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A bitter plant with a sweet future? A comprehensive review of an oriental medicinal plant: *Andrographis paniculata*

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Abstract *Andrographis paniculata* (Burm.f) Nees is one of the most popular and important medicinal plant of the Orient, and South East Asia. It finds mention in various forms in Indian, Chinese, Malay, Thai, Unani, and Japanese systems of medicine. The plant exhibits anti-cancer, anti-inflammatory, anti-diabetic, anti-hypertensive, anti-venom, cholestatic, hepatoprotective, anti-thrombotic, anti-retroviral, anti-microbial, anti-pyretic, anti-malarial, anti-oxidant, immunomodulatory, and cardioprotective effects. The major active principles contributing to biological activity are diterpene lactones, but flavonoids, xanthones and caffeic acid derivatives also contribute to anti-oxidant, anti-proliferative, anti-atherosclerotic, and anti-malarial effects. As a result of its wide spectrum of pharmacological activity, almost impeccable safety profile, being a widely cultivated medicinal plant, we have collected and compiled various facets of this plant. Extensive datamining of the phytochemistry and pharmacology of *Andrographis paniculata* revealed more than 50 diterpene lactones, 30 flavonoids, 8 quinic acid derivatives, and 4 xanthones. This review contains information on around 80 isolated compounds, out of which more than half of the compounds have no reported pharmacological activity. Though there are some good reviews available on *Andrographis paniculata*, the authors of the earlier reviews focused on one or two aspects of the plant and none have attempted to integrate the available information on this plant. This provided us the much needed impetus, warranting a full-fledged and complete review on *Andrographis paniculata,* one of the most popular and important Oriental medicinal plant.

Keywords *Andrographis paniculata* · Ent labdanes · Flavonoids · Quinic acid derivatives · Ethnopharmacology

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Introduction

Approximately 90% people in developing countries rely on traditional or alternative and complementary medicines, based largely on different species of plants, and plant products which is their basic medical aid (Fabricant and Farnsworth 2001). This is especially true for Asian, African or South American countries

where the majority of the population still depends on plant as source of medicines. Of the more than 120 pure pharmaceutical chemicals isolated from about 100 plant species, currently in use as drugs, 40 are obtained from tropical species (Farnsworth and Soejarto 1991). However, fewer than 5% of tropical forest plant species have been examined for chemical compounds and medicinal values (Zakrzewski 2002).

From the last decade or so, rapid strides in high throughput screening, laboratory techniques, molecular, pharmacological, tissue culture techniques, and germplasm conservation initiatives etc. have resulted in a huge number of publications of *Andrographis paniculata*. A recent review of literature with the keywords *Andrographis paniculata* on "PubMed", returned around 250 results. Of research describing pharmacological aspects in laboratory animals the majority of citations are from the year 2000 onwards. It was observed that research articles published from years 1951 to 1989 on *Andrographis paniculata* and andrographolides numbered around 13, whereas from the years 1990 to 1999, research publications on this plant was around 36. In the next decade (2000–2010) the number of publications jumped to 169 from 36. The objective of this review is to generate a strong opinion on *Andrographis paniculata* encouraging the scientific community to unravel further potential uses of this medicinal plant for various diseases. It is expected that with a gradual shift to sophisticated techniques of extraction, the discovery of hitherto unknown phytochemicals and extension of pharmacological spectrum of activity of this plant is a distinct possibility, throwing up exciting challenges to researchers.

Mishra et al. (2007) has written a commentary with their focus on the phytochemistry and pharmacological aspects of *Andrographis paniculata*. They have also thrown light on the safety issues of the plant extract with mention of some studies. The authors have not given information on the agricultural advancements, neither have they listed out the clinical studies. So the end result is whether the said review will serve its purpose to researchers, agriculturists, small scale herbal industries and it is hard to justify it. Chao and Lin (2010) has also performed an excellent review on the isolation and identification of *Andrographis paniculata.* The review article's thrust is on phytochemistry and occasionally has explained the pharmacological part also. A brief information is also available on the preclinical studies of various extracts of the plant. Fractionation of some bioactive extracts especially the ethyl acetate has been dealt with elaborately. It is worthwhile to mention here that the review did not mention the safety issues, the agronomical practices and clinical trials of the plant and hence may serve only academic purpose interests and may not generate an interest to diverse set of readers. Hence it is hoped that this review would serve as a cornerstone to the researchers, agronomists, and even small scale herbal industries and integrate the available information on *Andrographis paniculata* in order to realize the full pharmacological, agricultural and industrial potentials of this exciting medicinal plant.

Botanical description, habitat and parts used

Andrographis paniculata (Burm.f.) Nees is commonly known as "Hempedu Bumi", which literally means "bile of the earth", and "pokok cerita" in Malaysia, "Sambiloto" in Indonesia belonging to Family Acanthaceae. It is also known as "The Creat" in English and "King of bitters". It is an annual, branched, herbaceous plant with a tap root, and grows to a height of around 90–110 cm. The leaves are 3–7 × 1–2.5 cm in size, green, simple, opposite, lanceolate and glabrous with a short petiole. Flowers are white in colour with reddish-purple spots on the petals. The stems are dark green in colour 0.5–1 m height and 2–6 mm in diameter with presence of longitudinal furrows and wings on the angles of young plants. Seeds are small, numerous, abundant and yellowish brown in color. Generally the aerial parts of the plant [including leaves and stems] (WHO, Geneva 2002) are used as traditional medicine since the major active principle andrographolide is present in higher quantities in the aerial parts, but roots (Dua et al. 2004) have also been used but with limited success.

The plant can be abundantly found in South India, North India, Sri Lanka, Islands of Java and Borneo, Malaysia, Brunei, Thailand. The popularity, medicinal use and economic feasibility prompted farmers to cultivate extensively in China, Thailand, and Malaysia of late. In Malaysia, Forest Research Institute Malaysia (FRIM) and Malaysian Agriculture Development Institute (MARDI) in a concerted approach have made cultivation of dedicated nurseries a great success by maintaining different cultivars.

Cultivation and harvesting

Andrographis paniculata is mainly cultivated as rainy season crop. With the onset of monsoon, plant grows luxuriantly and starts flowering with the moderation in temperature. Flowering and fruiting starts from around 2–3 months from planting. Maximum herb biomass can be obtained in 90–100 days beyond which leaves start shedding. At the time of flowering, the active principle andrographolide is high in leaves. Since the whole plant contains active principles, entire harvested material is dried in shade and powdered.

About 400 gm. seed are sufficient for one hectare. Application of sufficient quantities of nitrogen, and phosphorus (Chauhan and Gyanendra 2003) per hectare will increase the herb yield. Addition of 5–7 tons of vermicompost and manure are necessary for raising nursery. The plants at flowering stage (around 90–120 days after sowing) are cut at the base leaving 10–15 cm stem for plant regeneration. About 50–60 days after first harvest, final harvest (Maheshwari et al. 2002) is performed. The yield varies between 3 and 4 tons dry herb/hectare (Seema et al. 2003).

Recent developments in agronomic techniques for mass production, maximising yield of active principles, conservation and management

Tissue culture techniques

The increase in demand for andrographolide by the pharmaceutical industries is putting undue pressure on the existing wild populations. However, the commercial exploitation of this plant is hampered due to its limited availability (Kanjilal et al. 2002). The increasing demand of andrographolide in Indian as well as international markets has motivated Indian farmers to start commercial cultivation of this medicinal plant (Kanjilal et al. 2002), but still there is a gap between supply and demand which can only be bridged by using non conventional methods or tissue culture related techniques. Micropropagation is the proven method for efficient in vitro propagation of medicinal and aromatic plants and for commercial exploitation of valuable plant-derived pharmaceuticals (Bajaj et al. 1988; Purohit et al. 1994; Pattnaik and Chand 1996; Rout 2002; Faisal et al. 2005). Purkayastha et al. 2008, reported that in about 90 days, 60–70 shoots were obtained from a single nodal explant and the nodal explants from primary shoots further regenerated equivalent number of shoots, demonstrating high frequency regeneration potential. The plantlets were successfully transferred to soil after hardening with a 92% survival rate. The process from the initiation of shoot buds to the transplantation of regenerants to soil is complete within 8–9 weeks.

Newer techniques of sustainable agriculture is being advocated strongly these days, using reduced fertilizers and pesticides which in turn help preserve and conserve soil health, fertility, a pollution free environment have to be given maximum importance in developing countries. One of the methods of maximizing yield under sustainable modes of agriculture is through the use of microbial inoculants. It was shown that the seeds of *Andrographis paniculata* when raised in polythene bags containing soil inoculated with isolates of *Glomus leptotichum* and *Glomus intraradices* species of arbuscular mycorrhizal fungi, showed an increase in plant growth and andrographolide concentration (Chiramel et al. 2006) over those grown in the absence of the inoculation of soil with the fungi. The main effect of mycorrhizal fungi in improving plant growth (Marulanda et al. 2003; Nowak 2004; Chen et al. 2005; Khalafallah and Abo-Ghalia 2008) s thought to be due to improved uptake of nutrients. This is especially true for phosphorus (Smith et al. 2003) due to the exploration by the external hyphae of the soil beyond root hair zone when phosphorus is depleted. Increased phosphorus uptake has been attributed not only to increased surface area of absorption but also to enhanced hyphal translocation (Turk et al. 2006). Enhanced plant biomass and phosphorus uptake (Chen et al. 2005) due to arbuscular mycorrhizal fungal inoculation has been reported by earlier workers in forest tree species and a few medicinal plants (Vasanthakrishna et al. 1995; Sailo and Bagyaraj 2005).

The conventional vegetative propagation of *Andrographis paniculata* is a tedious process and too slow to meet the commercial quantities required. Moreover, a high degree of variability among the seed-derived progenies and a scanty and delayed rooting of seedlings curb its propagation via seeds. Hence a study on the in vitro propagation of *Andrographis paniculata* (Burm. f.) Wallichi ex Nees through somatic embryogenesis, and influence of 2,4-dichlorophenoxyacetic

acid (2,4-D) on induction, maturation, and conversion of somatic embryos was investigated by Martin (2004). This study reported aṉ efficient protocol for plant regeneration from callus of *Andrographis paniculata* via somatic embryogenesis. Using this protocol, more than 1,800 plantlets can be produced from 1 g of callus within 200 days, facilitating rapid clonal propagation of this important medicinal plant.

Praveen et al. (2009) demonstrated the use of a cell culture technique to increase the yield of andrographolide from young leaves of *Andrographis paniculata*. Adventitious roots were induced directly from leaf explants using Murashige Skoog liquid medium with napththalene acetic acid and sucrose. The root cultures showed higher accumulation of biomass (fresh and dry weight) and andrographolide within 4 weeks. Seven-fold increment of fresh biomass was evident in suspension cultures along with 3.5-fold higher andrographolide compared to natural plants. These results demonstrated a great potentiality of adventitious root cultures for the production of andrographolides for commercial purposes on a large scale using bioreactor cultures.

The plant hormones or growth regulators influence their growth, differentiation and development (Aguiló et al. 2005). These growth regulatory compounds play a crucial role in the regulation and coordination of plant growth, morphogenesis, metabolism and role in biosynthesis of secondary metabolites (Jaleel et al. 2006). In a study performed by Anuradha et al. (2010), growth regulators like Abscisic acid and Gibberellic acid were used to study the effect on the andrographolide content and antioxidant potentials of *Andrographis paniculata*. These were applied by means of foliar spray during morning hours. A significant enhancement in nonenzymatic antioxidant contents was observed in all the plants under growth regulator treatments. Ascorbic acid and α-tocopherol content was increased significantly under the growth regulator treatments in leaves, stem and roots of *Andrographis paniculata*. HPLC analysis used to quantify the andrographolide content showed an increase in andrographolide content of growth regulator treated plants compared to control.

A few studies have focused on the agronomic traits of this plant. A study undertaken by Bhan et al. (2006), with the objective of evaluating *Andrographis paniculata* for growth behaviour, yield and andrographolide content for optimization of yield among different collections from ten locations in India. The parameters of interest that were recorded were total andrographolide concentration in leaves, dry herbage yield per plant, and leaf/stem ratio. The results demonstrate that the plant achieved highest growth at Jammu adapting to the subtropical conditions and was significantly superior with respect to the production of total andrographolides and dry herbage yield. Harvesting at 100 days after transplantation (DAT) was recommended.

