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Review on Andrographis Paniculata wall. ex Nees: Its Traditional Use, Secondary Metabolite Production, Phytochemistry, Pharmacology and Products Developed

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Review Article

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ABSTRACT

Andrographis paniculata Wall. ex Nees (Kalmegh) (AP) belongs to the family Acanthaceae and has a wide range of medicinal and pharmacological applications. It is one of the nineteen species of the genus *Andrographis*, which is indigenous to India. Various studies have covered the pharmacological aspects of the plant, however a wholesome review integrating the phytochemistry, pharmacology, secondary metabolite production and related challenges, patents and commercial products developed and their impact on the price of andrographolides has not been written so far. The increase in the andrographolide content of AP still needs to be handled using more economical methods. Rhizosphere microbe interventions can offer an economical solution. Our comprehensive review throws light on each of these aspects and will be very helpful in designing further research and product development.

INTRODUCTION

Andrographis paniculata Wall. ex Nees (Kalmegh) (AP) belongs to the family Acanthaceae and has a wide range of medicinal and pharmacological applications. It is one of the nineteen species of the genus *Andrographis*, which is indigenous to India. The herb is distributed in tropical Asian countries with hot and humid climate; however, it can be cultivated in subtropical regions during the monsoon season[1]. The herb is mentioned in the Indian Pharmacopoeia as a predominant constituent of at least 26 Ayurvedic formulations used to treat liver ailments. A number of bioactive components like andrographolides and polyphenols are reported in AP[2]. Among these, andrographolides are pharmacologically the most active compounds; however, andrographolide content in conventionally propagated or wild plants is estimated to be only 2%-3%.The conventional vegetative propagation of AP is too slow to meet the demand of pharmaceutical industries which is increasing annually at a rate of 3.1%. The plant is usually collected from wild sources for domestic consumption. Owing to its multivariate medicinal uses, the plant is placed at 17th position among 32 prioritized medicinal plants of India with a demand of 2197.3 tons[3]. The myriads of potential therapeutic effects of andrographolides have contributed to its rising price and market demand. Quality dry leaves of the plant are sold for as much as US $5/kg, whilst the purified andrographolide and its derivatives could reach up to the US $ 100,000/kg. The latest pricing by Sigma-Aldrich Corporation (USA) in 2016 for 100 and 500 mg packages of andrographolide (98%) is US $36.20 and the US $135.00 respectively. Though the plant is in great demand among the pharmaceutical countries all over the world, most work is confined to pharmacological activities and secondary metabolites of the plants. The review is the first complete work on AP and it is believed that this will be of great help in understanding and bridging the gap between production and technology development related to the plant.

MORPHOLOGY AND DISTRIBUTION

AP, popularly known as the King of Bitters, Chirayetah and Kalmegh in English, Urdu and Hindi languages respectively, is one of the most commonly used plants in the traditional systems of Unani and Ayurvedic medicines **(Figure 1)**. The plant grows in hedgerows throughout the plains of India and also cultivated in gardens. It is an annual, 1-3 ft high, profusely branched erect herb, 0.5 to 1 m in height with a tap root. Leaves are green, lanceolate, 3-7 cm × 1-2.3 cm in size, glabrous with slightly undulating margin. Flowers are small and solitary; corolla whitish or light pink in color with hairs. Fruit, a capsule is linear-oblong and acute at

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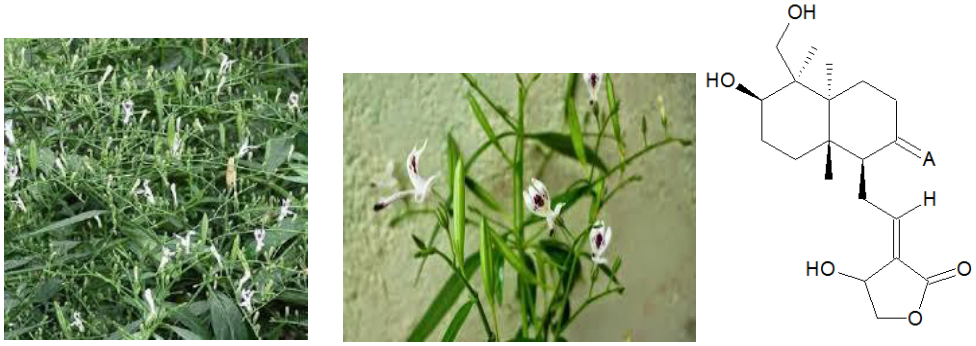
both the ends. The plant is abundantly available in South East Asiancountries, Sri Lanka, Pakistan, and Indonesia and cultivated extensively in China, Thailand, East and West Indies and Mauritius[1]. It is found in wild throughout the plains of India especially in Tamil Nadu, Karnataka, Maharashtra, Orissa, Uttar Pradesh and Uttarakhand.



a. b. c.

Figure 1. Andrographis paniculata (a) Plant; (b) Flowers and fruits; (c) Andrographolide

TRADITIONAL USES OF AP

AP has been reported to have antibacterial, antifungal, antiviral, choleretic, hypoglycemic, hypocholesterolemic, and adaptogenic effects[4]. In the Unani system of medicine, the plant is believed to possess anti-inflammatory, antipyretic, emmenagogue, astringent, gastric and liver tonic, diuretic, carminative and antihelmintic properties. It is also recommended for curing leprosy, gonorrhea, and various skin related disorders, owing to its "blood purifying" activity. Juice or an infusion of fresh leaves is effective in curing irregular bowel habits, and loss of appetite, mostly in infants[5]. Leaves and roots of the plant are also used in general debility, dyspepsia associated with gaseous distension, and in advanced stages of dysentery. In Chinese Medicine, the herb derived from the leaves or aerial parts of AP is known as Chuanxinlian, Yijianxi or Lanhelian and is known to possess similar properties, as described in Ayurveda, Unani or Traditional Indian Medicine. Various preparations and compound formulas of the herb have been used to treat infectious and non-infectious diseases, with significant efficacy reported in case of epidemic encephalitis B, neonatal subcutaneous annular ulcer, vaginitis, cervical erosion, pelvic inflammation, herpes zoster, chicken pox, mumps, neurodermatitis, eczema, and burns[6,7].

GENETIC DIVERSITY STUDY OF AP

Sabu et al.[8] demonstrated a moderate level of genetic variation among different Andrographis accessions from India and other countries of tropical Asia. Product synthesis did not correlate with isozyme pattern or sources of origin. The lower yield of andrographolide content might be related to different genotypes, different growing conditions of the plants and post-harvest treatments of the plant material. Padmesh et al.[9] determined the intraspecific variability in AP. They identified 5 major groups based on the geographical distribution that generally reflected expected trends between the genotypes. Andrographolide showed quantitative variations among the accessions which could not be correlated with allelic variation. The data revealed moderate variation within the species as shown by 61 polymorphic RAPD (random amplification of polymorphic DNA) products ranging in size from 310 to 3500 base pair out of the 73 products generated (83.56%). In Thailand, Tongdonae [10]

Studied the genetic diversity of AP using morphological characters and isozyme patterns and found that all accessions were significantly different. Classification based on morphological characters did not correlate with isozyme patterns and sources of origin. Wijarat et al.[11] studied different accessions of AP in Thailand and found that they were genetically similar; however, the study also suggested a close proximity between the AP accessions of Thailand and India. Prathanturarug et al.[12] reported that the morphological characteristics and andrographolide contents were stable among regenerants micro propagated from the same donor plant.

