (CCl_4) challenge preserved anti-oxidant enzymes-catalase and superoxide dismutase activities in erythrocytes whereas lipid peroxidation, alanine transaminase, aspartate transaminase and plasma thiobarbituric acid reactive substances were restored to values comparable with that obtained for control group that did not receive CCl_4. Andrographolide, 14-deoxy-11, 12-didehydroandrographolide were traceable in rat plasma following an oral dose of methanol extract (1 g/kg body weight) of the dried leaves, suggesting that these diterpenes may be responsible for the observed anti-oxidant activity[34].

1.7. Immunostimulant activity

Ethanol extract of the fresh plant and purified diterpenes- androgrpholide and neoandrographolide induced significant (P<0.001) stimulation of anti-body and delayed hypersensitivity response to sheep red blood cells in mice. The plant preparations also stimulated non-specific immune response of the animals measured in terms of macrophage migration index, phagocytosis of 14C-leucine labeled *E. coli* and proliferation of splenic lymphocytes. The stimulation of both antigen specific and non-specific immune response was, however, of lower order with andrographolide and neoandrographolide than with the ethanol extract, suggesting that substances other than these diterpenes present in the extract may also play a role in immunostimulator[35]. Dichloromethane fraction of the methanol extract of fresh whole plant significantly enhanced the human peripheral blood lymphocytes proliferation expressed as percentage stimulation index versus control by 52% at low concentrations. Whereas the methanol extract, the petroleum ether fraction and aqueous fraction of the methanol extract caused 18%, 18% and 4% increase in human peripheral blood lymphocytes proliferation respectively, suggesting that the immunostimulatory compounds of the methanol extract are concentrated in the dichloromethane fraction. This observation led to the screening of three diterpenes-andrographolide, 14-deoxyandrographolide and 14-deoxy-11, 12-didehydroandrographolide isolated from the dichloromethane fraction. At a concentration of 1 μmol/L, all the three compounds showed moderate increase in human peripheral blood lymphocytes proliferation with andrographolide showing the highest increase (14%)[36].

1.8. Cytotoxicity

The methanol extract, petroleum ether, dichlomethane fraction and aqueous fraction of the methanol extract were screened for anti-proliferation activity against HT-29 (colon cancer) cells. The methanol extract inhibited the proliferation of HT-29 cells by 50% at a concentration of 10 μg/mL. The petroleum ether and dichloromethane fractions inhibited proliferation of HT-29 cells with a GI_{50} value of 46 μg/mL and 10 μg/mL respectively. The aqueous extract did not inhibit the proliferation of HT-29 cells. Of all the diterpenes isolated from the dichlomethane fraction, only adrographolide inhibited the proliferation of all cancer cells screened. 14-deoxy-andrographolide showed moderate inhibition against the proliferation of two cancer cell out of the entire cell screened. 14-deoxy-11, 12-didehydroandrographolide did not inhibit the proliferation of any of the cancer cell line tested[36]. These findings are in consonance with earlier reports, that demonstrated the cytotoxic activity of andrographolide against human epidermoid carcinoma and lymphocytic leukaemia cells[19]. The growth inhibitory activity of the methanol extract of the aerial parts of *A. paniculata* and some of the isolated compounds on mouse myeloid leukemia cells has also been reported[37]. The *in vitro* anti-cancer activity of andrographolide and its semi-synthetic analogues-3, 19-isopropylideneandrographolide, 14-acetyl-3, 19-isopropylideneandrographolide and 14-acetylandrographolide were screened for anti-tumor activity against MCF-7 human breast cancer and HCT-116 colon cancer cell lines. 19-isopropylideneandrographolide and 14-acetylandrographolide showed cytotoxic activity against the two cell lines tested and they were equally potent when compared to parent andrographolide. In a similar study at the national cancer institute in the USA, 19-isopropylideneandrographolide and 14-acetylandrographolide were also screened and found to be cytotoxic against 60 human cancer cell lines[38]. Xanthones isolated from the chloroform fraction of the roots were screened for cytotoxicity and the results showed that all the compounds have IC_{50} values greater than 16 μg/mL, thus exhibiting non-cytotoxic behavior as per WHO criteria[22].