Prathanturarug et al. (2007) has reported the variation in growth and diterpene lactone content among field-cultivated *Andrographis paniculata*. They isolated the elite individual plants containing high amounts of andrographolide and 14-deoxy-11,12-didehydroandrographolide which would serve as a favorable measure in selection of appropriate plant material for the phytomedicine production, standardized compounds and phytomass. They also identified and selected superior plants for further breeding experiments. Micropropagation or Clonal propagation techniques using shoot tip and nodal segments (Sharma et al. 2010) are beneficial for mass-scale multiplication and conservation of an endangered or threatened and medicinally important species within short period and limited space.

For the regeneration of a whole plant from a cell or from a callus mass cytodifferentiation is not enough and there should be differentiation leading to organogenesis. This may occur through shoot bud differentiation (organogenesis) or through somatic embryogenesis. In the former, shoot buds (monopolar structures) were formed while in the latter, somatic embryos (bipolar structures) were formed both leading to regeneration of whole plant (Sharma et al. 2010).

It is useful to preserve the natural germplasm and also apply modern tissue culture techniques in mass cultivation and commercial feasibility. It would also be beneficial to develop suitable genetic markers for propagation and breeding programs to support natural conservation of various species of this plant. Propagation through tissue culture techniques provides sustainable development solutions for mass propagation of plants in general and endangered plants in particular. The powerful techniques of plant cell and tissue culture, recombinant DNA and bioprocessing technologies have offered mankind a great opportunity to exploit the medicinal plants under in vitro conditions.

Genetic markers and gene based techniques

Genetic markers are useful for identifying plant at genomic level (Srivastava and Mishra 2009) and establish new standards in standardization and quality control of botanicals. These markers are highly polymorphic in nature, show codominant inheritance, and occur frequently in genome. They are unbiased to environmental conditions or management practices and at the same time easily available, highly reproducible and allows easy exchange of information between laboratories. Gene-based initiatives for authentic identification of this plant and identify it from the other 28 species of *Andrographis paniculata* (Lattoo et al. 2008) will go a long way in differentiating the medically useful from the other less medically useful ones.

DNA-based molecular markers (Srivastava and Mishra 2009) have been used extensively for a wide range of applications. These applications include study of genetic variation, cultivar identification, genotyping, cross-breeding studies, identification of disease-resistant genes, identification of quantitative-trait loci, diversity analysis of exotic germplasms, sex identification of dioeceous plants, phylogenetic analysis, etc. This is especially useful in case of those that are frequently substituted or adulterated with other species or varieties that are morphologically and/or phytochemically indistinguishable.

DNA sequencing (Pereira et al. 2008) can also be used as a definitive means for identifying species of medicinal importance and can used for correct botanical identification and authentification of crude plant material as part of standardization and quality control procedures (WHO 2002). Variations due to transversion, insertion or deletion can be assessed directly and information on a defined locus can be obtained.

DNA microarray was developed in response to the need for a high-throughput, efficient and comprehensive strategy that can simultaneously measure all the genes or a large defined subset, encoded by a genome. DNA microarray can also be used for studying herb-drug interactions (Berman 2000), for discovery of new diagnostic indicators (Izuka et al. 2003), investigating the mechanism of action (DeFeudis et al. 2003) and the mechanisms underlying these interactions in terms of molecular pharmacology (Coldren et al. 2003), identification of genes involved in drug sensitivity or resistance, correct botanical identification and authentification of crude plant drugs etc.

Marker assisted breeding involves the use of DNA markers linked with DNA sequences of interest. Inheritance pattern of sequences/traits can be confirmed even prior to expression using Restriction Fragment Length Polymorphism (RFLP), RAPD, Amplified Fragment Length Polymorphism (AFLP) and minisatellite markers (Yu et al. 1991; Ma et al. 1994). Thus marker assisted breeding could be of use in producing a higher content of the principle marker andrographolide, or an increase in herb biomass.

Random Amplification of Polymorphic DNA (RAPD) analysis is a sophisticated and effective technique to measure the magnitude of diversity and discriminate between genotypes. A dominant marker, Random Amplification of Polymorphic DNA-based molecular markers have been found to be useful in differentiating different accessions of *Andrographis paniculata* (Lattoo et al. 2008) collected from different geographical regions. Germplasm analysis to study genetic diversity and effective conservation and management of germplasm is another important area in which a lot of efforts have been put in (Lattoo et al. 2008). The congruence of RAPD markers with the morphological descriptors provides a viable alternative to characterize the germplasm of *Andrographis paniculata* for optimum genetic improvement and effective conservation of its genetic resources. A better understanding of distribution of genetic variation at the intraspecific level would help identify superior genotypes for cultivar upgradation as well as to evolve strategies for the establishment of effective in situ and ex situ conservation programmes (Padmesh et al. 1999).

Fingerprinting of medicinal plants is also being carried out extensively. This information has potential in strategic planning of future breeding towards crop sustainability. DNA-based techniques have been widely used for authentication of plant species of medicinal importance.

Extraction techniques

Extraction procedure plays an important role in preserving the active constituents of plant extract and hence the pharmacological activity. The selection of extraction procedure ultimately depends on the nature of the active constituents of interest to be extracted. So it is very important to choose a proper

extraction technique such that the active constituents of interest are maintained in the natural state, but especially, in herbal industries timing, extraction efficiency, automation etc. are valid points for consideration. Conventional extraction of active constituents from *Andrographis paniculata* has been performed by maceration, Soxhlet extraction and ultrasonic extraction (Mukherjee 2002) and their large scale application has been severely limited by several disadvantages.

A dynamic microwave-assisted extraction with on line coupling with HPLC using flow injection interface to analyse andrographolide and dehydroandrographolide has been reported by Chen et al. (2007a). This approach offers several advantages and bypasses many of the problems associated with more traditional approaches. By tweaking several experimental parameters, the optimized extraction conditions of extraction were found to yield mean recoveries of 97.8 and 98.7% for andrographolide and dehydroandrographolide respectively. This technique was demonstrated to generate a higher extraction yield in a shorter time when compared with ultrasonic extraction official in the Chinese Pharmacopoeia. In addition, only small quantities of solvent (5 ml) and sample (10 mg) were required.The authors reported rapid analysis time, economy in solvent use, and reduced cost of analysis. The reliability and repeatability of the analysis were improved, with analysis and sample extraction taking place in a closed automated system, the associated risks of sample loss and contamination were reported to be minimal as well.

Presently supercritical fluid extraction (Lang and Wai 2001) is fast gaining momentum and has the most important application in the extraction of natural products. With increasing trends of herbal product use (Tonthubthimthong et al. 2001; Song et al. 1992; Ambrosino et al. 1999), this technique may well become the gold standard for extraction in the future.

A few studies have been carried out to determine the extraction efficiency of supercritical fluid extraction technique on the yield of andrographolides. Ge et al. (2002) reported the use of supercritical carbondioxide, and carbondioxide-ethanol mixture to extract andrographolide in a custom made extractor unit. He used orthogonal experiments to determine optimum conditions however the study had several short comings. The study failed to explain the effect of particle size and the supercritical solvent flow rate. The study also could not explain the effect of each operating parameter. Moreover, the geometry of the extractor, the supercritical solvent flow pattern, and the ethanol concentration in the supercritical solvent were not reported. The experiments conducted proved that at high temperature and pressure, the extraction rate and yield were higher, but the andrographolide content in the extracts was very low. But an enhanced extract yield and the andrographolide content with addition of ethanol as co-solvent was reported.

Kumoro and Hasan (2007), examined the effect of the solvent flow rate, pressure, and temperature on the supercritical carbondioxide extraction of andrographolide. During the course of experiments, the operating pressures were varied from 7.5 to 20 MPa, temperatures varied from 30 to 60°C, and the flow rates were varied from 0.5 to 4 ml/min. The highest yield within a set of experimental conditions occurred at 10 MPa, 40°C, and a flow rate of 2 ml/min for a 3 g sample of *Andrographis paniculata* ground-dried leaves. The measured extraction rate was found to be about 0.0174 g of andrographolide per gram of andrographolide present in the leaves per hour of operation.

Cui et al. (2009) compared extracts of different sample preparation procedures like microwave-assisted, marinated extraction, reflux, ultrasonic extraction of *Andrographis paniculata* using andrographolide and dehydroandrographolide as reference compounds. The authors reported a simple and timesaving extraction method i.e microwave-assisted extraction with a more effective fingerprint containing additional spectral information namely an enhanced fingerprint. According to the author enhanced fingerprint in comparison with conventional fingerprint with additional spectral information provides more advantages. Enhanced fingerprint displays more peaks which cannot be detected at a fixed wavelength but have UV responses at other wavelengths; Secondly, in quality assessment, conventional fingerprint might give different chromatographic patterns at different wavelengths, whereas enhanced fingerprint technique is more objective and credible.

Analytical techniques

Several methods have been reported for the quantitative determination of lactones in *Andrographis*

paniculata, including TLC, HPTLC, Liquid chromatography, micellar electrokinetic capillary chromatography (MECC), high speed counter current chromatography (Du et al. 2003), Ultra Performance Liquid Chromatography (UPLC).

A High-performance liquid chromatography coupled with on-line solid phase extraction and ultraviolet detection was developed by Chen et al. (2007b) for determining andrographolide and dehydroandrographolide in rabbit plasma. Plasma samples (100 µl) were injected directly into a C_{18} column and the mobile phase consisted of methanol:acetonitrile:water (50:10:40; v/v). The UV detection was performed at 225 nm. The calibration curves showed excellent linear relationship ($R \geq 0.9993$). The inter- and intra-day precisions of two analytes were in the range of 1.2–6.5% and the accuracies were between 92.0 and 102.1%. Their recoveries were all greater than 94%. The limits of detection were 0.019 µg/ml for andrographolide and 0.022 µg/ml for dehydroandrographolide.

Cui et al. (2009) reported on the comparison of conventional and enhanced fingerpoint profile of andrographolide and dehydroandrographolide by HPLC DAD. The precision, stability and repeatability of method were evaluated by the analysis of five successive injections of the same sample solution and were found to be good. It was seen that compared with conventional fingerprints, enhanced fingerprint contains more peaks, especially in the retention time of 15–25. Moreover, the signals in enhanced fingerprint are much stronger than those in conventional fingerprints. Hence compared with enhanced fingerprint, the classification ability and discrimination power of conventional chromatographic fingerprint is limited to perform further quality control. This study underlines the fact that the most relevant factor on the quality of *Andrographis paniculata* was the collecting location and the harvesting time of the plant. In order to get consistent raw materials of *Andrographis paniculata*, the collecting location and harvest time should be fixed.

Kavuri et al. (2010) reported a simple, rapid, precise, accurate, robust and stability indicative Ultra Performance Liquid Chromatography method with UV detection for the quantitative estimation of andrographolide and 14-deoxy 11, 12-didehydroandrographolide from *Andographis paniculata*. The method for quantification of andrographolide and 14-deoxy, 11-12 didehydoandrographolide was validated with regard to its selectivity, linearity, precision, accuracy, stability, robustness, limit of detection and quantification. The ultra fast LC method showed good linearity, accuracy and satisfactory precision with run time of less than 3 min. The developed and validated method was found to be suitable to analyze lower as well as higher amounts of andrographolide and 14-deoxy 11, 12-didehydroandrographolide in plant raw material, crude plant extracts and various dosage forms.

A summary of reported methods is presented in Table 1. Simple HPLC and HPTLC methods are also used for estimating amount of andrographolide, 14-deoxy-11,12 didehydroandrographolide, and neoandrographolide using reverse phase technique with UV detection at 230 nm have been used.

Ethnopharmacology

Andrographis paniculata has been used for centuries together in Asia to treat fevers, sore throat, gastric infections, upper respiratory tract infections, and many other chronic and infectious disorders. The plant which is known as "Kalmegh" in Ayurvedic texts is an important constituent in majority of Ayurvedic preparations and is official in the Ayurvedic Pharmacopoeia. More importantly, *Andrographis paniculata* has also been included in "WHO monographs on selected medicinal plants" (WHO 2002), which serves as an authority and ready reckoner for health officials, scientists, doctors and pharmacists and will also be appreciated by the lay man. The monographs play an essential role in promoting the safe and proper use of plants as medicines globally.