Padmesh et Al.[9] analyzed 52 accessions (displaying morphological and chemical variation) of AP from India, Thailand, Malaysia and Indonesia for intraspecific variability following RAPD analysis. Molecular analysis revealed moderate variation within the species. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) followed by cluster analysis resulted in five major groups among genotypes and it was noted that AP-48 (Thailand) had a close resemblance to AP-38 (Tamil Nadu) and AP-29 (Assam). Results indicated that RAPD could be effectively used for assessing genetic diversity and provide prospective value in breeding. Latoo et al used a combination of RAPD (6 highly polymorphic primers) and Mahalanbios D2 statistics to elucidate genetic diversity and quantify variation in metric traits among 53 accessions of AP belonging to five ecogeographic zones. The study showed a correlation between breeding systems, morphometric markers, and RAPD markers. The investigation

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suggested that various accessions available in different ecogeographic zones of India may have originated from native places of wild abundance. The self-pollinating structure of AP results in its minimized genetic diversity. Valdiani et al.[13] studied intraspecific crossability in AP as an alternative strategy to increase heterosis and save the plant from extinction.

PRODUCTION AND PROPAGATION OF AP IN INDIA

The production volume of AP achieved by commercial agriculture is not exactly available in the literature. However, the total production volume achieved from wild growing plants is about 5,000 tons per year, mainly from the states of Madhya Pradesh, Uttar Pradesh, West Bengal and Bihar[14]. Propagation of AP occurs through seeds. Owing to the problems of physical and innate dormancy, the seed germination is low and infrequent. In addition to this, the plant has a long seed germination period, which further worsens the probability of germination, because of greater chances of seed contamination which may result in weak seedlings. So far, use of plant growth regulators, mechanical scarification, and chemical treatments has been employed to break seed dormancy and prevent contamination[14].

MICROBES PRESENT IN THE RHIZOSPHERE OF AP

Soil microorganisms constitute world's largest reservoir of biological diversity and are crucial to the functioning of terrestrial ecosystems. The rhizosphere, a narrow zone, adjacent to and influenced by, living plant roots[15], is a site of high microbial activity in and around roots in soil[16]. It harbors a great diversity of microorganisms affecting plant growth and health[17,18]. A comprehensive study of the establishment of microfungi in the non-rhizosphere, rhizosphere and rhizosphere plane of AP was done by Dwivedi and Singh[19]. *Rhizopus nigricans, Aspergillus flavus, A. terreus, A. luchuensis, A .niger, Penicillium citrinum, Paecilomyces fusisporus* and *Fusarium poae* were frequently isolated from non-rhizosphere while *A. niger, A. flavus, Trichoderma lignorum* and *P. citrinum* were of significant occurrence in the rhizosphere of AP. It was found during the study that *Rhizopus nigricans* appeared during a later stage of plant growth in the non-rhizosphere, rhizosphere and rhizosphere plane of AP. Amongst aspergilla, only *A. flavus* and *A .niger* colonized the rhizoplane and others were confined to non-rhizosphere and rhizosphere regions. The abundance of these species fluctuated at different stages of plant growth. Another important finding of the study was that *T. lignorum* was isolated with high frequency from rhizoplane and rhizosphere than from non-rhizosphere; however, there was no definite trend with increasing plant growth. The aspergilla species *A. terreus* and *A. luchuensis* and *Penicillium* species were less frequently isolated from rhizosphere and never from rhizoplane, which could be attributed to the effect of root exudates on these species. Arunachalam and Gayathri[20] isolated *endophytic* bacteria (unidentified) from the rhizosphere, which showed antimicrobial, enzymatic and plant growth promoting activities.

Phytochemistry

The herb is mentioned in the Indian Pharmacopoeia as a predominant constituent of at least 26 Ayurvedic formulations used to treat liver ailments. A number of bioactive components like andrographolides and polyphenols are reported in AP. The main chemical constituents of AP are diterpenoids (which contain hydroxyl, α,β unsaturated-ϒ lactone, and exomethylene groups in their chemical structures) and flavonoids. Among these, *andrographolides* are pharmacologically the most active compounds, while the rhizomes are a harbor for several flavonoids[21]. Flavonoids mainly exist in roots but can also be isolated from leaves. The aerial parts of the plant mainly contain alkanes, ketones, and aldehydes. Initially it was thought that the lactone *andrographolide* alone was the bitter principle in the leaves, however, later it was found that the leaves contained two bitter principles- andrographolide and kalmeghin **(Table 1)**. Rao et al.[22] isolated seven andrographolides from the aerial part of AP; among the non-andrographolide compounds, they isolated nine 2'-oxygenated flavonoids, which occur rarely in nature. Andrographis species are known for the profuse production of 2'-oxygenated flavonoids and andrographolides,which can thus be used as chemotaxonomic markers for *Andrographis* species as the production of these markers, is confined to *Andrographis* species in *Acanthaceae* family.

Table 1. Major compounds identified in different extracts of various parts of Andrographis paniculata, and their cited biological activities.

	Phytocompounds	Molecular Weight	Plant Part	Extract type	Activity	Reference
	Andrographolide type					
1	Andrographolide	350.44	Aerial ; Leaves	Methanol; Dichloromethane; 50% ethanol; Hot water extract (70-75 degree C)	Cell differentiation inducing activity on mouse myeloid lukemia cells; Cardiovascular activity; Against Inflammatory Bowel Disease; Anti- cancer; Inhibition of thrombin induced platelet aggregation	[92, 93, 94,95, 96]
2	14-epi-Andrographolide	351	Aerial	Methanol	Cell differentiation inducing activity on mouse myeloid lukemia cells	[93]
3	Isoandrographolide	350.44	Aerial	Methanol	Cell differentiation inducing activity on mouse myeloid lukemia cells	[93]