1.9. Antidiabetic activity

Andrographolide and 14-deoxy-11,12-didehydroandrographolide isolated from the alcoholic extract of the aerial parts of *A. paniculata* reduced the phenotypes indicating diabetic nephropathy in MES-13 cells, which include secretion of extracellular matrix protein

fibronectin, cytokine TGF-β, states of oxidative stress, and apoptosis marker caspase-3. Compound 14-deoxy-11,12-didehydroandrographolide showed more potent activity than andrographolide in the reduction of apoptosis marker caspase-3, fibrosis marker cytokine TGF-β, and plasminogen activator inhibitor-1. Both compounds also reduced reactive oxygen species in the MES-13 cells[39].

The aqueous extract (50 mg/kg) of *A. paniculata* raw material produced a significant (P<0.05) reduction (52.9%) in blood glucose level in streptozocin-induced hyperglycaemic rats. Freeze dried material of *A. paniculata* (6.25 mg/kg body weight), however, produced a more significant (P<0.001) reduction (61.81%) in blood glucose level. The results further showed that the aqueous extract of *A. paniculata* did not produce significant reduction in blood glucose level in normoglycemic rats[40].

2. Anti-protozoan activity

Four xanthones isolated from fractions of the roots were screened for anti-plasmodial activity against *Plasmodium falciparum*, only compound 1,2-dihydroxy-6,8-dimethoxy-xantone possessed substantial anti-plasmodial activity against *Plasmodium falciparum* with an IC_{50} value of 4 μg/mL. This compound also exhibited *in vivo* antimalarial activity in mice infected with *Plasmodium berghei*, where it produced substantial reduction (62%) in parasitemia[22]. This study involving the root fraction showed more anti-malarial activity when compared with a previous study with fractions isolated from the leaves[41]. Andrographolide, noeandrographolide, deoxyandrographolide and andrographoside isolated from the leaves have been shown to have some activity against *Plasmodium berghei* NK65 in *Mastomys natalensis*[42].

2.1. Insecticidal activity

The ovicidal and larvicidal activity of the crude leaf extracts of *A. paniculata* with five different solvents, namely, benzene, hexane, ethylacetate, methanol, and chloroform were tested against the early third instar larvae of *Culex quinquefasciatus* (Say) and *Aedes aegypti* (Linn). The benzene, hexane, ethylacetate, methanol and chloroform extract were found to be more effective against *Culex quinquefasciatus* than *Aedes aegypti*. The LC_{50} were 112.19, 137.48, 118.67, 102.05, 91.20 mg/L and 119.58, 146.34, 124.24, 110.12, 99.54 mg/L respectively. The methanol and ethyl acetate extract were found to be most effective for ovicidal activity against the two mosquito species. The extract of methanol and ehtylacetate also exerted 100% mortality at 200 mg/L against *Culex quinquefasciatus* and at 250 mg/L against *Aedes aegypti*[43].

2.1.1. Anti- infective activity

The efficacy of the leaf extract of *A. paniculata* in the treatment of the symptoms of uncomplicated upper respiratory tract infection has been reported. The findings obtained in a randomized double blind placebo controlled clinical evaluation, using the visual analogue scale for quantification of symptoms, showed that Kalmcold treatment significantly (P<0.05) decreased all the symptoms score except for ear ache whereas symptoms remained unchanged or got worse after Day 3 of the study period for the placebo group. The study revealed that Kalmcold was 2.1 times or 52.7% more effective than placebo in reducing symptoms of uncomplicated upper respiratory tract infection[44]. *A. paniculata* extract SHA-10 (1 200 mg/day) administered for a period of five days significantly (P<0.05) reduced the intensity of the symptoms (tiredness, sleeplessness, sore throat and nasal secretion) in uncomplicated common cold beginning at Day 2 of treatment over placebo group. At Day 4, a significant decrease in the intensity of all the symptoms (headache, tiredness, ear ache, sleeplessness, sore throat, nasal secretion, phlegm, frequency and intensity of cough) was observed for *A. paniculata* group[45].