In Southern India, the whole plant of *Andrographis paniculata* is widely employed either orally or applied locally, or both as an antidote against snake bites (Russell 1980) of cobra, Russell's viper, Banded Krait etc. and this has verified experimentally by Samy et al. (2008). The expressed juice of the leaves of *Andrographis paniculata* alone or together with cardamom, cloves and cinnamon made into small globules and prescribed as a common household remedy for griping, irregular stools, loss of appetite, flatulence, and diarrhoea of children. In Eastern India, the tribals of Santal, Kheria and Moora, Khatra region of Bankura district, West Bengal, India, use the infusion of the whole plant to recover from fever (Mandal et al. 2001). Decoction or infusion of the leaves has been

Table 1 Summary of analytical methods for determination of andrographolides

Analytical technique	Analyte	Sample type	LOQ	References
HPLC	AG, DeAG, NeAG	Methanol extract	1 μg/ml	Jain et al. (2000)
MECC	AG, DeAG, NeAG	10% ethanol extract	–	Cheung et al. (2001)
MECC	AG, DeAG, NeAG	60% ethanol extract	–	Zhao et al. (2002)
HPLC	AG	Tablets in 50% methanol	50 ng/ml	Li and Fitzloff (2002)
HPLC	AG	Methanol extract, calibration std of AG prepared in rabbit serum	5 μg/ml	Kumaran et al. (2003)
HPLC	AG, NeAG	Methanol extract	–	Pholphana et al. (2004)
HPLC	AG, Iso AG, NeAG, DeAG	Methanol extract	0.5–100 μg/ml	Li and Fitzloff (2004)
HPLC	AG	90% ethanol extract	–	Wongkittipong et al. (2004)
HPLC	AG, NeAG	Hexane, chloroform, water and methanol extracts	–	Srivastava et al. (2004)
HPLC	AG, DeAG	Methanol extract	10 μg/ml	Akowuah et al. (2006)
FIS	AG	Ethanol extract/capsules in 95% ethanol	1.5 μg/ml	Ruengsitagoon et al. (2006)
HPLC–MS	AG		9.9–320.0 ng/ml	Gu et al. (2007)
HPLC	AG	Tablets in methanol	0.05 μg/ml	Suo et al. (2007)
HPLC	AG, DeAG	40% ethanol extract	AG-1.7 μg/ml DeAG-1.9 μg/ml	Chen et al. (2007b)
HPLC–DAD	AG, DeAG	85% ethanol and 70% ethanol	–	Cui et al. (2009)
UPLC	AG, DeAG	Herb and tablet in 60% methanol	AG- 0.06 μg/ml DeAG- 0.03 μg/ml	Kavuri et al. (2010)

AG andrographolide, *DeAG* 14-deoxy-11,12-didehydroandrographolide, *NeAG* neoandrographolide, *MECC* micellar electrokinetic capillary chromatography, *FIS* flow injection spectrophotometry

used with satisfactory results in sluggish liver, neuralgia, certain forms of dyspepsia associated with gaseous distension of the bowels, in general debility, as a blood purifier (Girach et al. 1994), in convalescence after fevers and in advanced stages of dysentery. During the influenza epidemic in India around 1919, the plant was claimed to be highly efficacious in arresting the progress of the disease. The leaves and roots are also used as febrifuge, tonic, stomachic, cholagogue and anthelminthic. It is used indigenously in medicine particularly as bitter tonic, curing fevers, dysentery and eliminating intestinal worms (Singh et al. 2010). The plant is used to relieve griping, irregular stools and loss of appetite in case of infants (Kirtikar and Basu 1935). It is also reported to heal peptic ulcer (Kirtikar and Basu 1935).

Ancient Chinese physicians used it to treat inflammatory conditions, fever, cold, laryngitis and have been described as a cold property herb (Huang and Wu 2002) to get rid of body heat and dispose toxins from body. In Malaysian folk medicine the decoction of fresh leaves has been used for centuries as an antidiabetic and antihypertensive (Burkill 1966). In Scandinavian countries, it is commonly used for the prevention and relief of cold (Cáceres et al. 1999) (Spasov et al. 2004) and sore throat. In Thailand, it is included as one of the important medicinal plant used in the primary healthcare system.

The most surprising and useful feature of this plant is a broad pharmacological activity ranging from antidiabetic (Rammohan et al. 2006, 2008a, b, c) anticancer (Kumar 2003), immunostimulatory (Xu et al. 2007), antihypertensive (Zhang et al. 2006), anti-inflammatory (Shen et al. 2002), antiviral including anti HIV (Reddy et al. 2005; Wiart et al. 2005), anti malarial (Dua et al. 2004), cardioprotective (Ojha et al. 2009), renoprotective (Singha et al. 2007), hepatoprotective (Singha et al. 2007) and anti thrombotic effects (Thisoda et al. 2006).

Phytochemistry

The use of aerial parts of *Andrographis paniculata* has been described for its myriad uses. There are wide differences in the composition of phytochemicals with respect to part used, geography, season and time of harvesting etc. Andrographolides are present in high levels just before the flowering season (after 90 days) which gradually falls. The aerial parts (leaves and stems) contain the highest proportion of available diterpene lactones of which the major one contributing to pharmacological activity is andrographolide, and roots the lowest. The other lactones are 14-deoxy-11-andrographolide, 14-deoxyandrographolide, neoandrographolide, 14-deoxy-11,12 didehydroandrographolide, andrographon, andragraphan, deoxyandrographiside, andrographiside, andrographosterol etc. It is worthy to note here that a majority of the active constituents of *Andrographis paniculata* represent the *ent labdane* class occurring together with some flavonoids also. Quinic acid derivatives and xanthones (only roots) are also present as minor constituents of *Andrographis paniculata*. A few of the reported works on phytochemistry are mentioned here.

A study by Matsuda et al. (1994) on the ethyl acetate fraction of methanol extract of *Andrographis paniculata* was found to contain a new series of six ent-labdane diterpenoids, two diterpene glucosides, and four diterpene dimers along with already known compounds. They were found to be potent cell differentiation inducers (Table 2) and may be used in the treatment of cancer.

Reddy et al. (2003), reported the isolation of a flavone, 5-hydroxy-7′,2′,6′-trimethoxyflavone and a 23-carbon terpenoid, 14-deoxy-15-isopropylidene-11,12-didehydroandrographolide together with five known flavonoids (7-Omethyldihydrowogonin, 7-O-methylwogonin, skullcapflavone I 2′-methyl ether, 7-O-methylwogonin 5-O-glucoside,and skullcapflavone I 2′-O-glucoside) and four known diterpenoids (14-deoxy-11,12-didehydroandrographolide, andrographolide, isoandrographolide and neoandrographolide). Rao et al. (2004), isolated and identified 5,7, 20,30-tetramethoxyflavanone and 5-hydroxy-7,20,30-trimethoxyflavone.

Reddy et al. (2005), reported a novel bis-andrographolide ether along with five known known flavonoids (7-Omethyldihydrowogonin, 7-O-methylwogonin, skullcapflavone I 20-methyl ether, 7-O-methylwogonin 5-O-glucoside, skullcapflavone I 20-O-glucoside and four known labdane type diterpenoids (14-deoxy-11,12-didehydroandrographolide, andrographolide, isoandrographolide, and neoandrographolide) isolated from the methanolic extract of aerial parts of *Andrographis paniculata* and structure was established by spectral data.

Pramanick et al. (2006), reported the isolation of new labdane type diterpenoid, andropanolide, from the methanolic extract of leaves of *Andrographis paniculata*, along with seven known diterpenoids (andrographolide, andrograpanin,isoandrographolide, 14-deoxy-11, 12-didehydroandrographolide,14-deoxyandrographolide, isoandrographolide, neoandrographolide). Another new diterpene, andrographolactone, isolated from the aerial parts of ethanolic extracts was found to have cytotoxic effects on two human cancer cell lines (Wang et al. 2009). Recently Xu et al. (2010) reported the yield of one novel diterpene (13R, 14R) 3, 13, 14, 19-tetrahydroxy-ent-labda-8, 11-dien-16, 15-olide from the ethanol extract of the leaves of *Andrographis paniculata*, which has a cis-diol group in the lactone moiety, and 3, 19-isopropylidene-14-deoxy-ent-labda-8, 13-dien-16,15-olide, together with eight known diterpenoids (andrographolide, neoandrographolide, andrograpanin, 14-deoxyandrographolide, 14-deoxy-11,12-didehydroandrographolide, 14-deoxyandrographoside, andrographoside and 8-methylandrographolide).

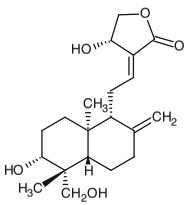
The isolated compounds from *Andrographis paniculata* and their activity are listed in Table 2.

Pharmacology

Andrographis paniculata is having wide range of pharmacological effects. It is also interesting to note that from 1982 to 1999 the pharmacological effects reported centered on antiretroviral (Basak et al. 1999), antioxidative (Das et al. 2009; Lin et al. 2009; Akowuah et al. 2009), antimalarial (Najila et al. 2002), anticancer (Singh et al. 2001; Rajagopal et al. 2003; Kumar et al. 2004; Madamanchi et al. 2008; Lee et al. 2010c), immunomodulatory (Sheeja and Kuttan 2007b), antihypertensive (Zhang and Tan, 1999, Zhang and Tan1998), anti-psychotic (Mandal et al. 2001), anti-inflammatory (Xia et al. 2004; Chandrasekaran et al. 2010; Chao et al. 2009a, b, Qin et al. 2006), anti-thrombotic (Thisoda et al. 2006), antidiabetic (Reyes et al. 2006; Yu et al. 2008, Verma and

Table 2 Phytoconstituents isolated from *Andrographis paniculata*

Chemical constituents: ent labdane diterpenoids
Activity reported in literature

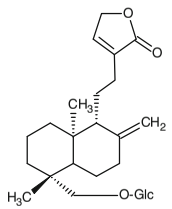


Andrographolide

Part: Leaves, aerial parts, whole plants, and roots

Extract: MeOH*, EtOH$^{\#@}$, hexane^, acetone–water¤

Preclinical pharmacology: Anti retroviral (Wiart et al. 2005; Reddy et al. 2005); Anti proliferative and pro apoptotic (Yang et al. 2010; Zhou et al. 2008a); Antiinflammatory (Wang et al. 2004; Hidalgo et al. 2005; Qin et al. 2006; Li et al. 2009; Bao et al. 2009; Parichatikanond et al. 2010); Radiosensitiser (Hung et al. 2010); cholestatic (Lee et al. 2010b); Immunomodulatory (Panossian et al. 2002; Burgos et al. 2005a, b; Iruretagoyena et al. 2005; Xu et al. 2007; Carretta et al. 2009; Naik and Hule 2009; Wang et al. 2010); Antidiabetic (Zhang and Tan 2000; Yu et al. 2008; Rammohan et al. 2006, 2008a, b, c; Lee et al. 2010a); Anti angiogenic (Sheeja and Kuttan 2007a); Anti thrombotic (Thisoda et al. 2006); Anti-urothelial (Sheeja and Kuttan 2006); Anti-leishmaniasis (Sinha et al. 2000); Hepatoprotective (Handa and Sharma 1990a, b); Analgesic, antipyretic and anti-ulcerogenic (Madav et al. 1995); protective activity against alcohol-induced hepatic and renal toxicity (Singha et al. 2007); Cardioprotective (Woo et al. 2008)



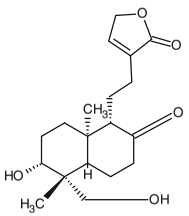
Neoandrographolide

Part: Leaves, aerial parts, whole plants, and roots

Extract: MeOH*, acetone–water¤, ethanol^{+}

Preclinical pharmacology: Anti-inflammatory (Batkhuu et al. 2002; Liu et al. 2007; Parichatikanond et al. 2010); Anti-parasitic (Misra et al. 1992); Hepatoprotective (Chander et al. 1995; Kapil et al. 1993); Chemosensitiser (Pfisterer et al. 2010); Anti-Herpes-Simplex virus (Wiart et al. 2005); Antioxidant (Kamdem et al. 2002)

References: Fujita* et al. (1984); Zou* et al. (2010); Pramanick* et al. (2006;) Chen^{+} et al. (2006); Shen¤ et al. (2006); Rao*et al. (2004); Wu* et al. (2008); Chen^{+} et al. (2008); Reddy* et al. (2003); Matsuda et al. (1994); Xu^{+} (2010)



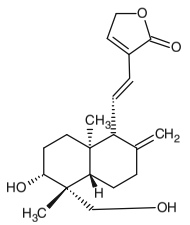
14-deoxyandrographolide

Part: Leaves, aerial parts, whole plants

Extract: MeOH*, acetone–water¤, EtOH$^{\#}$, Hexane^

Preclinical pharmacology: Hepatoprotective (Roy et al. 2010); Uterine smooth muscle relaxant (Burgos et al. 2003); Immunomodulator (Naik and Hule 2009); platelet activating factor antagonist (Burgos et al. 2005a); Vasorelaxant and Antihypertensive (Zhang and Tan 1999, 1998)

References: Matsuda* et al. (1994); Chen$^{\#}$ et al. (2006); Wu* et al. (2008); Rao* et al. (2004); Shen¤ et al. (2006); Chen$^{\#}$ et al. (2008); Pramanick* et al. (2006); Fujita* et al. (1984); Reddy^ et al. (2005); Xu$^{\#}$ et al. (2010)



14-deoxy 11,12-didehydroandrographolide

Part: Leaves, aerial parts, whole plants.