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4	14-deoxyandrographolide	334.45	Aerial	Methanol; Dichloromethane	Cell differentiation inducing activity on mouse myeloid lukemia cells; Cardiovascular activity; immu-nostimulatory, anti- atherosclerotic,anti-hepatotoxic , anticancer	[93,94, 95, 97,98]
5	14-Deoxy-12methoxyandrographolide	365	Aerial	Methanol	Cell differentiation inducing activity on mouse myeloid lukemia cells	[93]
6	12-epi-14-Deoxy-12methoxyandrographolide	365	Aerial	Methanol	Cell differentiation inducing activity on mouse myeloid lukemia cells	[93]
7	14-Deoxy-12-hydroxyandrographolide	351	Aerial	Methanol; Dichloromethane	Cell differentiation inducing activity on mouse myeloid lukemia cells; Cardiovascular activity	[93, 94]
8	14-Deoxy-11-hydroxyandrographolide	351	Aerial	Methanol	Cell differentiation inducing activity on mouse myeloid lukemia cells	[93]
9	14-deoxy-11-oxoandrographolide	348	Aerial	95% ethanol	Antileishmaniasis	[99,100]
10	6'-Acetylneoandrographolide	523	Aerial	Methanol	NF	[93]
11	3, 4-Dideoxyandrographolide	318	Aerial	95% ethanol	NF	[99]
12	Bisandrographolide A	665	Aerial	Methanol	NF	[93]
13	Bisandrographolide B	665	Aerial	Methanol	NF	[93]
14	Bisandrographolide D	697	Aerial	Methanol	NF	[93]
15	14-deoxy-11, 12-didehydroandrographolide	332	Whole Plant; Aerial Part	Methanol, Dichloromethane; Hot water extract (70-75 degree C)	Anti-inflammatory; Cardiovascular activity;immu-nostimulatory, anti-atherosclerotic, and anti-hepatotoxic activities, Inhibition of thrombin induced platelet aggregation, Anti-inflammatory, anticancer, antihypertensive	[93-98, 101]
16	14-deoxy-14,15-didehydroandrographolide	Whole Plant	Whole Plant	Methanol 95% ethanol, 80%	Cytotoxic, antitumor, NF-κB- dependent trans-activation	[98]
17	Neoandrographolide	480	Aerial Part, Roots	95% ethanol, 80% ethanol; 50% ethanol; Dichloromethane	Cardiovascular activity; anti-inflammatory and anti-hepatoxic properties	[94, 98-99]
18	Andrograpanin	318.45	Leaves	ethanol	Anti-inflammatory	[102]
19	Isoandrographolide	305.44	Whole Plant	Methanol	NF	[93, 102-103]
	Non- Andrographolide type					
	Glycosides					
20	Andrographiside	512.58	Whole Plant	Methanol	Hepatoprotective	[97, 104]
21	14-Deoxyandrographiside	496	Aerial part	95% ethanol	NF	[99]
22	Andropanoside	496.59	Whole plant	Methanol	NF	[105]
	Flavonoids					
23	Andrographic acid	364.43	Aerial Part	70% acetone	NF	[103]
24	Andrographidine A	462.44	Aerial Part,Roots,	ethanol,Methanol, 80%	NF	[106-107]
25	Andrographidine B	330	Roots	Methanol, 80% ethanol	NF	[106-107]
26	Andrographidine C	298	Roots	Methanol, 80% ethanol	NF	[106-107]
27	Andrographidine D	358	Roots	Methanol	NF	[106]
28	Andrographidine E	328	Roots	Methanol	NF	[106]
29	Andrographidine F	537	Roots	Methanol	NF	[106]
30	Andrographidine G	477.13	Whole Plant	Methanol	NF	[106]
	Labdanic Acids					
31	Magnesium andrographate	571	Aerial Part	70% acetone, Hydrophillic	NF	[108]
32	Disodium andrographate	409.16	Aerial Part	70% acetone;Hydrophillic	NF	[108]
33	dipotassium andrographate 19-O-D-	603.131	Aerial Part	70% acetone;	NF	[108]
34	Glucoside	603.131	Aerial Part	Hydrophillic	NF	[108]

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35	Andrographic acid	364.438	Aerial Part	Methanol	NF	[103]
36	Beta-sitosterol	414.17	Whole plant	Methanol	NF	[97]
37	Beta-daucosterol	576.84	Roots	80% ethanol	NF	[107]
38	Oleanolic acid	456.7	Roots	80% ethanol	NF	[107]
39	Squalene	410	Leaves	Ethanol	Antioxifdant, antitumour	[110]
40	Phytol	296	Leaves	Ethanol	Anti-inflammatory	[110]
	Flavonoids					
41	5-hydroxy-7,8,2',3'-tetramethoxyflavone	358	Whole plant, Roots	Methanol	NF	[109]
42	5-Hydroxy-7,2',3'-trimethoxyflavone	328.1	Whole plant	Methanol	NF	[109]
43	7,8 -Dimethoxy-5-flavone	298	Roots	Methanol	NF	[106]
45	5, 5'-dihydroxy-7, 8, 2'-trimetroxyflavone	345.095	Roots	80% ethanol	NF	[107]
46	5-hydroxy-7, 8, 2', 6'-tetramethoxyflavone	358	Roots	80% ethanol	NF	[07]
47	5, 3'-dihydroxy-7, 8, 4'-trimethoxyflavone	343	Roots	80% ethanol	NF	[107]
48	2'-hydroxy-5, 7, 8-trimethoxyflavone	329.2	Roots	80% ethanol	NF	[107]
49	5-hydroxy-7, 8, 2', 3', 4'-pentamethoxyflavone	389	Roots	80% ethanol	NF	[107]
50	Wightin	345	Roots	80% ethanol	NF	[107]
51	5, 2', 6'-trihydroxy-7-methoxyflavone 2'-O-beta-D-glucopyranoside	463	Roots	80% ethanol	NF	[107]
52	5, 7, 8, 2'-tetramethoxyflavone	501	Roots	80% ethanol	NF	[107]
53	5-hydroxy-7, 8-dimethoxyflavanone / 7-O-methyldihydrowogonin	298.29	Roots, Whole Plant	80% ethanol, Methanol	NF	[107, 109]
54	5, 2'-dihydroxy-7, 8-dimethoxyflavone	314.29	Roots	80% ethanol	NF	[107]
55	5-hydroxy-7, 8, 2', 5'-tetramethoxyflavone	520.48	Roots	80% ethanol	NF	[107]
56	5-hydroxy-7, 8, 2', 3'-tetramethoxyflavone	357.341	Roots	80% ethanol	NF	[107]
57	5-hydroxy-7, 8, 2'-trimethoxyflavone	328.32	Roots	80% ethanol	NF	[107]
58	5, 4'-dihydroxy-7, 8, 2', 3'-tetramethoxyflavone	374.341	Roots	80% ethanol	NF	[107]
59	(2S)-5,7,2',3'-tetramethoxyflavanone	344	Whole Plant	Methanol	NF	[109]
60	Dihydroskullcapflavone I	317.1021	Whole plant	Methanol	NF	[109]
61	5-Hydroxy-7,8,2 ,5 -tetramethoxyflavone-5-O-b -D-glucopyranoside	521.17	Whole plant	Methanol	NF	[111]
62	Acanthoside B	580.57	Whole plant	Methanol	NF	[105]
63	Procumbide	362.12	Whole plant	Methanol	NF	[105]
64	Procumboside	242.31	Whole plant	Methanol	NF	[105]
65	Curvifloruside F	494.5	Whole plant	Methanol	NF	[105]
	Phenyl propanoids					
66	Trans-cinnamic acid	148.158	Roots	80% ethanol	NF	[107]
67	4-hydroxy-2-methoxycinnamaldehyde	178.184	Roots	80% ethanol	NF	[107]
	Phenols					
68	Chlorogenic acid	354.308	Aerial part	50% ethanol	Anti-inflammatory	[107]

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70	Caffeic acid	180.57	Whole plant	80% ethanol	Hepatoprotective	[112-113]
71	Dicaffeoylquinic acid	516.45	Whole plant	80% ethanol	anti-atherosclerotic,Hepatoprotective	[114-115]
72	Gallic acid	170.119	Whole plant	80% ethanol	Antioxidant, hepatoprotective	[112,116]
73	Ferulic acid	194.18	Whole plant	80% ethanol	Antioxidant, hepatoprotective	[106, 117]

CURRENT APPROACHES TO INCREASE ANDROGRAPHOLIDE PRODUCTION IN AP

The current yield of andrographolide is too low to meet the commercial goal of plant cell-based bioprocess for the production of most secondary metabolites. Thus, in order to increase the yield, various techniques in plant cell-based processes are being critically studied. However, most often trials with plant cell cultures fail to produce the desired products. One of the main problems in increasing the production of secondary metabolites is the lack of basic knowledge of the biosynthetic routes and mechanisms responsible for the production of plant metabolites. The problem of limited productivity of desired metabolites due to lack of particular precursors can be tackled through biotransformation, using an exogenous supply of biosynthetic precursors; further, genetic manipulation, and metabolic engineering may improve the accumulation of compounds. Andrographolide content varies within plant parts and with the geographical distribution. The andrographolide content in conventionally propagated or wild plants is estimated to be only 2%-3%[23]. The conventional vegetative propagation of AP is excessively slow to meet the demand of pharmaceutical industries. Variability among the seed derived progenies and delayed rooting of seedlings confines propagation through seeds[24].