2.1.2. Anti -angiogenic activity

Ethanol extract of the whole plant of *A. paniculata* and its major component andrographolide were screened for anti-angiogenic activity using both the *in vitro* and *in vivo* models. Intraperitoneal administration of the ethanol extract and andrographolide significantly (P<0.001) inhibited the B16F-10 melanoma cell line induced capillary formation in C57BL/6 mice by 35.96% and 31.1% respectively. Treatment with the ethanol extract and andrographolide significantly (P<0.001) reduced serum levels of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, NO and granulocyte-macrophage colony-stimulating factor and the most potent angiogenic factor vascular endothelial growth factor compared with control. Vascular endothelial growth factor mRNA levels of expression in B16F-10 cell line showed a reduced level of expression in the presence of ethanol extract and andrographolide. Ethanol extract and andrographolide elevated antiangiogenic factors such as TIMP-1 and IL-2 compared to control. Treatment with ethanol extract (10 μg/mL) and andrographolide (0.25 μg/mL) inhibited micro vessel sprouting from rat thoraxic aorta induced by B16F10 melanoma conditioned medium[46].

2.1.3. Hepato -renal protective activity

Andrograholides and arabinogalactan proteins isolated from the herbs of *A. paniculata* were screened for hepato-renal protective activity against ethanol-induced toxicity in mice. Intraperitoneal pretreatment of mice with andrograholides (500 mg/kg body weight of mice) and arabinogalactan (125 mg/kg body weight of mice) for 7 d, before intraperitoneal injection of ethanol (7.5 mg/kg body weight) minimized toxicity as revealed by different enzyme assay in the liver and kidney tissues. Both andrograholides and arabinogalactan significantly (P<0.001) reduced levels of glutamic-oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase and LP enzymes in liver and kidney in a comparable manner with the reference

standard Silymarin when compared to the ethanol treated group[23].

2.1.4. Liver enzyme modulation

Both andrographolide and 14-deoxy-11, 12-didehydroandrographolide inhibited mRNA and protein expression of *CYP1A2*, *CYP2D6*, and *CYP3A4* in HepG2 hepatoma cells. The lowest concentration (0.3 μm) of both diterpenoids produced a more than 50% reduction in the mRNA and protein expression of *CYP3A4* and this reduction was consistent with the enzyme activity. Both compounds also reduced the ability of dexamethasone to induce *CYP3A4* expression[47]. Andrographolide induced enhanced expression of *CYP1* in PAH-responsive C57BL/6 male mice and did not alter *CYP1* expression in the PAH-non-responsive DBA/2 male mice, intact or ovariectomized females and orchiectomized male mice. However treatment with testosterone restored the effect of andrographolide on *CYP1* in both orchiectomized males and ovariectomized females. This observation suggests a role for a male hormone system as a crucial mediator of the modulation of *CYP1* expression by andrographolide[48]. Andrographolide and *A. paniculata* extract significantly (P<0.05) increased the clearance and reduced the area under concentration-time curve of theophylline (1 mg/kg) in the blood of male Sprague Dawley rats. The elimination half-life and mean residence time of theophylline were shortened by 14% and 17%, respectively, in the andrographolide treated rat in the presence of high dose theophylline (5 mg/kg). However, theophylline (5 mg/kg) accumulated in the blood of rats pretreated with *A. paniculata* extract. This suggests that some herbal constituents contained in *A. paniculata* extract may interact with theophylline and retard its elimination when administered at a high dose. This creates the need for people taking *A. paniculata* extract to be alerted to the possibility of herb-drug interaction[49].

2.1.5. Sex hormone/function modulation

Oral administration of the extract of the leaves in doses of 200, 600 and 2000 mg/kg body weight (*i.e.* 30, 90 and 300 fold higher than its daily therapeutic dose in humans) to pregnant rats for a period of 19 d for the 200 mg/kg group and 11 d for the 600 and 2000 mg/kg group respectively did not show any effect on the elevated level of progesterone in the blood plasma of pregnant rats when compared with control groups. This suggests that *A. paniculata* at therapeutic doses cannot induce abortion[50]. Andrographolide (50 mg/kg body weight) administered to male ICR mice significantly (P<0.05) decreased the mounting latency at 120 min and 180 min and increased the mounting frequency at 180 min after treatment, suggesting an improvement in sexual functions. Pre-incubation of endothelium-intact aortic strip with andrographolide for 10 min before adding nor-epinephrine resulted in a significant reduction in nor-epinephrine effect on aortic strip tension, an observation which suggests that andrographolide improves sexual function by causing smooth muscle relaxation and increasing blood flow to the penis. Also, chronic once daily treatment of male mice with androgapholide (50 mg/kg) for 2, 4, 6 or 8 weeks significantly (P<0.05) increased serum testosterone level at Week 4 and this level declined back to normal (pretreatment levels) at Week 6 and 8 with continued treatment. Furthermore, andrographolide (50 mg/kg) was shown to have no significant effect on sperm count and motility[51].