Extract: MeOH*, EtOH$^{\#}$, Hexane^

Preclinical pharmacology: Cholestatic (Lee et al. 2010b); Vasorelaxant and Antihypertensive (Yoopan et al. 2007; Zhang et al. 1998; Zhang and Tan 1998); Anti Herpes (Wiart et al. 2005); Vasorelaxant and Antihypertensive (Zhang and Tan 1999); Antioxidant and hepatoprotective (Akowuah et al. 2009); Antithrombotic (Thisoda et al. 2006); Cytotoxic (Tan et al. 2005); Anti retroviral (Reddy et al. 2005); Anti-diabetic (Lee et al. 2010a)

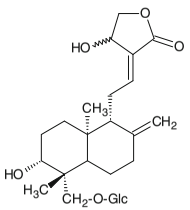
References: Chen* et al. (2008); Fujita* et al. (1984); Xu$^{\#}$ et al. (2010); Reddy* et al. (2003); Wu* et al. (2008); Rao* et al. (2004); Chen* et al. (2006); Matsuda* et al. (1994); Zou* et al. (2010); Reddy^ et al. (2005)

Table 2 continued

Chemical constituents: ent labdane diterpenoids
Activity reported in literature

Anticancer Shi et al. (2008; Cheung et al. 2005; Sheeja and Kuttan 2007b; Han et al. 2006; Ji et al. 2007; Manikam and Stanslas 2009; Zhou et al. 2006; Liang et al. 2008; Jiang et al. 2007; Shi et al. 2009; Lee et al. 2010c; Burgos et al. 2005b; Zhou et al. 2010); Inhibition of Ebstein Barr-virus (Lin et al. 2008); Anti-influenza (Chen et al. 2009; Ko et al. 2006)

References: Zou* et al. (2010); Reddy* et al. (2003); Matsuda* et al. (1994); Chen# et al. (2006); Wu* et al. (2008); Xu@ et al. (2010); Rao* et al. (2004); Reddy^ et al. (2005); Shen¤ et al. (2006); Chen# et al. (2008); Pramanick* et al. (2006); Fujita* et al. (1984)



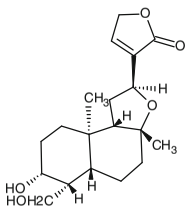
Andrographiside

Part: Leaves, aerial parts, and whole plant

Extract: MeOH*, EtOH¤, acetone–water+,

Preclinical pharmacology: Hepatoprotective (Kapil et al. 1993)

References: Zou et al. (2010); Matsuda* et al. (1994); Chen¤ et al. (2006); Shen+ et al. 2006; Chen¤ et al. (2008); Xu¤ et al. (2010); Rao* et al. (2004); Seth* et al. (2010)



Isoandrographolide

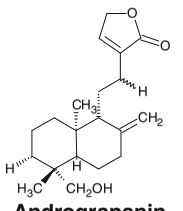
Part: Leaves, aerial parts, roots, and whole plants

Extract: MeOH*, EtOH^, acetone–water+

Preclinical pharmacology: Differentiation inducer (Matsuda et al. 1994); Inhibits growth of *Bacillus subtilis* (Shen et al. 2006); Antiinflammatory and anticancer (Han G, Chinese Patent, CN1785177A)

Antiproliferative (He et al. 2010); Cytotoxic (Li et al. 2007)

References: Wu* et al. (2008); Reddy* et al. (2003); Chen^ et al. (2008); Pramanick* et al. (2006); Matsuda* et al. (1994); Shen+ et al. (2006)



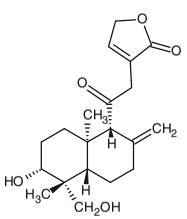
Andrograpanin

Part: Leaves, aerial parts

Extract: EtOH*, ^95%v/v EtOH; Hexane¤

Preclinical pharmacology: 1. Antiinflammatory (Ji* et al. 2005; Liu^ et al. 2008)

References: Ji* et al. (2005); Liu^ et al. (2008); Reddy¤ et al. (2005)



14-deoxy-11-oxo-Andrographolide

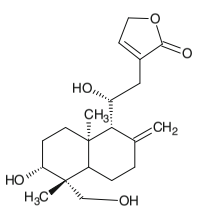
Part: Leaves and aerial parts

Extract: MeOH*, acetone–water^

Preclinical pharmacology:

Antileishmaniasis (Lala et al. 2003)

References: Shen* et al. (2006); Fujita^ et al. (1984)



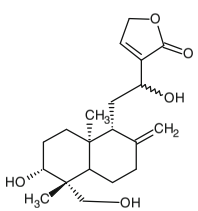
14-deoxy-11-hydroxy-andrographolide

Part: Aerial parts and whole plant

Extract: MeOH*, acetone–water^

Preclinical pharmacology: Cell differentiation inducer (Matsuda et al. 1994)

References: Matsuda* et al. (1994); Shen^ et al. (2006); Rao* et al. (2004)



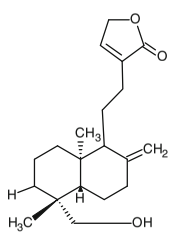
14-deoxy-12-hydroxy-andrographolide

Part: Aerial parts

Extract: MeOH*, acetone–water^

Preclinical pharmacology: Anti-microbial (Shen et al. 2006)

References: Shen^ et al. (2006); Matsuda* et al. (1994)



3,14-dideoxyandrographolide

Part: Aerial parts

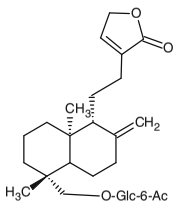
Extract: EtOH

Preclinical pharmacology: Anti-proliferative (Chen et al. 2008)

References: Chen et al. (2008)

Table 2 continued

Chemical constituents: ent labdane diterpenoids
Activity reported in literature



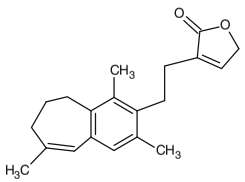
6´-acetylneoandrographolide

Part: Aerial part

Extract: MeOH

Preclinical pharmacology: Cell differentiation inducer (Matsuda et al. 1994)

References: Matsuda et al. (1994)



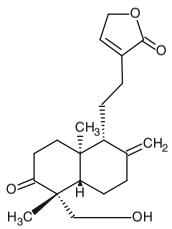
Andrographolactone

Part: Aerial parts

Extract: EtOH

Preclinical pharmacology: Cytotoxic (Wang et al. 2009)

References: Wang et al. (2009)



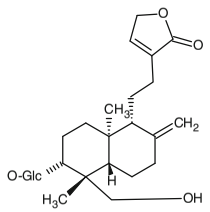
3-oxo-14-deoxyandrographolide

Part: Aerial parts

Extract: EtOH

Preclinical pharmacology: Anti-proliferative (Chen et al. 2008)

References: Chen et al. (2008)



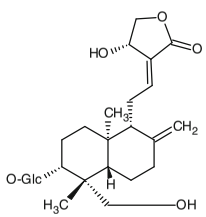
3-O-beta-D-glucopyranosyl 14,19-dideoxyandrographolide

Part: Aerial parts

Extract: Acetone–water

Preclinical pharmacology: Antimicrobial (Shen et al. 2006)

References: Shen et al. (2006)



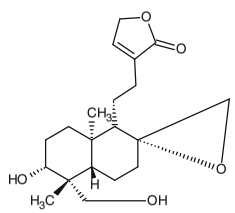
3-O-beta-D-glucopyranosyl-andrographolide

Part: Aerial parts

Extract: Acetone–water

Preclinical pharmacology: Antimicrobial (Shen et al. 2006)

References: Shen et al. (2006)



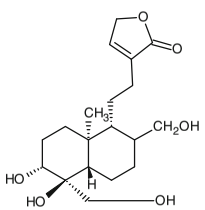
8,17-epoxy-14-deoxyandrographolide

Part: Aerial parts

Extract: Acetone–water

Preclinical pharmacology: Antimicrobial (Shen et al. 2006)

References: Shen et al. (2006)



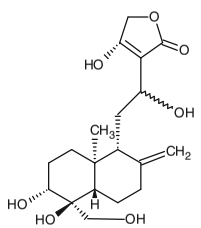
14-deoxy-17-beta-hydroxyandrographolide

Part: Aerial parts

Extract: Acetone–water

Preclinical pharmacology: Antimicrobial (Shen et al. 2006)

References: Shen et al. (2006)



12-hydroxyandrographolide

Part: Aerial parts

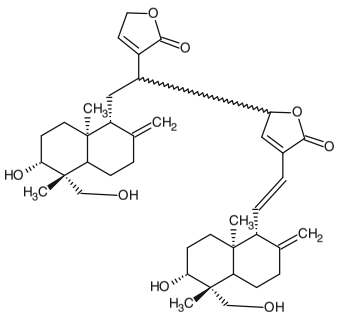
Extract: EtOH

Preclinical pharmacology: Anti-proliferative (Chen et al. 2008)

References: Chen et al. (2008)

Table 2 continued

Chemical constituents: ent labdane diterpenoids
Activity reported in literature



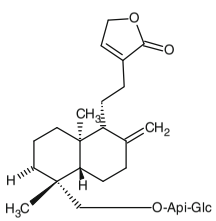
Bisandrographolide A

Part: Aerial parts

Extract: MeOH

Preclinical pharmacology: Cell differentiation inducer (Matsuda et al. 1994); Transient Receptor Potential Channel Vanilloid-4 (TRPV-4) activator/analgesic and antiinflammatory (Smith et al. 2006)

References: Matsuda et al. (1994)



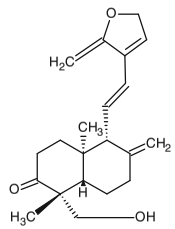
19-O-[beta-D-apiofuranosyl-beta-D-glucopyranoyl]-3,14-dideoxyandrographolide

Part: Aerial parts

Extract: Acetone–water

Preclinical pharmacology: Anti-microbial (Shen et al. 2006)

References: Shen et al. (2006)



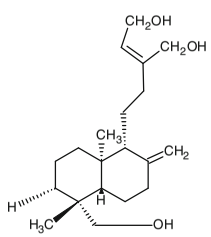
3-oxo-14-deoxy-11,12-didehydroandrographolide

Part: Aerial parts

Extract: EtOH

Preclinical pharmacology: Anti-proliferative (Chen et al. 2008)

References: Chen et al. (2008)



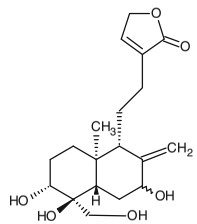
8(17),13-ent-labda-diene-15,16,19-triol

Part: Aerial parts

Extract: EtOH

Preclinical pharmacology: Anti-proliferative (Chen et al. 2008)

References: Chen et al. (2008)



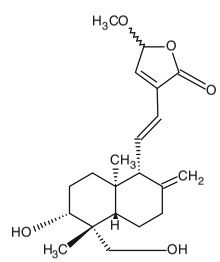
7-hydroxy- 14-deoxyandrographolide

Part: Aerial parts

Extract: EtOH

Preclinical pharmacology: Anti-proliferative (Chen et al. 2008)

References: Chen et al. (2008)



15-methoxy-3,19-dihydroxy-8(17)11,13-ent-labda-trien-16,15-olide

Part: Aerial parts

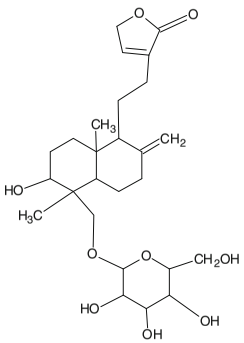
Extract: EtOH

Preclinical pharmacology: Anti-proliferative (Chen et al. 2008)

References: Chen et al. (2008)