Thus attempts were made by many researchers to increase the quantity of andrographolide in (AP) plant parts using different inducers. Recently, the leaf biomass and the production of andrographolide compounds in AP were significantly increased after exogenous treatment with the synthetic cytokinin-1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU) at 0 (water), 5, or 10 mg L-1, for 7 days. CPPU significantly enhanced the formation of axillary bud and promoted branching by 4.6-5.6-fold, resulting in higher fresh weight (FW) and dry weight (DW). Though CPPU at 10 mg L-1 slightly caused leaf stress and chlorophyll reduction, a 5 mg L-1 CPPU enhanced the andrographolide content[25].

Further, to meet the overgrowing demand of andrographolide by pharmaceutical companies, attempts were also made to multiply AP through tissue culture. Andrographolide induction in callus treated by Naphthalene Acetic Acid was achieved by Alwar and Subramaniyan[26] Valdiani et al.[27] investigated the genetic mechanisms controlling the biosynthesis of andrographolides using a diallele analysis. The high-performance liquid chromatographic analysis confirmed that the biosynthesis of andrographolides was considerably increased through interspecific hybridization. Though there has been a continuous upsurge of technique intensive artificial means of increasing secondary metabolite production in AP. However, enhancement of andrographolide content through natural amendments is still unachieved. Rhizosphere microbes promote plant growth either through improved nutrient acquisition or hormonal stimulation or suppression of pathogens[28]. Several specific bacterial or fungal species are found to be associated with the rhizosphere of certain medicinal plants.

Species such as *Azospirillum, Azotobacter*, and Pseudomonas are found in the rhizosphere of *Catharanthus roseus*, Coleus forskohlii, Ocimum sanctum and Aloe vera while the occurrence of arbuscular mycorrhizal fungus (AMF) species such as *Glomus coronatum, G. mosseae, G. etunicatum, G. geosporum, G. viscosum* and *G. rubiforme* has been reported in the rhizosphere of *Smilax aspera* and *Helichrysum litoreum*[29]. A study by Rajasekar and Elango[30] demonstrated that a combination of PGPR strains Azospirillum, Azotobacter, Pseudomonas and Bacillus significantly increased plant height, root length, and alkaloid content in Withania somnifera. Though there have been a number of successful case studies of increase in secondary metabolite content either directly or by increasing the plant biomass, however, such explorations with sustainable results are still meager in AP.

BIOAVAILABILITY OF ANDROGRAPHOLIDE IN VIVO SYSTEMS

Natural Bioavailability

The Biopharmaceutics Classification System (BCS), classified drugs into 4 categories, class I (high solubility, high permeability); class II (low solubility high permeability); class III (low solubility low permeability), and class 1V (high solubility, low permeability). Andrographolide belongs to class III, as per BCS[31]. The absolute bioavailability of AP was reported to be 2.67% and the drug metabolized to a sulfonate 14-deoxy-12-sulfo- andrographolide in duodenum and jejunum. The poor oral bioavailability of andrographolide is attributed to its high lipophilicity (log P value = 2.632 ± 0.135), low aqueous solubility (3.29 ± 0.73 mg/ml) rapid transformation and efflux by P-glycoprotein[32].

Distribution-major Organ of Absorption

The pharmacokinetic and oral bioavailability of AP has been thoroughly worked out by Panossian et al.[33]. The study gave a systematic insight on its absorption, bioavailability, pharmacokinetics and elimination in rats and human systems. Andrographolide

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was quickly absorbed from the gastro-intestinal tract into the blood with an absorption half-life of about 25 minutes. It then bound extensively to blood proteins and redistributed between blood and tissues within 1-2 hours. The maximum concentration of drug was achieved after 1.36 hours of its administration [33].

Formulations and Drug Delivery Systems Tried

Liu et al. [34] designed a biocompatible microemulsion of AP (BMAP) containing both fat-soluble and water-soluble constituents. The pharmacokinetic results of the microemulsion showed that the AUC0-7 and AUC0→∞ values of BMAP were 2.267 and 27.156 μg·mL(-1)·h(-1) respectively, and were about 1.41-fold and 6.30-fold greater than that of ethanol extraction, respectively. Self-micro emulsifying formulations composed of AP extract (11.1%), Capryol 90 (40%), Cremophor RH 40 (40%) and Labrasol (8.9%) were developed in liquid and pellet forms to improve the bioavailability of andrographolide in in vivo systems. The pharmacokinetic parameters like maximum concentration (Cmax), time to reach maximum concentration (Tmax) and the under concentration time curve (AUC0-12) were determined. The optimized formulations showed a significant increase in the dissolution of andrographolide, 97.64 % and 97.74 % within 15 min from liquid and pellet formulations respectively as compared to the crude extract powder (10% within 2 h) ($p<0.05$). The AUC0-12 of andrographolide (for the same dose equivalent of 35mg/kg of andrographolide) from liquid and pelleted formulations was 9 and 26 fold higher as compared to the unformulated extract. The Cmax of andrographolide in liquid SMEDDS and SMEDDS pellets was 6 fold and 5 fold respectively; however, the Tmax of andrographolide from liquid and pelleted SMEDDS was similar to that of AP extract. The formulations were found to improve the dissolution and oral bioavailability of andrographolide thus enabling a reduction in the dose of the sparingly soluble AP extract[35]. Engineered nano-systems prepared by pre-electrolyte deposition of chitosan biopolymer on AP with poly lactic –co-glycolic acid (PLGA) were developed for smart recovery in hepatotoxic conditions. A rapid in vitro dissolution of the nano-system occurred up to 8 hours followed by a sustained effect for 432 hours. The nano system showed improved biocompatibility and permeability necessary for pre-oral activity. A pre-treatment with the drug resulted in increased activity in the CCl4 damaged antioxidant enzymes. It also reduced the release of pro-inflammatory cytokines IL-6 and TNFα in CCl4 damaged liver tissues, thus protecting the liver by inducing hepatocyte proliferation and modulating the release of tissue- repair mediators[36]. *Chellampillai* and *Pawar*[37] developed a system of pH-sensitive nanoparticles by a nanoprecipitation technique using Eudragit ,EPO (cationic poly methacrylate Copolymer) for improving the bioavailability of andrographolide. The smaller size of the nanoparticle (255 ± 9 nm) as compared to the pure drug (49,461 ± 7 nm) increased its oral bioavailability 2.2 fold as compared to pure drug.

Metabolism-major Metabolites

The reports on the metabolism of andrographolide in rats and human systems have been meager. The metabolic adducts of andrographolide can be categorized as sulfonic acid or sulfate type adducts, glucuronide conjugates, and creatinine adducts. Though sulfonic acid or sulfate type adducts have been mainly identified; seven glucuronide conjugates and two creatinine adducts (14-deoxy-12-(creatinine-5-yl)-andrographolide-19-O--D-glucuronide 14-deoxy-12-(creatinine-5-yl)-andrographolide-19-O-D-glucuronide B) were also reported in the metabolic transformation[38,39]. The major metabolite of andrographolide in rats was identified as 14-deoxy-12(R)-sulfo andrographolide which has anti-inflammatory effects[40,41]. About ten andrographolide metabolites, mainly sulfonic acid adducts and sulfate compounds have been isolated and identified. Eleven new urinary metabolites of andrographolide in human were identified as 3-O-sulfate and 19-O-β-glucuronide conjugates[42]. Six metabolites of andrographolide identified as sulfate ester compounds were reported by He et al.[40]. Four sulfonate metabolites of andrographolide, 14-deoxy-12(R)- sulfo andrographolide, 14-deoxy-12(S)-sulfo andrographolide, 14-deoxy-12(R)- 9S andrographolide and 14-sulfo isoandrographolide were identified by He et al.[40].The studies suggest that metabolism of andrographolide takes place through multiple pathways.