2.1.6. Toxicity

The safety of *A. paniculata* extract (Kalmcold) in genotoxic tests has been reported and also the LD_{50} value has been determined to be more than 5 g/kg rat body weight in an oral acute toxicity study[52]. Testicular toxicity as assessed by reproductive organ weight, testicular histology, ultra structural analysis of leydig cells and testosterone levels was not found after 60 d treatment of Sprague Dawley rats with ethanol extract of the dried herbs of *A. paniculata* at doses of 20, 200 and 1000 mg/kg suggesting the relative safe toxicity profile[53,54].

3. Conclusion

A. paniculata has been extensively used as traditional medicine in India, China and Southeast Asia. The aerial parts possess most of the medicinal properties and are used to treat snakebites, insect stings, fever, sore throat, cough and stomachache. Phytochemical study revealed that diterpenoid lactones which are the major phytochemical constituents and flavonoids have been isolated from the aerial parts of this specie. Miscellaneous compounds such as xanthones, rare noriridoids and trace/macro elements have been isolated from the roots. Different types of formulations, extracts and pure compounds obtained from this plant have been shown to possess biological activities including anti-microbial, anti-inflammatory, anti-oxidant, anti-diabetic, cytotoxicity, immune modulatory, sex hormone modulatory, liver enzyme modulatory, anti-malaria, anti-angiogenic and hepato-renal protective activity. Diterpenoid lactones including the bitter andrographolide are pure compounds derived from this plant with most promising biological activities. This review has provided a robust insight into the phytochemistry, medicinal uses and pharmacology of *A. paniculata*. Nonetheless, further study on the phytochemistry and mechanism of action of the pure compounds are necessary to fully understand the phytochemical profile and the complex pharmacological effects of this plant. In addition, clinical and laboratory studies on the toxicity of all the

plant part extracts and other pure phytochemicals isolated from this plant are also important to ensure its safety and eligibility as source of modern medicine.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This work was in part supported by a US- Senior Fulbright Award granted to Dr. A. Falodun for study at University of Mississippi, USA, CIESCs for the Fulbright award. University of Benin is also highly acknowledged.

Comments

Background

A. paniculata is a medicinal plant belonging to the family Acanthaceae. The plant has been reported for the treatment of various diseases in Asia, America and Africa. The plant has been reviewed for its phytochemistry, pharmacological and toxicity. Diterpenes, flavonoids, xanthones, noriridoides and other miscellaneous compounds have been isolated from the plant. Extract and pure compounds of the plant have been reported for their pharmacological activities. However there are lesser studies upon its toxicological aspects.

Research frontiers

This is a literature-survey based review article including the phytochemistry, pharmacology and a brief introduction to toxicology. Since the plant is reported to exhibit the promising medicinal value, but there is insufficient study upon its phytochemical, pharmacological and toxicological aspects.

Related reports

This review is based on literature study on scientific journals and books from library and electronic sources since 1952 till 2013.

Innovations & breakthroughs

The present review represents a number of structures of the compounds isolated from the palnt species. Furthermore it gives better idea about the specific compound or the extract for a specific biological activity. And it gives good knowledge about the traditional therapeutic use of the plant all around the world.

Applications

This review can help in carrying out the research work upon the plant specific extract and it may also help to study the toxicological effects of the extracts and pure compounds isolated from the plant species.

Peer review

The present review is well-written and precisely summarised. The structures of the compounds and differernt biological activities of the pure compounds and extracts as well have been represented in a good way. This type of review may lead to further research upon the plant species.

References

[1] Farnsworth NR, Soejarto DD. Global importance of medicinal plants. In: Akerelev O, Heywood V, Synge H, editors. *The conservation of medicinal plants*. Cambridge: Cambridge University Press; 1991, p. 25-51.