Table 2 continued

Ent labdane diterpenoids
Activity not reported in literature



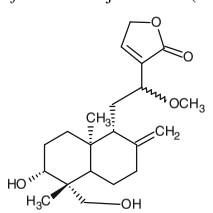
Andropanoside

Part: Leaves and aerial parts

Extract: MeOH*, acetone–water^{+}

Preclinical pharmacology: No reported activity

References: Fujita* et al. (1984); Shen^{+} et al. (2006)



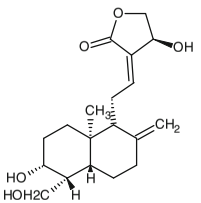
14-deoxy-12-methoxy-andrographolide

Part: Leaves, aerial parts, whole plant

Extract: MeOH*, acetone–water^

Preclinical pharmacology: No activity reported

References: Wu* et al. (2008); Shen^ et al. (2006); Fujita* et al. (1984); Matsuda* et al. (1994)



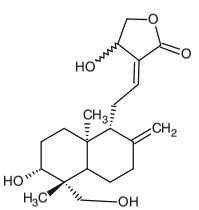
Andropanolide

Part: Leaves

Extract: MeOH

Preclinical pharmacology: No reported activity

References: Pramanick et al. (2006)



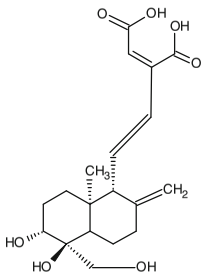
14-epi andrographolide

Part: Aerial part

Extract: MeOH

Preclinical pharmacology: No activity reported

References: Matsuda et al. (1994)



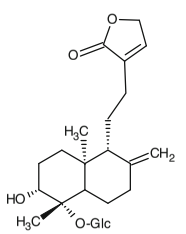
Andrographic acid

Part: Whole plant

Extract: MeOH

Preclinical pharmacology: No reported activity

References: Li et al. (2007)



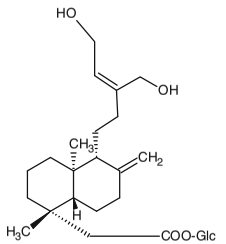
deoxyandrographiside

Part: Aerial parts and whole plants

Extract: MeOH*, EtOH^

Preclinical pharmacology: No reported activity

References: Wu* et al. (2008); Zou^ et al. (2010); Chen^ et al. (2006)



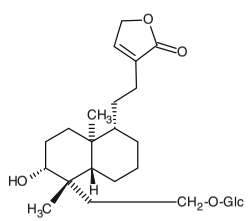
Andrographatoside

Part: Aerial parts

Extract: Acetone–water

Preclinical pharmacology: No reported activity

References: Shen et al. (2006)



14-deoxyandrographiside

Part: Aerial parts and whole plants

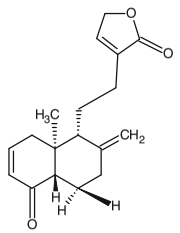
Extract: EtOH

Preclinical pharmacology: No reported activity

References: Kulyal et al. (2010)

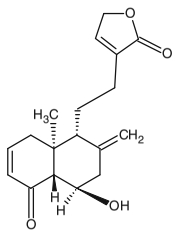
Table 2 continued

Ent labdane diterpenoids
Activity not reported in literature



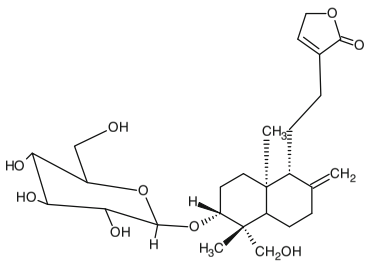
19-nor andrographolide B

Part: Aerial parts
Extract: EtOH
Preclinical pharmacology: No reported activity
References: Zhang et al. (2006)



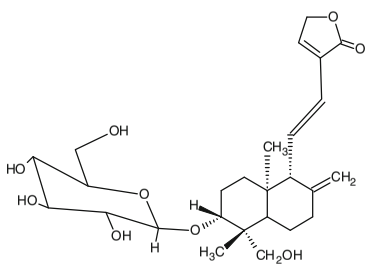
19-nor andrographolide C

Part: Aerial parts
Extract: EtOH
Preclinical pharmacology: No reported activity
References: Zhang et al. (2006)



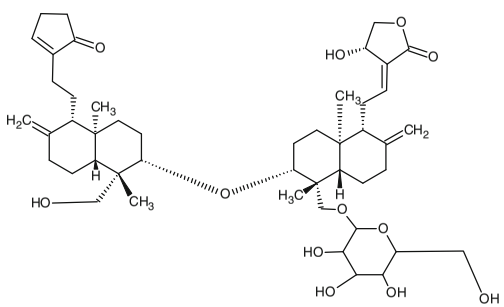
3-O-beta-D-glucosyl-14-deoxyandrographiside

Part: Aerial parts
Extract: EtOH
Preclinical pharmacology: No activity reported
References: Zhou et al. (2008a)



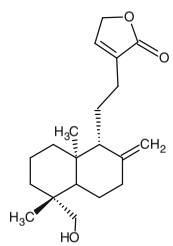
3-O-beta-D-glucosyl-14-deoxy-11,12-didehydroandrographiside

Part: Aerial parts
Extract: EtOH
Preclinical pharmacology: No activity reported
References: Zhou et al. (2008b)



Bisandrographolide ether

Part: Aerial parts
Extract: Hexane
Preclinical pharmacology: No reported activity
References: Reddy et al. (2005)

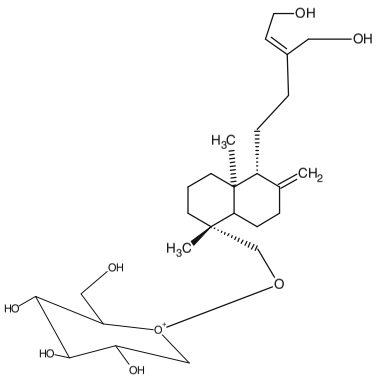


19-hydroxy-ent-labda-8(17),13-dien-15,16-olide

Part: Aerial parts
Extract: EtOH
Preclinical pharmacology: No activity reported
References: Chen et al. (2006)

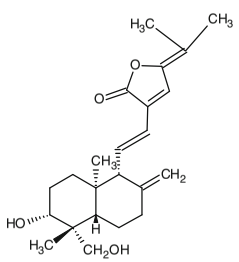
Table 2 continued

Ent labdane diterpenoids
Activity not reported in literature



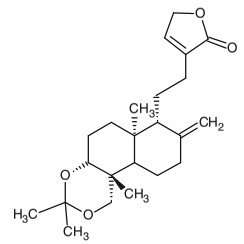
19-O-beta-D-glucopyranosyl-ent-labda-8(17),13-dien-15,16,19-triol

Part: Aerial parts
Extract: MeOH
Preclinical pharmacology: No activity reported
References: Zou et al. (2010)



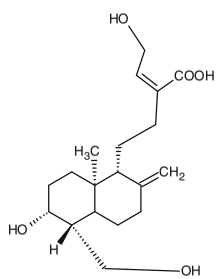
14-deoxy-15-isopropylidene-11,12-didehydroandrographolide

Part: Aerial parts
Extract: MeOH
Preclinical pharmacology: No activity reported
References: Reddy et al. (2003)



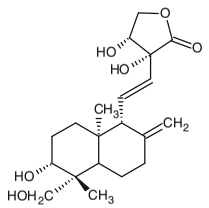
3,19-isopropylidene-14-deoxy-ent-labda-8(17),13-dien-16,15-olide

Part: Aerial parts
Extract: EtOH
Preclinical pharmacology: No activity reported
References: Xu et al. (2010)



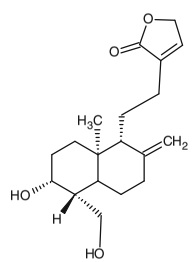
3,15,19-trihydroxy-ent-labda-8(17),13-dien-16-oic acid

Part: Aerial parts
Extract: EtOH
Preclinical pharmacology: No activity reported
References: Chen et al. (2006)



3,13,14,19-tetrahydroxy-ent-labda-8(17),11-dien-16,15-olide

Part: Aerial parts
Extract: EtOH
Preclinical pharmacology: No activity reported
References: Xu et al. (2010)

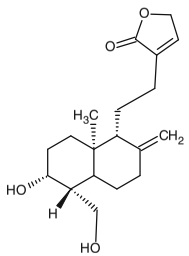


3,18,19-trihydroxy-ent-labda-8(17),13-dien-16,15-olide

Part: Aerial parts
Extract: EtOH
Preclinical pharmacology: No activity reported
References: Chen et al. (2006)

Table 2 continued

Ent labdane diterpenoids
Activity not reported in literature



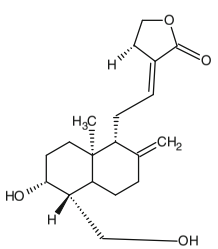
3,18,19-trihydroxy-ent-labda-8(17),13-dien-16,15-olide

Part: Aerial parts

Extract: EtOH

Preclinical pharmacology: No activity reported

References: Chen et al. (2006)



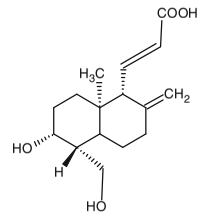
3,19-dihydroxy-ent-labda-8(17),12-dien-16,15-olide

Part: Aerial parts

Extract: EtOH

Preclinical pharmacology: No activity reported

References: Chen et al. (2006)



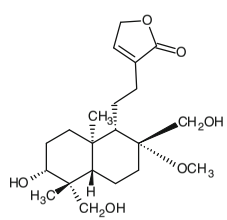
3,19-dihydroxy-14,15,16-trinor-ent-labda-8(17),11-dien-13-oic acid

Part: Aerial parts

Extract: EtOH

Preclinical pharmacology: No activity reported

References: Chen et al. (2006)



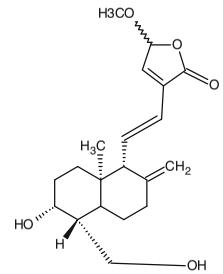
8-methoxyl-14-deoxy-17-beta-hydroxyandrographolide

Part: Aerial parts

Extract: MeOH

Preclinical pharmacology: No activity reported

References: Ma et al. (2010)



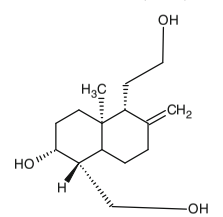
3,19-dihydroxy-15-methoxy-ent-labda-8(17),11,13-trien-16,15-olide

Part: Aerial parts

Extract: EtOH

Preclinical pharmacology: No activity reported

References: Chen et al. (2006)



13,14,15,16-tetranor-ent-labda-8(17)-ene-3,12,19-triol

Part: Aerial parts

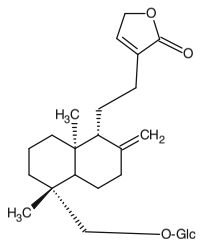
Extract: EtOH

Preclinical pharmacology: No activity reported

References: Chen et al. (2006)

Table 2 continued

Ent labdane diterpenoids
Activity not reported in literature



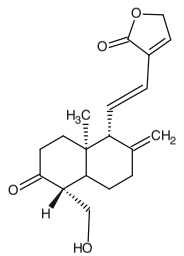
19-[(beta-D-glucopyranosyl)oxy -19-oxo-ent-labda-8(17),13-dien-16,15-olide

Part: Aerial parts

Extract: EtOH

Preclinical pharmacology: No activity reported

References: Chen et al. (2006)



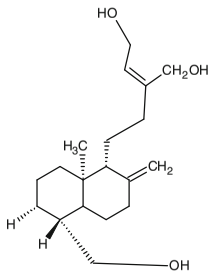
19-hydroxy-3-oxo-ent-labda-8(17),11,13-trien-16,15-olide

Part: Aerial parts

Extract: EtOH

Preclinical pharmacology: No activity reported

References: Chen et al. (2006)



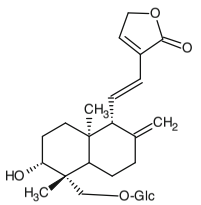
ent-labda-8(17),13-dien-15,16,19-triol

Part: Aerial parts

Extract: EtOH

Preclinical pharmacology: No activity reported

References: Chen et al. (2006)



14-deoxy-11,12-didehydroandrographiside

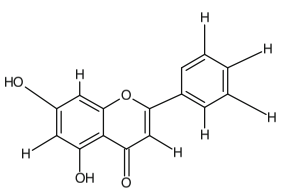
Part: Aerial parts

Extract: EtOH

Preclinical pharmacology: No activity reported

References: Matsuda et al. (1994)