Excretion/Elimination

Intensive drug metabolism was the major elimination route and the drug could not be detected after the eighth hour of administration. About 8.2% of the compound was eliminated through urine within 72 hrs of administration, the elimination rate being, 0.028 ml/min. More than 90% of the compound was eliminated through other ways, probably through metabolic transformation.The highest rate of elimination took place in the interval of 6-24 hrs[33].

PHARMACOLOGY

Anti-inflammatory Activity

Andrographolide was found to inhibit the inflammatory responses produced by rat neutrophils. Pretreatment with the compound (0.1 ± 10 mM) prevented N-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced neutrophil adhesion and transmigration in a concentration-dependent manner. Further, the andrographolide pretreatment significantly reduced fMLP-induced production of H_2O_2 and O_2 and also decreased fMLP-induced expression of both CD11 and CD18, an essential integrin-mediated in neutrophil adhesion and transmigration. The study suggested that andrographolide prevented the reactive oxygen species (ROS) production through part modulation of protein kinase C-dependent pathway and thus affected the downregulation of Mac-1, essential for neutrophil adhesion and transmigration[43]. Another anti-inflammatory mechanism of andrographolide **(Figure 2)** involves inhibition of the activation of NF-kappaB, suppression of inducible nitric oxide synthase (iNOS) expression and

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inhibition of COX-2 expression in human fibroblast cells[44]. Andrographolide reduced the intensity of the peritoneal inflammation produced by acetic acid in mice, indicating its ability to inhibit the permeability of small blood vessels. It also affects important inflammatory mediators, such as eicosanoids and platelet-activating factor (PAF) in a dose-dependent manner (IC50 ~ 5 μM) [45]. Another major phytoconstituent of AP, 14-Deoxy-11,12-didehydroandrographolide, significantly inhibited the expression of proinflammatory cytokines/chemokines (TNF-α, IL-1β, IL-6, CCL-2/MCP-1, IFN-α, IFN-β, IFN-γ, MIP-1α, MIP-1β in lungs of mice infected with pathogenic influenza viruses, H_5N_1 [46].

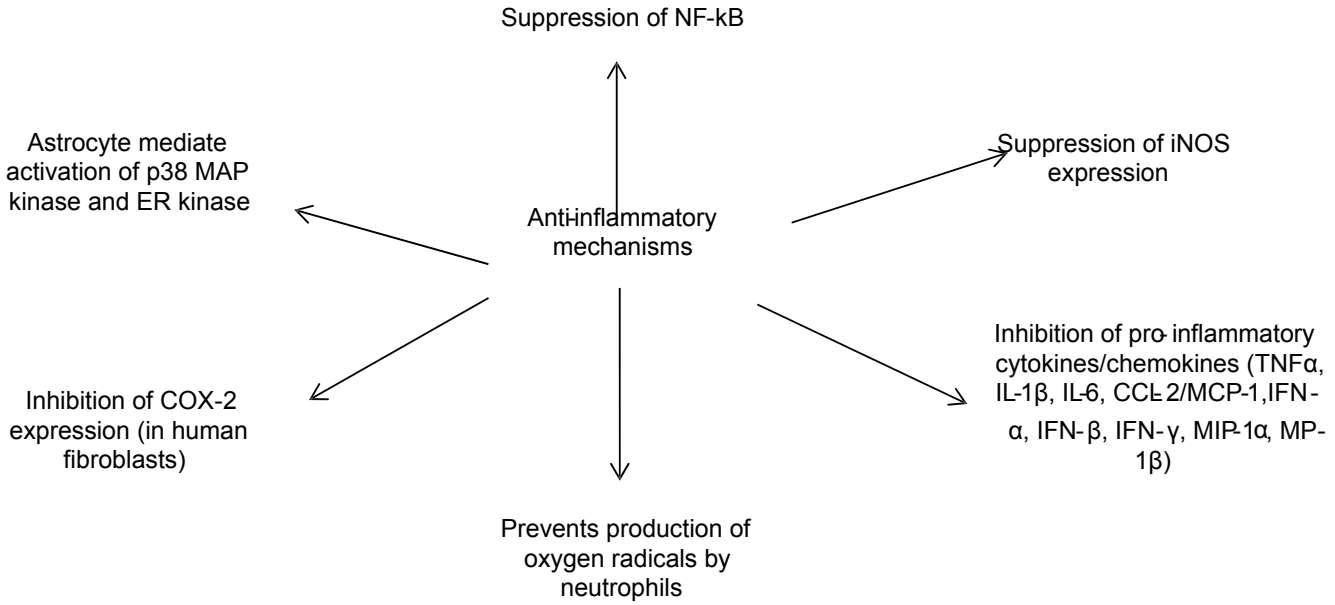

Figure 2. Anti-inflammatory mechanisms of *Andrographis paniculata*

Neuroprotective

The neuroprotective effects of andrographolide were studied on RSC96 cells in vitro. The RSC96 cell line consisting of immortalized rat Schwann cell line were treated with varying concentrations of andrographolide (0 to 50 μM), prior to the MTT assay. Cell proliferation, morphology, synthesis and nerve-specific gene expression were studied and andrographolide was found to be most effective between concentration range 0.78 and 12.5 μM. The treatment increased DNA content and promoted the gene expression of glial cell line-derived neurotrophic factor, brain-derived neurotrophic factor, ciliary neurotrophic factor, and the specific Schwann cell marker S100β ($P<0.05$). Andrographolide accelerated the proliferation of RSC96 cells without altering the Schwann cell phenotype [47] .In another study, andrographolide potently activated NF-E2-related factor 2 (Nrf2) and also upregulated heme oxygenase-1 (HO-1) expression in primary astrocytes. Andrographolide reduced Nrf2, ubiquitination efficiency, and turnover rate, followed by upregulation of Nrf2 mRNA between 8 and 24 h. HO-1 is a known gene target of transcription factor Nrf2, which is critically involved in cellular defense against oxidative stress[48]. Andrographolide recuperated the cognitive impairment in the social species Octodon degus (the only wild-type South American rodent that develops Alzheimer's-like pathology with age), a natural model of Alzheimer's disease. The treatment resulted in the recovery of spatial memory and learning performance, recovery of synaptic basal transmission, partial or complete protection of certain synaptic proteins and reduction of phosphorylated tau protein and amyloid beta aggregate maturation in aged degus[49]. In a similar study, andrographolide increased neural progenitor cell proliferation and the number of immature neurons in the hippocampus of 2- and 10-month-old mice compared to age-matched control mice. It also stimulated neurogenesis increasing the number of newborn dentate granule neurons. The effect of andrographolide on APPswe/PS1ΔE9 transgenic mouse model of Alzheimer's disease showed an increased cell proliferation and density of immature neurons in the dentate gyrus. Concomitantly the increase in neurogenesis, also induced activation of the Wnt signaling pathway in the hippocampus of wild-type and APPswe/PS1ΔE9 mice, evident by increased levels of β-catenin, the inactive form of GSK-3β, and NeuroD1, a Wnt target gene involved in neurogenesis[50].