[2] Latto SK, Khan S, Dhar AK, Chaudhry DK, Gupta KK, Sharma PR. Genetics and mechanism of induced male sterility in *Andrographis paniculata* (Berm.f.) Nees and its significance. *Curr Sci* 2006; **91**: 515-519.

[3] Li J, Huang W, Zhang H, Wang X, Zhou H. Synthesis of andrographolide derivatives and their TNF-alpha and IL-6 expression inhibitory activities. *Bioorg Med Chem Lett* 2007; **17**: 6891-6894.

[4] Mishra SK, Sangwan NS, Sangwan RS. *Andrographis paniculata* (Kalmegh): a review. *Pharmacognosy Rev* 2007; **1**: 283-298.

[5] Khare CP. *Andrographis paniculata*. In: KhareKhare CP, editor. *Indian medicinal plants, an Illustrated Dictionary*. New Delhi, India: Springer; 2007, p. 2, 49-50.

[6] Chopra RN. *Glossary of Indian medicinal plants*. New Delhi: Council for Scientific and Industrial Research; 1980, p. 18.

[7] Jarukamjorn K, Kondo S, Chatuphonprasert W, Sakuma T, Kawasaki Y, Emito N. Gender-associated modulation of inducible CYP1A1 expression by andrographolide in mouse liver. *Eur J Pharm Sci* 2010; **39**: 394-401.

[8] Chaturvedi GN, Tomar GS, Tiwari SK, Singh KP. Clinical studies on Kalmegh (*Andrographis paniculata* Nees) in infective hepatitis. *J Int Inst Ayurveda* 1983; **2**: 208-211.

[9] Balu S, Alagesaboopathi C. Anti-inflammatory activities of some species of *Andrographis* Wall. *Anc Sci Life* 1993; **13**: 180-184.

[10] Saxena S, Jain DC, Bhakuni RS, Sharma RP. Chemistry and pharmacology of *Andrographis* species. *Indian Drugs* 1998; **35**: 458-467.

[11] Perry LM. *Medicinal plants of East and Southeast Asia: attributed properties and uses*. Cambridge: MIT Press; 1980.

[12] Deng WL. Preliminary studies on the pharmacology of the *Andrographis* product dihydroandrographolide sodium succinate. *Newslett Clin Herb Med* 1978; **8**: 26-28.

[13] Alagesaboopathi C, Dwrakan P, Ramachandran VS. *Andrographis paniculata* Nee in tribal medicine of Tamil Nadu. *Anc Sci Life* 1999; **19**: 28-30.

[14] Panossian A, Davtyan T, Gukassyan N, Gukasova G, Mamikonyan G, Gabrielian E, et al. Effect of andrographolide and Kan Jang fixed combination of extract SHA-10 and extract SHE-3 on proliferation of human lymphocytes, production of cytokines and immune activation markers in blood cell culture. *Phytomedicine* 2002; **9**: 598-605.

[15] Bensky D, Gamble A. *Chinese medicine material medica*. Revised edition. Vista: Eastland press; 1993, p. 95.

[16] Poolsup N, Suthisisang C, Prathanturarug S, Asawamekin A, Chanchareon U. *Andrographis paniculata* in the symptomatic treatment of uncomplicated upper respiratory tract infection: systematic review of randomized controlled trials. *J Clin Pharm Ther* 2004; **29**(1): 37-45.

[17] Phosphane N, Rangkadilok N, Thongnest S, Ruchirawat M, Ruchirawat J. Determination and variation of three active diterpenoids in *Andrographis paniculata* (Burm.f.) Nees. *Phytochem Anal* 2004; **15**: 365-371.

[18] Li WK, Fitzloff JF. HPLC-PDA determination of bioactive diterpenoids from plant materials and commercial products of *Andrographis paniculata*. *J Liq Chromatogr Relat Technol* 2004; **27**: 2407-2420.

[19] Siripong P, Kongkathip B, Preechanukool K, Picha P, Tunsuwan K, Taylor WC. Cytotoxic diterpenoid constituents from *Andrographis paniculata* Nees leaves. *Sci Asia* 1992; **18**: 187-194.