Flavonoids
Activity reported in literature



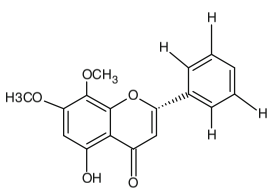
apigenin

Part: Whole plant

Extract: MeOH

Preclinical pharmacology: Anti-platelet aggregator (Wu et al. 2008)

References: Wu et al. (2008)



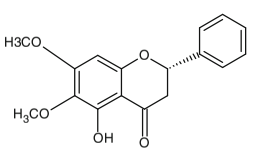
7-O-methylwogonin

Part: Roots, Whole plants

Extract: MeOH*, Hexane^,

Preclinical pharmacology: Antiplatelet-aggregatory (Wu et al. 2008)

References: Rao* et al. (2004); Wu* et al. (2008); Reddy^ et al. (2003)



Onysilin

Part: Whole plant

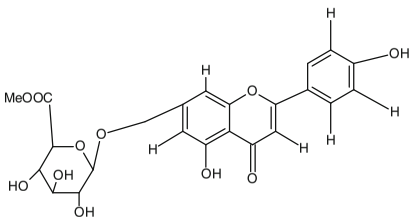
Extract: MeOH

Preclinical Pharmacology: Anti-platelet aggregator

References: Wu et al. (2008)

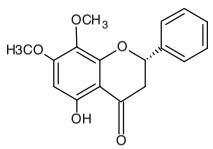
Table 2 continued

Flavonoids
Activity reported in literature



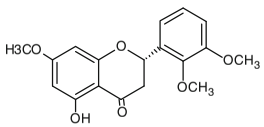
apigenin-7-O-beta-D-methylglucuronide

Part: Whole plant
Extract: MeOH
Preclinical pharmacology: No reported activity
References: Wu et al. (2008)



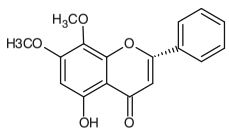
5-hydroxy-7,8-dimethoxyflavanone

Part: Aerial parts
Extract: Hexane
Preclinical pharmacology: No reported activity
References: Reddy et al. (2005)



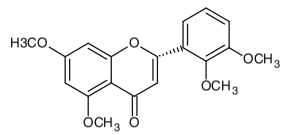
5-hydroxy-7,2',3'-trimethoxyflavone

Part: Aerial parts, roots
Extract: MeOH
Preclinical pharmacology: No reported activity
References: Reddy et al. (2003); Rao et al. (2004)



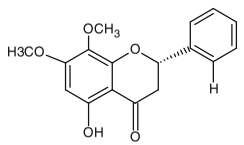
5-hydroxy-7,8-dimethoxyflavone

Part: Aerial parts
Extract: Hexane
Preclinical pharmacology: No reported activity
References: Reddy et al. (2005)



5,7, 2',3'-tetramethoxyflavanone

Part: Aerial parts, roots
Extract: MeOH
Preclinical pharmacology: No reported activity
References: Reddy et al. (2003); Rao et al. (2004)

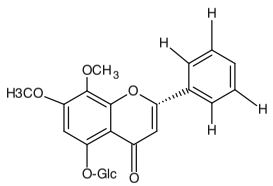


7-O-methyldihydrowogonin

Part: Roots, whole plants
Extract: Hexane^, MeOH*
Preclinical pharmacology: No reported activity
References: Reddy^ et al. (2003); Rao* et al. (2004)

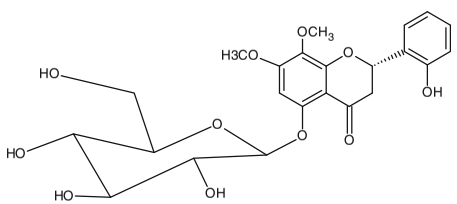
Table 2 continued

Flavonoids
Activity reported in literature



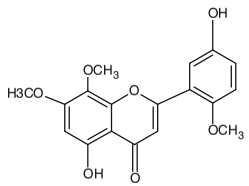
7-O-methylwogonin- 5-glucoside

Part: Roots, Whole plants
Extract: Hexane^, MeOH*
Preclinical pharmacology: No reported activity
References: Rao* et al. (2004); Reddy^ et al. (2003)



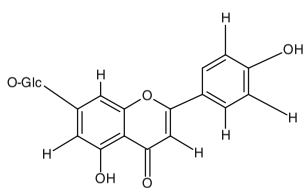
Andrographidine A

Part: Whole plants, roots
Extract: MeOH
Preclinical pharmacology: No activity reported
References: Kuroyanagi et al. (1987); Li et al. (2007)



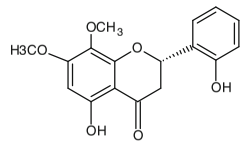
Andropaniculosin A

Part: Whole plants
Extract: MeOH
Preclinical pharmacology: No reported activity
References: (Wu et al. 2008)



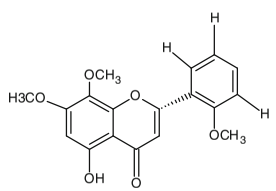
Cosmosiin

Part: Whole plant
Extract: MeOH
Preclinical pharmacology: No reported activity
References: Wu et al. (2008)



Dihydroskullcapflavone I

Part: Whole plant
Extract: MeOH
Preclinical pharmacology: No reported activity
References: Reddy^ et al. (2003). Rao* et al. (2004)

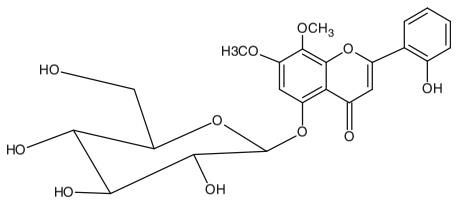


skullcapflavone-12'-methylether

Part: Whole plant
Extract: MeOH
Preclinical pharmacology: No reported activity
References: Rao et al. (2004)

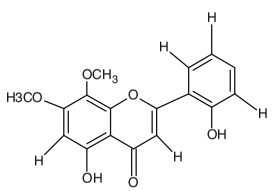
Table 2 continued

Flavonoids
Activity reported in literature



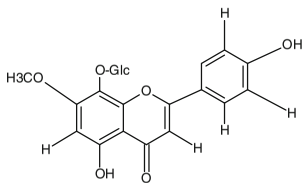
Andropaniculoside A

Part: Whole plants
Extract: MeOH
Preclinical pharmacology: No activity reported
References: Wu et al. (2008)



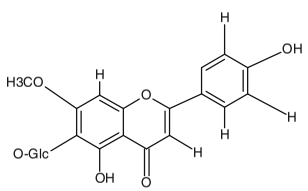
skullcapflavone I

Part: Whole plant
Extract: MeOH
Preclinical pharmacology: No reported activity
References: Wu et al. (2008)



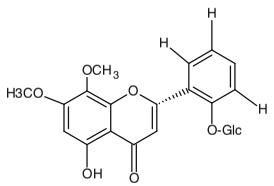
Isoswertisin

Part: Whole plant
Extract: MeOH
Preclinical pharmacology: No reported activity
References: Wu et al. (2008)



scutellarin-6-O-beta-D-glucoside-7-methylether

Part: Whole plant
Extract: MeOH
Preclinical pharmacology: No reported activity
References: Wu et al. (2008)

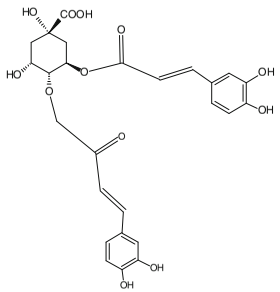


Skullcapflavone I 2'-glucoside

Part: Roots, whole plant
Extract: MeOH*, acetone^
Preclinical pharmacology: No reported activity
References: Rao* et al. (2004); Reddy^ et al. (2003)

Table 2 continued

Quinic acids
Activity reported in literature



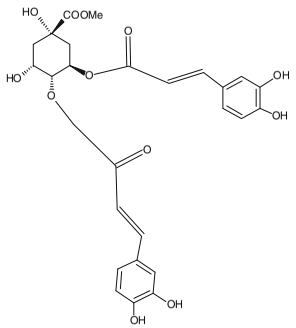
3,4-dicaffeoylquinic acid

Part: Whole plant

Extract: MeOH

Preclinical pharmacology: Anti-platelet aggregator

References: 1. Wu et al. (2008)



methyl-3,4-dicaffeoylquinate

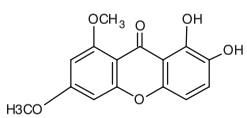
Part: Whole plant

Extract: MeOH

Preclinical pharmacology: No reported activity

References: Wu et al. (2008)

Xanthones
Activity reported in literature



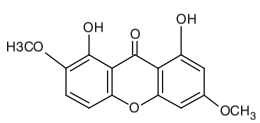
1,2-dihydroxy-6,8-dimethoxyxanthone

Part: Roots

Extract: Sequential extraction with pet. ether, MeOH, $CHCl_3$ and water

Preclinical pharmacology: Anti-malarial (Dua et al. 2004)

References: Dua et al. (2004)



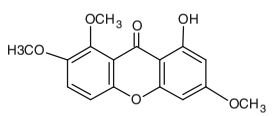
1,8-dihydroxy-3,7-dimethoxyxanthone

Part: Roots

Extract: Sequential extraction with pet. ether, MeOH, $CHCl_3$ and water

Preclinical pharmacology: Anti-malarial (Dua et al. 2004)

References: Dua et al. (2004)



3,7,8-trimethoxy-1-hydroxyxanthone

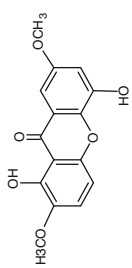
Part: Roots

Extract: Sequential extraction with pet. ether, MeOH, $CHCl_3$ and water

Preclinical pharmacology: Anti-malarial (Dua et al. 2004)

References: Dua et al. (2004)

Table 2 continued

Xanthones Activity reported in literature	 **4,8-dihydroxy-2,7-dimethoxyxanthone** *Part*: Roots *Extract*: Sequential extraction with pet. ether, MeOH, $CHCl_3$ and water *Preclinical pharmacology*: Anti-malarial (Dua et al. 2004) *References*: Dua et al. (2004)	

*,#,@,¤,^,+ denotes different solvents used for preparing extracts

Vinayak 2008), antidiabetic (Lee et al. 2010a), antiatherosclerotic (Chen et al. 2004), cholestatic (Lee et al. 2010a), respiratory conditions (Roxas and Jurenka 2007), analgesic, antipyretic and antiulcerogenic (Madav et al. 1995) studies. The screening techniques used in the previous decades were primal and involved simple methodologies. But with the advent of molecular biology and receptor pharmacology techniques, better isolation and separation techniques, sophisticated analytical equipments, enzyme inhibition studies, cutting edge research methods were employed resulting in discovery or elucidation of various mechanisms of action of *Andrographis paniculata* extract for a variety of conditions and disorders.

Preclinical pharmacology

A few studies on preclinical pharmacology of *Andrographis paniculata* on laboratory animals by various researchers are described here:

Kumar et al. (2004) reported an account of anticancer and immunomodulatory effects of dichloromethane fraction of the methanolic extract of the herb on in vitro cell growth assay at concentrations ranging from 0.1 to 100 μg/ml. The active constituents responsible for these effects were found to be andrographolide, 14-deoxyandrographolide and 14-deoxy-11,12-didehydroandrographolide.

The protective activity of andrographolide and arabinogalactan proteins against alcohol-induced hepatic and renal toxicity was studied in mice by Singha et al. (2007). It was observed that pretreatment of mice with andrographolide, arabinogalactan proteins and silymarin as a positive control at doses of 62.5, 125, 250 and 500 mg/kg intraperitoneally administered every alternate day for 7 days reduced the toxicity in comparison to ethanol treated group. Andrographolide and arabinogalactan proteins were found to be the bioactive compounds responsible for protection against alcohol-induced hepatic and renal toxicity.

A study was carried out to evaluate the immunomodulatory activities of an ethanolic extract of *Andrographis paniculata* and andrographolide by Xu et al. (2007). The extract and andrographolide was administered at 25, 50 mg/kg body weight and 1, 4 mg/kg body weight respectively by oral route in mice immunised with an inactivated *Salmonella*

typhimurium vaccine. Mice vaccinated with either one or two doses of killed *Salmonella typhimurium* vaccine were administered two different quantities of ethanolic extract of *Andrographis paniculata* and andrographolide for 14 days in mice immunised with one dose of the vaccine, and for 28 days in mice immunised with two doses of vaccine. The results showed enhanced Immunoglobulin G antibody levels against *S. typhimurium* by both ethanolic extract of *Andrographis paniculata* and andrographolide. There was also a demonstrated increase in the production of Interferon-γ following stimulation with the bacterial lysate, indicating an induction of *Salmonella*-specific cell-mediated response/immune response.