Antioxidant Activity

Aqueous-methanol (50%) extract of AP (100, 200 400 mg/kg b.w. oral), prevented isoproterenol (85 mg/kg b.w. subcutaneous), induced increase in lipid peroxidation and increased the activities of antioxidant enzymes viz. superoxide dismutase, catalase, glutathione peroxidase and the levels of reduced glutathione in hearts. Moreover, the extract prevented the leakage of lactate

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dehydrogenase from the heart and recouped the heart from isoproterenol induced myocardial ischemic injury[51]. The methanol extract of AP leaves showed substantial scavenging of DPPH, H_2O_2, and linoleate free radicals at a concentration of 100mg/ml[52]. The antioxidant activity of AP ethanol extract (10mg/ml) of leaves, stems, and fruits were evaluated for the red cell hemolysis assay, free radical scavenging, and superoxide dismutase activities. Leaf extracts showed the highest antioxidant potential followed by stem and fruit. However, highest scavenging (88.13%) of free DPPH free radical was observed in fruit extracts[53]. AP ethanol extract showed remarkable (in vitro) DPPH free radical scavenging activity with an IC50 value of 57.08µmol/L. The ferric reducing antioxidant potential (FRAP) extract was 4676.20, mimicking those of BHT, at 5228.6. Nitric oxide (NO) radical scavenging activity (172.9%) was comparable to vitamin C at 183.7%. Malondialdehyde and nitric oxide levels in colon homogenate of AP ethanol extract treated rat were significantly lower at 500 mg/kg, while significant SOD activity was observed at a similar concentration. AP ethanol extract (80%) exerted an anti-inflammatory (pelvic) effect by downregulation of NF-κB pathway. The extract reduced the pathogens- induced excessive production of cytokines and chemokines e IL-1β, IL-6, CXCL-1, MCP-1, and RANTES, in the uterus and fallopian tube of treated rats [54]. Protective effect of andrographolide against H_2O_2 induced cell death, reactive oxygen species, and lipid peroxidation was observed in HepG2 cells. It was found that andrographolide leads to activation of p38 MAP kinase, via adenosine A2A receptor signaling, which resulted in enhanced expression of Nrf-2, its translocation to nucleus and activation of HO-1. Andrographolide also activated adenylate cyclase resulting in cAMP formation which in turn activated protein kinase A and inhibited GSK-3β by phosphorylation. Inactivated GSK-3β led to retention of Nrf-2 in the nucleus resulting in sustained expression of HO-1 by binding to its antioxidant response element (ARE) [55].

Anticancer Activity

The anticancer activity of andrographolide has been demonstrated in several types of cancers **(Figure 3)**. Andrographolide inhibited invasive ability of A549 cells through down-regulation of PI3K/Akt signaling and inactivation of c-Jun/c-Fos followed by a reduction in MMP-7 expression[56]. It also enhanced chemosensitivity of tumor cells to doxorubicin through inhibition of STAT3 activity, which suggested a potential therapeutic strategy using andrographolide in combination with conventional chemotherapeutic agents for treatment of cancer[57]. Andrographolide induced cell cycle arrest[58] and triggered apoptosis in human cancer cells. Andrographolide also inhibited DU145 cell growth in vitro and in vivo via suppression of the IL-6 signaling pathway[59].

Andrographolide inhibited expression of androgen receptor (AR) and prostate cancer cell growth and induced apoptosis. Andrographolide downregulated the AR expression at both mRNA and protein levels, prevented its nuclear translocation, and inhibited transactivation of its target genes. It also prevented the binding of Hsp90 to AR, resulting in proteasome-mediated AR degradation. Furthermore, andrographolide decreased the expression of androgen target genes such as prostate-specific antigen (PSA) and inhibited castration-resistant C4-2 cell growth by reducing AR expression and activity[60]. The inhibition of hepatoma tumor growth induced by andrographolide (10mg/kg) was found in a xenograft mouse tumor model in vivo. The miRNA chip analysis showed an increased expression of 22 miRNAs, whereas the expression of other 10 miRNAs decreased after treatment. Functional annotation of the target genes based on the differentially expressed miRNAs suggested that the majority of the genes were involved in a variety of signaling pathways, including miRNAs in cancer, mitogen-activated protein kinases (MAPKs) and focal adhesion[61]. Yang et al. [62] studied the cytotoxic effect of andrographolide on human T-ALL (T-cell acute lymphoblastic leukemia) cells. It was found that 10 µg/mL compound could significantly induce Jurkat cells' apoptosis, depending on the inhibition of PI3K/AKT pathway. Synergistic anticancer effects of andrographolide and paclitaxel (PTX) (widely used in chemotherapy for cancer treatment) were studied against A549 NSCLC (non-small cell lung cancer) cells. The study demonstrated the effects of 24-48 h treatment with 0.48-60.75 nM PTX and 5.10-328.0 µM andrographolide on cell cycle and intracellular reactive oxygen species (ROS), cellular proliferation and apoptosis. The antitumor efficacy of 20 mg/kg PTX with 100 mg/kg andrographolide was studied in a xenograft murine model. The combination inhibited the growth of A549 transplanted tumors by 98% [63] Andrographolide showed a time- and concentration- dependent inhibitory effect on highly proliferative MDA-MB-231 breast cancer cell proliferation, however, the treatment did not affect normal breast epithelial cells, MCF-10A (>80 %). Increased production of reactive oxygen species (ROS) with a corresponding decrease in mitochondrial membrane potential (MMP), externalization of phosphatidylserine was observed, while the population of apoptotic cells increased with prolonged exposure to andrographolide. Additionally, caspase-3 and caspase-9 were activated while Bax and Apaf-1 expression were significantly increased with a corresponding decrease in Bcl-2 and Bcl-xL expression in andrographolide-treated cells[64]. Furthermore, andrographolide was also reported to inhibit prostate cancer cells (LNCaP, C4-2b, and PC), by targeting cell cycle regulators, CXCR3 and CXCR7 chemokine receptors[65]. 14-Deoxy-11,12-didehydroandrographolide (14-DDA), a major diterpenoid of AP, induced the formation of endoplasmic reticulum (ER) vacuoles and autophagosomes, with concurrent upregulation of LC3-II in the breast carcinoma cells. The mechanism of action involved increase in cytosolic calcium concentration leading to a collapse in mitochondrial membrane potential in LC3-II cells. The ER stress pathway, was significantly upregulated, DDIT3 knockdown suppressed the formation of both ER vacuoles and autophagosomes, indicating that 14-DDA-induced ER stress and autophagy is dependent on this transcription factor[66]. The inhibitory effects of andrographolide on the growth of multiple myelomas (MM) cells and its possible impact on the nuclear factor

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(NF)-κB signaling pathway were studied by Gao and Wang[67]. Andrographolide reduced the proliferation and enhanced cellular apoptosis, caspase-9/3 activation of MM cells, and downregulated the expression of TLR4 and NF-κB protein.