[20] Reddy MK, Reddy MV, Gunasekar D, Murthy MM. Crux C, Bodo B. A flavones and an unusual 23-carbon terpenoid from *Andrographis paniculata*. *Phytochemistry* 2003; **62**: 1271-1275.

[21] Dua VK, Ojha VP, Roy R, Joshi BC, Valecha N, Devi CU, et al. Anti-malarial activity of some xanthones isolated from the roots of *Andrographis paniculata*. *J Ethnopharmacol* 2004; **95**: 247-251.

[22] Xu C, Chou GX, Wang CH, Wang ZT. Rare noriridoids from the roots of *Andrographis paniculata*. *Phytochemistry* 2012; **77**: 275-279.

[23] Singh PK, Roy S, Dey S. Protective activity of andrographolide and arabinogalactan proteins from *Andrographis paniculata* Nees. against ethanol-induced toxicity in mice. *J Ethnopharmacol* 2007; **111**: 13-21.

[24] Behera PR, Nayak P, Baric DP, Rautray TR, Thirunavoukkarasu M, Chand PK. ED-XRF spectrometric analysis of comparative elemental composition of *in vivo* and *in vitro* roots of *Andrographis paniculatata* (Burm.f.) Wall. ex Nees-a multi-medicinal herb. *Appl Radiat Isot* 2010; **68**: 2229-2236.

[25] Koteswara Rao Y, Vimalamma G, Rao CV, Tzeng YM. Flavonoids and andrographolides from *Andrographis paniculata*. *Phytochemistry* 2004; **65**: 2317-2321.

[26] Kuroyanagi M, Sato M, Ueno A, Nishi K. Flavonoids from *Andrographis paniculata*. *Chem Pharm Bull* 1987; **35**: 4429-4435.

[27] Singh PK, Roy S, Dey S. Antimicrobial activity of *Andrographis paniculata*. *Fitoterapia* 2003; **74**: 692-694.

[28] Chandrasekaran CV, Gupta A, Agarwal A. Effect of an extract of *Andrographis paniculata* leaves on inflammatory and allergic mediators *in vitro*. *J Ethnopharmacol* 2010; **129**: 203-207.

[29] Chandrasekaran CV, Thiyagarajan P, Deepak HB, Agarwal A. *In vitro* modulation of LPS/calcimycin induced inflammatory and allergic mediators by pure compounds of *Andrographis paniculata* (King of bitters) extract. *Int Immunupharmacol* 2011; **11**: 70-84.

[30] Parichatikanond W, Suthisisang C, Dhepakson P, Herunsalee A. Study of anti-inflammatory activities of the pure compounds from *Andrographis paniculata* (Burm.f.) Nees and their effects on gene expression. *Int Immunopharmacol* 2010; **10**: 1361-1373.

[31] Liu J, Wang ZT, Ge BX. Andrograpanin, isolated from *Andrographis paniculata*, exhibits anti-inflammatory property in lipopolysaccharide-induced macrophage cells through down-regulating the p38 MAKs signaling pathways. *Int Immunopharmacol* 2008; **8**: 951-958.

[32] Neogy S, Das S, Mahapatra SK, Mandal N, Roy S. Amelioratory effect of *Andrographis paniculata* Nees on liver, kidney, heart, lung and spleen during nicotine induced oxidative stress. *Environ Toxicol Pharmacol* 2008; **25**: 321-328.

[33] Akowuah GA, Zhari I, Norhayati I, Mariam A. HPLC and HPTLC densitometric determination of Andrographolides and antioxidant potential of *Andrographis paniculata*. *J Food Compost Anal* 2006; **19**: 118-126.

[34] Akowuah GA, Zhari I, Mariam A, Yam MF. Absorption of andrographolides from *Andrographis paniculata* and its effect on CCl_4-induced oxidative stress in rats. *Food Chem Toxicol* 2009; **47**: 2321-2326.

[35] Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS. Immunistimulant agents from *Andrographis paniculata*. *J Nat Prod* 1993; **56**: 995-999.

[36] Kumar RA, Sridevi K, Kumar NV, Nanduri S, Rajagopal S. Anticancer and immunostimulatory compounds from *Andrographis paniculata*. *J Ethnopharmacol* 2004; **92**: 291-295.