Sheeja et al. (2007) performed a study on the anti-angiogenic activity of ethanolic extract of *Andrographis paniculata* and andrographolide on mice. The ip administration of extract and andrographolide at 10 mg/dose/animal and 500 μg/dose/animals respectively significantly inhibited the B16F-10 melanoma cell line induced capillary formation in C57BL6 mice. The serum cytokine profile showed an upward trend in levels of the proinflammatory cytokines such as InterLeukin-1β, InterLeukin-6, Tumour Necrosis Factor-α, Granulocyte Macrophage-Colony Stimulating Factor and the most potent angiogenic factor Vascular Endothelial Growth Factor in angiogenesis induced animals. Treatment with extract and andrographolide significantly reduced these elevated levels. The results demonstrate that crude ethanolic extract of *Andrographis paniculata* and andrographolide inhibited the tumor specific angiogenesis by regulating the production of various pro and anti-angiogenic factors such as pro-inflammatory cytokines, nitric oxide, Vascular Endothelial Growth Factor, InterLeukin-2 and Tissue Inhibitor of Metallo Proteinases-1.

The protective properties of andrographolide, and aqueous extract of *Andrographis paniculata* were investigated against nicotine induced liver, kidney, heart, lung and spleen toxicity by Neogy et al. (2008). Wistar rats administered nicotine intraperitoneally 1 mg/kg body weight/day), nicotine + andrographolide 250 mg/kg, nicotine + aqueous extract 250 mg/kg for a period of 7 days showed a significant increase in levels of lipid peroxidation, protein oxidation and the decreased antioxidant enzyme status noted in nicotine treated group as compared to vehicle treated group. The aqueous extract and andrographolide significantly reduced the lipid peroxidation, protein oxidation and increased the antioxidant enzyme status. This indicates that the herb and active constituent, andrographolide may act as protective agents against nicotine induced tissue injury.

Iruretagoyena et al. (2005) and coresearchers reported that andrographolide interferes with T cell activation and reduces experimental autoimmune encephalomyelitis. In a mouse model of autoimmune encephalomyelitis, andrographolide significantly reduces the disease symptoms in by inhibiting T cell and antibody responses directed towards myelin antigens. Andrographolide prevented ovalbumin-pulsed dendritic cells from activating either CD4+ or CD8+ T cell hybridomas. Thus treatment with andrographolide prevented processing and presentation of ovalbumin peptides on MHC molecules to ovalbumin-specific T cells. Moreover, andrographolide reduced the efficiency of dendritic cell maturation in response to lipopolysaccharide mediated inflammation. Andrographolide also caused in vitro inhibition of T cell activation leading to the suppression of the immune response in the experimental systems. Thus, IgG secretion against T cell-dependent antigen NP_{17}-BSA was significantly reduced by andrographolide treatment. Additionally andrographolide treatment significantly reduced both the incidence and clinical severity of experimental autoimmune encephalitis in C57BL/6 mice during early phase of disease. This ability of andrographolide to impair T cell activation could be the most probable reason. Observation of lymph node cellular suspensions from andrographolide treated mice showed reduced IFN-γ and IL-2 release in response to administration of myelin oligodendrocyte glycoprotein. Andrographolide-treated mice showed reduction in vivo T cell priming, which is probably responsible for the decreased delayed type hypersensitivity reactions and autoimmune encephalitis which may stem from the impairment on dendritic cell maturation and generation of peptide-MHC complexes. Hence andrographolide can be used to block T cell activation in vitro and in vivo which may be used as an immunosuppressive in the treatment of autoimmune diseases. Andrographolide could also interfere with neutrophils and microglial functions and these have already been implicated in the pathogenesis of inflammatory conditions. Antiapoptotic activity could also contribute to a reduction in the severity of autoimmune encephalitis by increasing neuronal resistance to cell death induced by local inflammation. Thus, andrographolide most likely

interferes with autoimmune encephalitis by preventing activation of autoreactive T cells and also by reducing inflammatory damage.

Xia et al. (2004) and colleagues reported the potent inhibition of Nuclear Factor Kappa B (NF-κB) by andrographolide. Andrographolide was found to act as a small molecular weight antagonist for NF-κB activation by covalently modifying reduced cysteine 62 of p50. A significant reduction in the peritoneal deposition of neutrophils induced by cytokines and LPS was observed in this study. Moreover a complete reversal of the mortality or increase in survival time from endotoxin shock was seen. A remarkable inhibition of leukocyte infiltration into bronchial airway lavage was also reported. Andrographolide covalently conjugates reduced cysteine 62 of p50, thus preventing NF-κB oligonucleotide binding to p50, inhibiting NF-κB transcriptional activity, and attenuating inflammation in various in vitro assays and in vivo models (Xia et al. 2004). Interference of andrographolide with reduced cysteine 62 evaluated against oxidized cysteine 62 shows that, cysteine 62 of p50 is less reduced in the cytoplasm, when there is a tendency for stronger reduction by redox factor-1 in the nucleus. This has now been proposed as essential for the NF-κB activation. Andrographolide mainly targets this critical regulatory site of p50, the reduced cysteine 62 of p50, for attenuation of nuclear NF-κB transcriptional activity. Hence the redox modulation of cysteine 62 for the interaction of p50 with NF-κB oligonucleotide follows the inhibition of NF-κB activation by covalent conjugation to reduced cysteine 62 of p50 by andrographolide. In addition, andrographolide also suppresses NO production and down-regulates leukocyte integrin Mac-1 causing inhibition of neutrophil adhesion and transmigration. Taken together the results of the animal models examined in this study have collectively demonstrated the therapeutic efficacy of andrographolide for treating various inflammatory disorders.

Andrographolide was analysed for the activation of NF-κB induced by platelet-activating factor and N-formyl-methionyl-leucyl-phenylalanine (fMLP) in HL-60 cells differentiated to neutrophils (Hidalgo et al. 2005). In this study a neutrophil-like dimethylsulfoxide was used as a cellular model of neutrophils. The results have shown that NF-κB activation by PAF and fMLP are strongly inhibited by andrographolide, an effect that is mediated by blocking the binding of NF-κB to DNA. The final step in NF-κB activation is its binding to DNA which was inhibited by andrographolide. It is well known now andrographolide interferes with a transcription factor, and also affects the expression of target genes controlled by NF-κB during inflammatory processes. Expression of COX_2 is reduced by NF-KB inhibitors in endothelial cells stimulated by PAF (Marrache et al. 2002) and in microglial cells andrographolide decreased COX_2 expression induced by LPS (Wang et al. 2004). Our results of decreased COX_2 expression after PAF or fMLP stimulation are in agreement with previous findings and demonstrate further that the inhibition of NF-κB binding to DNA, was able to decrease protein expression. Hence it was concluded that andrographolide exerts its anti-inflammatory effects by inhibiting NF-κB binding to DNA, thereby reducing the expression of proinflammatory proteins, such as COX_2.

The neuroprotective effect of andrographolide was investigated by Chan et al. (2010). It was found that andrographolide at a dose of 0.1 mg/kg administered i.p. 1 h after the surgical procedure, reduced infarct volume with a maximum reduction of approximately 50%. In addition, andrographolide suppressed the translocation of p65 from cytosol to nucleus, indicating reduced NF-κB activation. Andrographolide exhibited neuroprotective effects, with accompanying suppression of NF-κB and microglial activation, and reduction in the production of tumour necrosis factor-α and interleukin-1β, and pro-inflammatory factors such as prostaglandin E_2. These findings indicate the possible use of andrographolide in the treatment of cerebral stroke.

Hsieh et al. (2011) and coresearchers investigated the mechanism involved in andrographolide suppression of NF-κB signaling. They exposed the rat vascular smooth muscle cells (VSMCs) to various proinflammatory stimuli like Lipopolysaccharide, and IFN-γ. Andrographolide was shown to suppress LPS/IFN-γ-induced inducible nitric-oxide synthase and matrix metalloprotease 9 expression in rat VSMCs. Andrographolide also inhibited LPS/IFN-γ-induced p65 nuclear translocation, DNA binding activity, p65 Ser^{536} phosphorylation, and NF-κB reporter activity. These results demonstrate the protective anti-inflammatory actions of andrographolide which may be mediated through inhibition of transcription factor nuclear factor NF-κB.

Some of the reported pharmacological actions of *Andrographis paniculata* on commonly used laboratory animals are mentioned in Table 3.

Table 3 Pharmacological actions of *Andrographis paniculata* on commonly used laboratory animals

Activity	Animals/in vitro	Model	References
Hepatoprotective	Rats	Galactosamine model isolated rat hepatocytes trypan blue exclusion test	
	Common African rat	Oxygen uptake test *Plasmodium berghei* K173- induced hepatic damage	Chander et al. (1995)
Enzyme inhibition	Rats	Rat and human liver microsomes	Pekthong (2008)
	–	HepG2 cells	Ooi et al. (2011)
	Mouse	Hepatocyte primary culture	Kondo et al. (2011)
Antimalarial	In vivo	4-day suppressive test in common African rat	Misra et al. (1992)
Cell differentiation-inducing agent	In vitro test	Mouse myeloid leukemia cell line	(Matsuda et al. 1994)
Analgesic, antipyretic, antiulcerogenic	In vivo tests	Hot plate test (mice), acetic acid-induced writhing (mice) and Randall Selitto's test (rats)	Madav et al. (1995)
Antihypertensive	1. SHR and Wistar Kyoto rats	Plasma and lung ACE inhibition	Zhang and Tan (1996)
		Permanent cannulation of the jugular vein	Zhang and Tan (1998)
	2. In vitro on rats	Isolated rat atria	
Anti-HIV	In vitro studies	Enzyme inhibition studies	Basak et al. (1999)
Antimalarial	In vitro tests	Inhibition of *Plasmodium falciparum*	Rahman et al.(1999)
Antidiabetic	1. Rats	STZ model	Zhang and Tan (2000)
	2. Rats	STZ model	Yu et al. (2003)
	3. Rats	Glucose tolerance tests	Rammohan et al. (2006)
	4. Rats	High fat+low STZ insulin resistance model	(2008a, b, c)
	5. Rats	Adult STZ+Nicotinamide	
	6. In vitro tests, rats	T2DM model	
		In vitro alpha glucosidase, alpha amylase inhibition and oral tolerance test	
Immunomodulatory	In vivo tests on mice	Exposing pretreated mice to inactivated *S. typhimurium bacteria*	Xu et al. (2007)

Preclinical safety pharmacology

Numerous studies have been performed on the occurrence/presence of toxicity of *Andrographis paniculata* on rats, rabbits, and mice, but generally have been proved to be safe.

Akbarsha and Murugaian (2000) reported on the male reproductive toxicity on administering andrographolide at 25 and 50 mg/kg doses orally to Wistar albino rats for a period of 48 days. It was observed that sperm counts decreased, spermatozoa were not motile, and possessed abnormalities. The seminiferous epithelium was totally destroyed and in the seminiferous tubules fully differentiated spermatozoa were too limited. Thus a possible male reproductive toxicity effect could be considered. However a different study performed by Allan et al. (2009) demonstrated contrasting results. The researchers determined the effect of *Andrographis paniculata* extract Nees standardized to $\geq$10% andrographolide on male fertility in albino Wistar rats. The extract was orally administered at doses of 20, 200, and 1,000 mg/kg daily for 65 days prior to mating and 21 days during mating. The testosterone levels and fertility indices in treatment groups were found to be comparable with that of the control indicating no effect on fertility. Total sperm count and sperm motility were not affected. The testes and epididymies did not show any gross and histopathological changes. Hence the no-observed adverse effect level (NOAEL) of extract of *Andrographis paniculata* ($\geq$10% andrographolide) was found to be more than 1,000 mg/kg per day.

A recent study by Chandrasekaran et al. (2009) on the genotoxic potential and acute oral toxicity of standardized extract of *Andrographis paniculata* has concluded that at a highest concentration of 5,000 μg/ml the standardized extract did not induce mutations both in the presence and absence of S9 in *Salmonella typhimurium* mutant strains of TA98 and TAMix. In chromosome aberration and micronucleus studies the extract did not induce clastogenicity in CHO-K1 cells in vitro. Based on these results, it is evident that the extract is genotoxically safe. In the acute oral toxicity study, female rats treated at the highest dose of 5,000 mg/kg of the standardised extract failed to produce any signs of toxicity after 14 days.