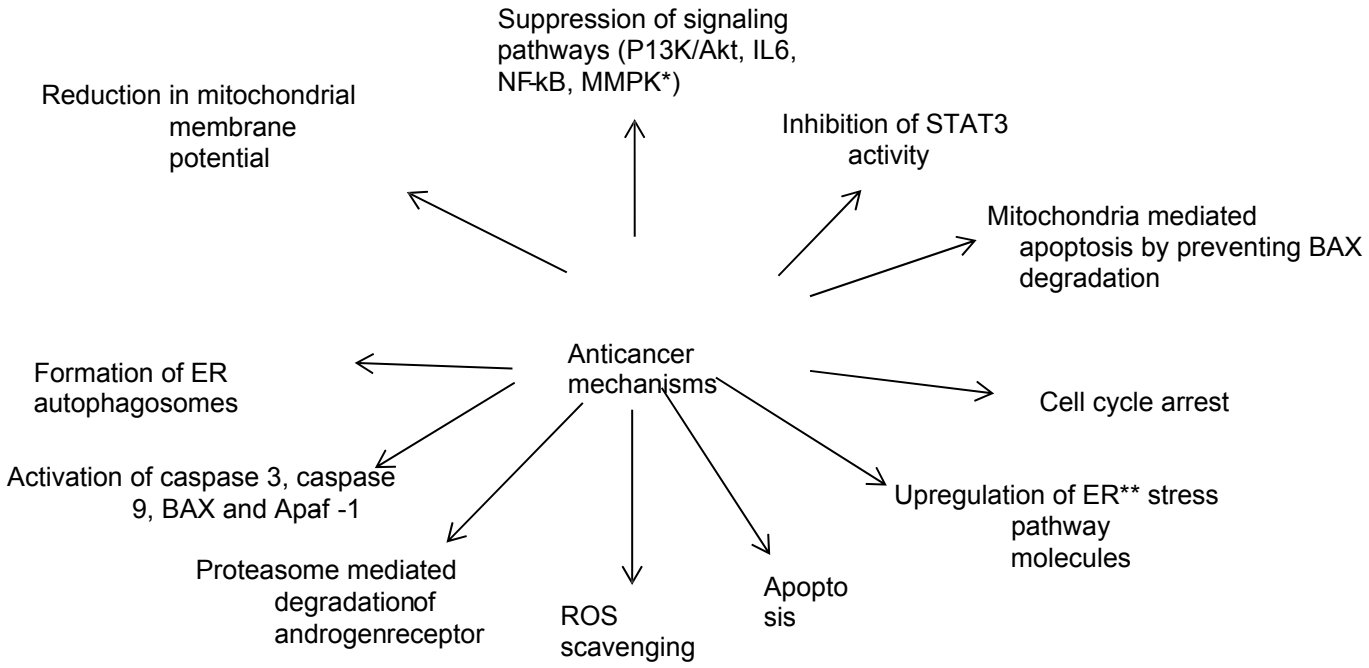

Figure 3. Anticancer mechanisms of *Andrographis paniculata*

*mitogen activated protein kinase

**endoplasmic reticulum

Anticancer activity of dichloromethane, petroleum ether and aqueous fractions of AP methanol extract were studied. The dichloromethane fraction significantly inhibited the proliferation of HT-29 (colon cancer) cells and augmented the proliferation of human peripheral blood lymphocytes (HPBLs) at low concentrations. Further fractionation of the dichloromethane extract resulted in the isolation of diterpene compounds, i.e. andrographolide, 14-deoxy-11, 12-didehydroandrographolide and 14-deoxyandrographolide. Andrographolide showed anticancer activity on various cancer cells, i.e CNS (U251), melanoma (M14), breast (NCI/ADR-RES), colon (SW620), prostate (DU145), and lung (H522) (Kumar et al.2004). AP ethanol extract tested on azoxymethane (AOM)-induced aberrant crypt foci (ACF) in vivo and in vitro, showed a significant reduction in the number of ACF of the treated rats. The expression of proliferating cell nuclear antigen (PCNA) and β-catenin protein was down-regulated in the AP-treated groups compared to the AOM group. The active fraction mainly containing diterpenoids 14-deoxy-11, 12-didehydroandrographolide, 14-deoxyandrographolide and andrographolide, in addition to amentoflavone and epicatechin, showed the significant antiproliferative effect on HT29, colon cancer cell line[68]. In vitro and in vivo studies on colorectal cancer indicated that andrographolide treatment significantly re-sensitized HCT116/5-FUR cells (HCT116 cells which are 5-FU resistant) to cytotoxicity of fluorouracil (5-FU). Mechanism analysis showed that Andrographolide/5-FU co-treatment enhanced apoptosis level of HCT116/5-FUR cells and increased level of BAX. The study demonstrated that Andrographolide could directly target to BAX (Biotin-Andrographolide pull-down and cellular thermal shift assay), and Andrographolide-BAX interaction prevented BAX degradation and enhanced mitochondria-mediated apoptosis[69]. The methanol extract of AP leaves was partitioned using various solvents and each fraction was tested for relative cytotoxicity against oral squamous cell carcinoma cell lines as compared to normal cells. The ethyl acetate fraction containing 14-deoxyandrographolide, andrographolide, neo andrographolide and deoxyandrographiside showed maximum cytotoxicity. Among the isolated compounds, andrographolide showed the greatest cytotoxicity and tumor specificity, also induced caspase-3 activation of HSC-2 oral squamous carcinoma cells[70]. Aqueous extract of AP elicited anti-invasion activities by suppressing TM4SF3 gene expression in esophageal cancer cells, EC-109 and KYSE-520. The extract inhibited the motility and invasion of esophageal cancer cells, which is the initial step of metastasis, without cytotoxicity[71].

Immunomodulatory Activity

Andrographolide, 14-deoxy-11, 12-didehydroandrographolide and 14-deoxyandrographolide isolated from dichloromethane fraction of methanol extract of aerial part of AP, increased proliferation of human peripheral blood lymphocytes (HPBL) by 14, 5 and 7%, respectively, at 1 μM concentration. All the three compounds enhanced the IL-2 induction in HPBLs. While andrographolide showed greater induction of IL-2, it was cytotoxic towards HPBLs at higher concentration [72].

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Antimicrobial Activity

The antimicrobial activity of aqueous extract, andrographolide and arabinogalactan proteins from AP were evaluated against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*. The aqueous extract showed significant antimicrobial activity, against *P. aeruginosa, E. coli*, and *C. albicans*, which was indicative of the synergistic effect of arabinogalactan proteins and andrographolides present in the extract[73]. The methanol extract of AP leaves (5.0 mg/disc) showed significant antimicrobial activity against *Bacillus cereus* and *S. aureus* in disc diffusion assay. HPLC estimation showed 73.40 mg/g andrographolide in the extract, which attributed to its antimicrobial activity[74].A similar study by Ahmed et al.[75] demonstrated antibacterial activity of methanol extract of AP through cup-plate agar diffusion method, against *S. aureus* (IMR S-277), *Streptococcus pyogenes* (IMR S-526), *Micrococcus luteus* (IMR B-7), *Proteus mirabilis* (IMR P-74) and *P. aeruginosa* (IMR P-84). The highest activity was observed against *S. aureus* (1 mg/ml), while least activity was observed against *P. aeruginosa*. Bioautography of MeOH extract revealed two prominent spots on the bioautogram against *S. aureus* and *P. mirabilis* which were used as an indicator organism and consequently led to the identification and subsequent isolation of two antibacterial compounds viz., 3-O-β-D-glucosyl-14-deoxyandrographolide and 14-deoxyandrographolide based on 1H- and 13C NMR spectra. In another study, aqueous ethanolic extract of AP showed anti-biofilm and antimicrobial activity against food-borne pathogen *Salmonella typhimurium* by perturbing its membrane integrity[76]. Silver nanoparticles using AP leaf extracts showed high antibacterial activity against Gram-positive *Enterococcus faecalis*. It was found that greater difference in zeta potential values between Gram-positive bacteria and Ag nanoparticles triggered better internalization of the particles. Thus the cell surface charge played a vital role in cell killing[77]. Another major phytoconstituent of AP, 14-Deoxy-11,12-didehydroandrographolide effectively reduced the mortality and weight loss of mice lethally challenged with A/chicken/Hubei/327/2004 (H5N1) or A/PR/8/34 (H1N1) influenza A viruses (IAV). Diterpenoid lactone, 3,19-isopropylideneandrographolide from AP, inhibited the herpes simplex virus type 1 (HSV-1) infection at the post-entry step[78].