[37] Matsuda T, Kuroyanagi M, Sugiyama S, Umehara K, Ueno A, Nishi K. Cell differentiation inducing diterpenes from *Andrographis paniculata* Nees. *Chem Pharm Bull* 1994; **42**: 1216-1225.

[38] Jade SR, Suburb GS, Matthews C, Hamzah AS, Lapis NH, Saad MS, et al. Semisynthesis and *in vitro* anticancer activities of andrographolide analogues. *Phytochemistry* 2007; **68**: 904-912.

[39] Lee MJ, Rao YK, Chen K, Lee YC, Chung YS, Teng YM. Andrographolide and 14-deoxy-11,12-didehydroandrographolide from *Andrographis paniculata* attenuates high glucose-induced fibrosis and apoptosis in murine renal mesangeal cell lines. *J Ethnopharmacol* 2010; **132**: 497-505.

[40] Husen R, Pihie AH, Nallappan M. Screening for antihyperglycaemic activity in several local herbs of Malaysia. *J Ethnopharmacol* 2004; **95**: 205-208.

[41] Dua VK, Oha VP, Biswas S, Valecha N, Singh N, SharmaVP. Antimalarial activity of different fractions isolated from the leaves of *Andrographis paniculata*. *J Med Aromat Plant Sci* 1999; **21**: 1069-1073.

[42] Misra P, Pal NL, Guru PY, Kariya JC, Srivastava V, Tandon JC.

Antimalarial activity of *Andrographis paniculata* (Kamelgh) against *Plasmodium berghei* NK65 in *Mastomys natalensis*. *Int J Pharm* 1992; **30**: 263-274.

[43] Govindarajan M. Evaluation of *Andrographis paniculata* Burm. f. (Family: Acanthaceae) extracts against *Culex quinquefasciatus* (Say) and *Aedes aegypti* (Linn). *Asian Pac J Trop Med* 2011; **4**: 176-181.

[44] Saxena RC, Singh R, Kumar P, Yadav SC, Neigh MP, Saxena VS, et al. A randomized double blind placebo controlled clinical evaluation of extract of *Andrographis paniculata* (Kalmcold]) in patients with uncomplicated upper respiratory tract infection. *Phytomedicine* 2010; **17**: 178-185.

[45] Ca′ceres DD, Hancke JL, Burgos RA, Sandberg F, Wikman GK. Use of visual analogue scale measurement (VAS) to assess the effectiveness of standardized *Andrographis paniculata* extract SHA-10 in reducing the symptoms of common cold. A randomized double blind-placebo study. *Phytomedicine* 1999; **6**: 217-223.

[46] Sheena K, Guruvayoorappan C, Kutta G. Antiangiogenic activity of *Andrographis paniculata* extract and andrographolide. *Int Immunopharmacol* 2007; **7**: 211-221.

[47] Obi JP, Kuroyanagi M, Suleiman SF, Muhammad TS, Tan ML. Andrographolide and 14-deoxy-11, 12-didehydroandrographolide inhibits cytochrome P450s in HepG2 hepatoma cells. *Life Sci* 2001; **88**: 447-454.

[48] Imsanguan P, Pongamphai S, Douglas S, Teppaitoon W. Supercritical antisolvent precipitation of andrographolide from *Andrographis paniculata* extracts: effect of pressure, temperature and CO_2 flow rate. *Powder Technol* 2010; **200**: 246-253.

[49] Chien CF, Wu YT, Lee WC, Lin LC, Tsai TH. Herb-drug interaction of *Andrographis paniculata* extract and andrographolide on the pharmacokinetics of theophylline in rats. *Chem Biol Interact* 2010; **184**: 458-465.

[50] Panossian A, Kochikian A, Gabrielian E, Muradian R, Stephanian H, Armenian F, et al. Effect of *Andrographis paniculata* extract on progesterone in blood plasma of pregnant rats. *Phytomedicine* 1999; **6**: 157-161.

[51] Sattayasai J, Srisuwan S, Arkaravichien T, Aromedee C. Effects of andrographolide on sexual functions, vascular reactivity and serum testosterone level in rodents. *Food Chem Toxicol* 2010; **48**: 1934-1938.

[52] Chandrasekaran CV, Thiyagarajan P, Sundarajan K, Goudar KS, Deepak M, Murali B, et al. Evaluation of the genotoxic potential and acute oral toxicity of standardized extract of *Andrographis paniculata* (Kalmcold). *Food Chem Toxicol* 2009; **47**: 1892-1902.