In a study performed by Sattayasai et al. (2010), the effects of andrographolide from Andrographis paniculata on sexual functions, vascular reactivity and serum testosterone level in experimental animals were observed. Andrographolide was administered orally in 5% DMSO at a dose of 50 mg/kg to male ICR mice and were mated with female mice. The mating behaviors, mounting latency and mounting frequency, were determined and compared with the standard reference drug sildenafil citrate. Administration of andrographolide significantly decreased the mounting latency at 120 and 180 min and increased mounting frequency at 180 min after treatment. Administration of 50 mg/kg andrographolide orally to male mice once daily for 2, 4, 6 or 8 weeks had no significant effects on sperm morphology and motility. Interestingly, at week 4, serum testosterone level in mice treated with andrographolide was significantly increased when compared to the control. It was concluded that the effects of andrographolide on vascular response to norepinephrine and testosterone level observed in this study might contribute to the sexual enhancing properties.

Guo et al. (1988) proved the lack of toxicity, on administering 500 mg/kg of extract and andrographolide for 20 days continuosly, for 10 days to mice. No effects on their appetite, growth or any untoward appearance on stool was found. The animals behaved normally and no altered blood counts were seen.

Rats and rabbits orally receiving 1 g/kg andrographolide or neoandrographolide for 7 days did not alter body weight, blood count, hepatic or renal functioning or functioning of other vital organs, demonstrating a clean toxicity record.

Cytochrome P_{450} plays an important role in the pharmacology of drugs and toxicology of xenobiotics. Understanding how drugs or xenobiotics induce or inhibit P_{450} activity is biologically relevant and ultimately leads to better models for predicting the actions of these agents. There was also an observed induction of of expression of CYP1A1 mRNA by andrographolide itself in a concentration-dependent manner in mouse hepatocyte primary culture (Jaruchotikamol et al. 2007).

Jarukamjorn et al. (2006) studied the impact of *Andrographis paniculata* crude extract on mouse hepatic cytochrome P_{450} enzymes by determining P_{450} content and P_{450}-associated activities by treating ICR male mice with 3-methylcholanthrene at 100 mg/kg/day and phenobarbital at 100 mg/kg/day intraperitoneally in normal saline consecutively daily for 3 days. *Andrographis paniculata* in distilled water (equivalent to 5 mg/kg/day andrographolide) was administered orally consecutively daily for 7, 14, 21, and 30 days respectively. Assessment of hepatic microsomal P_{450} activities by alkoxyresorufin *O*-dealkylations showed that both the aqueous and alcoholic extracts of *Andrographis paniculata* significantly increased ethoxyresorufin *O*-dealkylase and pentoxyresorufin *O*-dealkylase activities, while those of ethoxyresorufin *O*-dealkylase and pentoxyresorufin *O*-dealkylase activities suggested that *Andrographis paniculata* might affect hepatic cytochrome P450 enzymes of which CYP1A1 and CYP2B are the most responsive P_{450} isoforms. This study may serve as an invaluable guideline of rational administration and precaution for using of herbal medicines.

In another study by Pekthong et al. (2008), the inhibitory effect of *Andrographis paniculata* extract and andrographolide in the extract on hepatic cytochrome P_{450}s (CYPs) activities was examined using rat and human liver microsomes. It was found that the extract inhibited the catalytic activities of rat and human liver microsomal CYP1A2, CYP2C and human liver microsomal CYP3A4. These results suggest that the extract could act as an anticarcinogen in humans because of its specific inhibitory effect on CYP1A2 activity. The inhibitory effect of extract on CYP3A and 2C9 activities cannot be ruled out to cause drug–drug interactions, especially for CYP2C9 due to its low expression in human liver and because it is known to metabolize several narrow therapeutic index drugs.

Kondo et al. (2011) examined the effect of changes caused by andrographolide to intracellular GSH levels during the β-naphthoflavone-induced expression of CYP1A1 mRNA using mouse hepatocytes in primary culture. It was observed that the modification of inducible CYP1A1 mRNA expression by andrographolide was bimodal depending on treatment period and the modulation was retrieved by changing the intracellular GSH content. These results suggest that GSH status might be involved β-NF-induced CYP1A1 mRNA expression, and interaction of andrographolide with GSH might modulate the expression.

Another study carried out by Ooi et al. (2011) evaluated the effects of andrographolide and 14-deoxy-11, 12-didehydroandrographolide on CYP1A2, CYP2D6, and CYP3A4 expressions in HepG2 hepatoma cells. The mRNA and protein expression of the CYP.enzymes were performed by Quantitative RT-PCR and Western blot techniques. It was found that both diterpenoid active principles inhibited the mRNA and protein expressions of CYP1A2, CYP2D6, and CYP3A4. These findings suggest that patients known to take herbal preparation containing *Andrographis paniculata* Nees should be advised and cautioned of potential drug-herb interactions.

Clinical pharmacology

The standardized extract of *Andrographis paniculata* either alone or in combination with other plant extracts have been used in several clinical trials in recent years to ascertain its safety and efficacy on children, adult healthy humans, or adults with specific conditions/diseases. The studies of *Andrographis paniculata* either alone or in combination with other herbs are presented in Table 4. The standardized extract have been used in humans with specific conditions like ulcerative colitis, AIDS, common cold, upper respiratory tract infection with sinusitis, and arthritis and the safety and efficacy results have been promising. Few cases of adverse effects on humans were found to be self-limiting with nausea, diarrhea, metallic taste, allergic reaction, fatigue, headache, lymph node pain and lymphadenopathy. The adverse reports on human use were usually mild and infrequent. But then, most clinical trials were of short duration (less than 2 weeks), so the safety of the plant in chronic use could not be gauged. Furthermore, a systematic review undertaken by the Natural Standard Research Collaboration (Kligler et al. 2006) which compiled safety and efficacy results from 2 reviews and 7 clinical trials showed that out of 879 subjects exposed to the plant in

Table 4 Studies on the effects of *Andrographis paniculata* on healthy humans or patients with specific conditions/diseases

Type of clinical study	Number of subjects	Disease/condition	References
Phase 2 randomized double blind study	152 adults	Pharyngotonsilitis	Thamlikitkul et al. (1991)
Phase 2 randomised double blind-placebo study	158 adult male and female	Common cold	Cáceres et al. (1999)
Phase 1 dose-escalating study	18 adult male and female	HIV+	Calabrese et al. (2000)
Phase 2 double blind placebo-controlled study	200 adult male and female	URTI and sinusitis	Gabrielian et al. (2002)
Phase 2 randomized controlled three parallel group study	130 children of age 4–11 years	Uncomplicated common cold	Spasov et al. (2004)
Phase 1 open randomized parallel group study	14 adult males	Healthy	Mkrtchyan et al. (2005)
Phase 2 double blind placebo-controlled study	223 adults	Ulcerative colitis	Sandborn et al. (2009)
Phase 2 prospective randomized double blind and placebo-controlled	60 adults	Rheumatoid arthritis	Burgos et al. (2009)
Phase 2 randomized double blind placebo controlled	223 adults male and female	Uncomplicated URTI	Saxena et al. (2010)

any dosage form, only one case of anaphylactic shock was reported.

Saxena et al. (2010) evaluated the effect of an extract of *Andrographis paniculata*, in patients with uncomplicated upper respiratory tract infection (URTI). A total of 223 patients of both sexes were randomized in two groups which received either KalmCold (200 mg/day) or placebo in a double blind manner. The mean scores of all symptoms showed a decreasing trend from day 1 to day 3 but from day 3 to day 5 most of the symptoms in placebo treated group either remained unchanged (cough, headache, and earache) or got aggravated(sore throat and sleep disturbance) whereas in KalmCold TM treated group all symptoms showed a decreasing trend.

A prospective, randomized, double blind, and placebo-controlled study in patients with rheumatoid arthritis was performed by Burgos et al. (2009). The extract of *Andrographis paniculata* formulated as tablets (30% total andrographolides) were administered three times a day for 14 weeks, after a 2-week washout period to 60 patients with arthritis. It was observed that the intensity of joint pain decreased in the active vs placebo group at the end of treatment, although these differences were not statistically significant. A significant diminishing in tender joints, number of swollen joints, total grade of swollen joint, number of tender joints, total grade of swollen joints, total grade of tender joints was seen within the group with the active drug treatment. These improvements were also associated with a reduction of rheumatoid factor, IgA, and C4. These findings suggest that *Andrographis paniculata* could be a useful herbal treatment in the treatment of arthritis.

Moreover, very few reports on the incidences and propensity of the plant to cause frequent drug–drug interactions (Pekthong et al. 2008) are available which indicates its safety on administration in humans. It is hoped that with the demonstration of safety of *Andrographis paniculata* extracts in humans, the determination of effects of the extract in various diseases and condition is now possible, if the proof of concept preclinical studies demonstrate significant activity.

Conclusion

In promoting the proper use of medicinal products, a comprehensive road map must be charted supporting R&D, cultivation, production, trade and appropriate use in the community and healthcare sectors. Thus a joint, coordinated and holistic effort among scientists, farmers, manufacturers, traders, health care professionals and regulatory authorities is essential to propel the industry to comply with consumer expectations of quality, safety and efficacy in herbal products.

Although Ge et al. (2002) was the first to perform extraction of andrographolide using supercritical carbon dioxide as well as carbon dioxide and ethanol mixture, the technique itself is not new. But there is a need to suitably integrate this technique into research programs which will help reap rich dividends. It was shown that using supercritical fluids at high temperature and pressure, the extraction rate and yield were higher, but the yield of andrographolide in the extracts was very low and the simultaneous addition of ethanol as co-solvent enhanced the extract yield and the andrographolide content.

Previous works on the phytochemistry of *Andrographis paniculata* have established around 50 labdane diterpenoids and 30 flavonoids (Xu et al. 2010). More than 30 novel phytochemicals from *Andrographis paniculata* have been isolated, their structures elucidated using spectral and chromatographic techniques and found to have anticancer activity. It is interesting to note that in the isolation and identification of novel phytochemicals from *Andrographis paniculata*, the primary choice of extraction has been the conventional liquid–solid extraction technique of which Soxhlet extraction has been particularly favored. It is expected that with a gradual shift to the state of art, supercritical fluid extraction, the discovery of hitherto unknown phytochemicals and extension of pharmacological spectrum of activity of little known compounds of this plant is a distinct possibility, throwing up exciting challenges to researchers.

Datamining of available literature on *Andrographis paniculata* clearly shows an overwhelming therapeutic potential and can be safely regarded as one of the "modern world's panacea". Hence this high volume of research, keenly displays the interest of pharmacologists, medicinal chemists, and natural product chemists hot in pursuit to unravel the mysteries of this important medicinal plant. Moreover, quality control, maintaining batch to batch consistency and regulatory approval needs suitable means of redressal. Vital to the continuing research interests in *Andrographis paniculata* is a shift of focus from animal to humans.

This final frontier can also be conquered given the fact that more than nine clinical trials on the investigation of *Andrographis paniculata* for a variety of conditions have already been successfully completed with no report of serious adverse effects incidents or fatalities.

So with the increase of interest in this plant the demand for active principles is skyrocketing and hence the dependence on wild populations is increasing, putting pressure on the already fragile ecosystem. Hence tissue culture techniques for mass production, and gene based initiatives for maximizing the active principles are assuming massive thrusts. Ultimately there is a positive scenario for a tremendous increase in organized cultivation and farming serving as a definite source of income. With the increase in popularity of the plant, there is a demand for this plant in the market thus sustaining the small scale, medium scale herbal industries employing a steady workforce.

Hence future prospects of *Andrographis paniculata* will be clearly on an upswing as research groups world over will be looking to exploit this herb for the treatment of a variety of disorders, industries thriving on this plant, and eventually the agriculturist also reaping his benefits. It is imperative that detailed information presented as a review here might fill the vacuum and paucity of information on this plant, providing the much required stimulus to utilize this plant in generating and sustaining a possible means of livelihood. But still it is far from reaching the "extraordinary league" of select group of phytochemicals like, paclitaxel, docetaxel, vinblastine, vincristine, taxols, podophyllotoxin, cannabinoids, digitoxin, camptothecins, etc. which are already established drugs forming an important part of current drug therapy for various disorders.

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