[53] Burgos RA, Caballero EE, Sanchez NS, Schroeder RA, Wikman GK, Hancke JL. Testicular toxicity assessment of *Andrographis paniculata* dried extract in rats. *J Ethnopharmacol* 1997; **58**: 219-224.

[54] Balu S, Alagesaboopathi C, Elango V. Antipyretic activities of some species of *Andrographis* Wall. *Anc Sci Life* 1992; **12**: 399-402.

[55] Cheung HY, Cheung CS, Kong CK. Determination of bioactive diterpenoids from *Andrographis paniculata* by micellar electrokinetic chromatography. *J Chromatogr A* 2001; **930**: 171-176.

[56] Du QZ, Jerz G, Winterhalter P. Seperation of andrographolide and neoandrographolide from the leaves of *Andrographis paniculata* using high speed counter-current chromatography. *J Chromatogr A* 2003; **984**: 147-151.

[57] Kleipool RJ. Constituents of *Andrographis paniculata* Nees. *Nature* 1952; **169**: 33-34.

[58] Chan WR, Taylor IR, Willis CR, Bodden RL. The structure and stereochemistry of neoandrogrpholide, a diterpene glycoside from *Andrographis paniculata* Nees. *Tetrahedron* 1971; **27**: 5081-5091.

[59] Jain DC, Gupta MM, Saxena S, Kumar S. LC analysis of hepatoprotective diterpenoids from *Andrographis paniculata*. *J Pharm Biomed Anal* 2000; **22**: 705-709.

[60] Yang M, Wang J, Kong L. Quantitative analysis of four major diterpenoids in *Andrographis paniculata* by 1H NMR and its application for quality control of commercial preparations. *J Pharm Biomed Anal* 2012; **70**: 87-93.

[61] Wu D, Cao X, Wu S. Overlapping elution-extrusion counter-current chromatography: a novel method for efficient purification of natural cytotoxic andrographolides from *Andrographis paniculata*. *J Chromatography A* 2012; **1223**: 53-63.

[62] Aromdee C. Modifications of andrographolide to increase some biological activities: a patent review (2006-2011). *Expert Opin Ther Pat* 2012; **22**(2): 169-180.

[63] Zou QY, Li N, Dan C, Deng WL, Peng SL, Ding LS. A new *ent*-labdane diterpenoid from *Andrographis paniculata*. *Chin Chem Lett* 2010; **21**: 1091-1093.

[64] Ma XC, Gou ZP, Wang CY, Yao JH, Xin XL, Lin Y, et al. A new *ent*-labdane diterpenoid lactone from *Andrographis paniculata*. *Chin Chem Lett* 2010; **21**: 587-589.

[65] Xou C, Chou GX, Zhen TW. A new diterpene from the leaves of *Andrographis paniculata* Nees. *Fitoterapia* 2010; **81**: 610-613.

[66] Gupta KK, Taenia SC, Dhar KL, Atal CK. Flavoniods of *Andrographis paniculata*. *Phytochemistry* 1983; **22**: 314-315.

[67] Varma A, Padh H, Shrivastava N. Andrographolide: a new plant-derived antineoplastic entity on horizon. *Evid Based Complement Alternat Med* 2011; doi: 10.1093/ecam/nep135.

[68] Zhou B, Zhang D, Wu X. Biological activities and corresponding SARs of andrographolide and its derivatives. *Mini Rev Med Chem* 2013; **13**(2): 298-309.

[69] Lim JC, Chan TK, Ng DS, Sagineedu SR, Stanslas J, Wong WS. Andrographolide and its analogues: versatile bioactive molecules for combating inflammation and cancer. *Clin Exp Pharmacol Physiol* 2012; **39**(3): 300-310.

[70] Kishore PH, Reddy MV, Reddy MK, Gunasekar D, Caux C, Bodo B. Flavonoids from *Andrographis lineate*. *Phytochemistry* 2003; **63**: 457-461.

[71] Chaos WW, Lin BF. Isolation and identification of bioactive compounds in *Andrographis paniculata* (Chuanxinlian). *Chin Med* 2010; **5**: 17.