Hepatoprotective

Chen et al.[79] investigated the modulatory potency of AP ethanol extract and andrographolide on the expression and activity of cytochrome P450 (CYP) isozymes, glutathione S-transferases (GST), uridine diphosphate glucuronosyl transferases (UGT), and P-glycoprotein in rat liver. The effects of ethanol extract and andrographolide on tolbutamide pharmacokinetics in rats and on the hypoglycemic effect of tolbutamide in high-fat-diet-induced obese mice were also investigated. Rats were intra gastrically dosed with 2 g/kg/day extract or 50 mg/kg/day andrographolide for 5 days before administering a dose of 20 mg/kg tolbutamide. AP ethanol extract and andrographolide reduced the AUC0–12h (area under the plasma tolbutamide concentration versus time curve) of tolbutamide by 37% and 18%, respectively, compared to control. AP ethanol extract and andrographolide accelerated the metabolism rate of tolbutamide through increased expression and activity of drug-metabolizing enzymes without impairing the hypoglycemic effect of tolbutamide. Andrographolide was found to protect liver cells from H2O2 induced cell death by upregulation of Nrf-2/HO-1 mediated via adenosine A2a receptor signaling[79] .

MISCELLANEOUS

The antiplasmodial activity of xanthones isolated from roots of AP was reported by Dua et al [80]. A 4 µg/ml concentration of xanthone reduced the growth of *Plasmodium falciparum* by 50 %. In vivo anti-malarial sensitivity test on Swiss Albino mice with Plasmodium berghei infection using Peters' 4-day test gave substantial reduction (62%) in parasitaemia after treating the mice with 30 mg kg(-1) dose[80]. Andrographolide and neoandrographolide identified in the butanol fraction of alcoholic extract of AP showed significant antidiarrhoeal activity against *E. coli* enterotoxins in *in vivo* studies[81]. Bisandrographolide A from AP was reported to activate TRPV4 channels (transient receptor potential cation channel subfamily V member 4). TRPV4 is a nonselective cation channel which regulates the systemic osmotic pressure by the brain, vascular function, affects the liver, intestinal, renal and bladder function, skin barrier function and response of the skin to ultraviolet-B radiation. It also affects the growth and structural integrity of the skeleton, function of joints, airway- and lung function, retinal and inner ear function, and in pain. The channel is usually activated by osmotic, mechanical and chemical cues [82].

Clinical Trials

A double-blind, placebo-controlled, parallel-group clinical study was carried out to evaluate the effect of an AP extract SHA-10 fixed combination, Kan Jang, for the treatment of acute upper respiratory tract infections, including sinusitis. The trial included 95 individuals in the treatment group and 90 individuals in the placebo group, who followed medication for 5 days. The individual symptoms of a headache and nasal and throat symptoms together with general malaise showed the significant improvement while a cough and eye symptoms did not differ significantly between the groups [83]. Adverse effects and tolerance to a dry powder of aerial part of AP were studied in 20 patients with type 2 diabetes mellitus for a period of 12 weeks. The dosage started with 600 mg daily, which gradually increased to a maximum of 1.8 gm daily. No significant change was observed in body weight, blood pressure, liver function, renal function, cardiac enzymes, haemogram, serum electrolytes, blood cholesterol, serum triglycerides and blood hormone levels (triiodothyronine, thyroxine, thyrotropin, insulin and fasting cortisol) during the study period. However, there was a fall in HbA1c by 5.46% ($p<0.01$) and fasting sugar insulin by 20.93% ($p<0.003$)[84]. A randomized controlled clinical trial was carried out in 60 subjects with hypertriglyceridemia. They were divided into 3 groups and treated with low dose of AP

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extract (APE-L, andrographolide 71.64-72.36 mg/day), high dose of AP extract (APE-H, andrographolide 119.64-120.36 mg/day), and gemfibrozil 300 mg/day, for 8 weeks. APE-H 120 mg/day and gemfibrozil 300 mg/day caused a significant reduction of TG level (P=0.0442 and 0.0145, respectively) without causing any notable difference in the safety or tolerability among the treatment groups[85]. Another randomized, double-blind, placebo-controlled trial evaluated the efficacy of AP extract (HMPL-004) in 224 adults with mild-to-moderate ulcerative colitis. Patients were randomized to AP extract (HMPL-004) 1,200 mg or 1,800 mg daily or placebo for 8 weeks. At the end of the study, 38% of the patients receiving 1800 mg AP extract daily were in clinical remission[86]. A randomized, double-blind, multicentre, 8-week parallel group study was conducted using HMPL-004 1200 mg/day compared with 4500 mg/day of slow release mesalazine (mesalamine) granules in patients with mild-to-moderately active ulcerative colitis. Remission and response were observed in 28% and 74% of HMPL-004-treated patients and 24% and 71% of mesalazine-treated patients, respectively [87]. A randomized, double-blind placebo-controlled clinical study was conducted to evaluate the efficacy of KalmCold, an extract of AP, 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 comparison of the overall efficacy of KalmCold over placebo was was 2.1 times (52.7%) higher than placebo (p< or =0.05)[88]. One hundred and fifty-two adult patients with pharyngotonsillitis volunteered in the randomized double-blind study to assess the efficacy of AP. The volunteers randomly received either paracetamol or AP (3 g/day or 6 g/day) for 7 days. No significant difference in the clinical effects was noticed at the end of the study[89]. In a similar study, the effectiveness of AP SHA-10 extracts in reducing the prevalence and intensity common cold was compared with a placebo. A group of 158 adult patients of both sexes was divided into two equal size subgroups, one of which received AP dried extract (1200 mg/day) and the other a placebo for 5 days. The results suggested that AP had a high degree of effectiveness in reducing the prevalence and intensity of the symptoms of an uncomplicated common cold[90]. Two randomized double-blind, placebo-controlled parallel group clinical trials were performed to investigate the effect of a standardized extract (SHA-10) of AP fixed combination (Kan Jang) for the treatment of uncomplicated upper-respiratory tract infections. The trial included 46 patients in the pilot study and 179 patients in the phase III trial. Medication was given thrice daily for a minimum of 3 days and a maximum of 8 to 19 days for the pilot study, and for exactly three days in the phase III study. The total symptom score showed improvement in the pilot study (p=0.08), while the total symptom score and the total diagnosis score showed the significant efficacy of AP (p<0.0006)[91]. In another prospective, randomized, double-blind, and placebo-controlled study in patients with rheumatoid arthritis (RA), AP extract tablets (30% total andrographolides) were administered three times a day for 14 weeks, after a 2-week washout period to 60 patients with active RA. The intensity of joint pain decreased in the active vs placebo group at the end of treatment however the differences were not statistically significant [92]. A phase I dose-escalating clinical trial of andrographolide was conducted in 13 HIV-positive patients and 5 healthy volunteers. The objectives were to assess safety, tolerability, and effects of andrographolide on plasma virion HIV-1 RNA levels and CD4 (+) lymphocyte levels. A significant rise in the mean CD4(+) lymphocyte level of HIV subjects occurred after administration of 10 mg/kg andrographolide . There were no statistically significant changes in mean plasma HIV-1 RNA levels throughout the trial. The study suggested that andrographolide may inhibit HIV-induced cell cycle dysregulation, leading to a rise in CD4 (+) lymphocyte levels in HIV-1 infected individuals[93]. Patents Various patents of AP either in form of extracts, or synergistic herbal formulations have been filed or granted. Most of these patents are from Chinese inventors, while some are from Indian inventors **(Table 2)**. It should be mentioned here that AP is an important cold property herb used in traditional Chinese medicine, for its antipyretic properties[94]. Products various products containing AP extracts have been developed by different companies **(Figure 4)**. The majority of these products are immune boosting, while one product acts as a remedy for the respiratory tract, skin and intestine infections, and one as a hepatoprotective agent **(Table 3)**. Conclusion AP is used as a traditional medicine in India, China, and other Asian countries and has an array of medicinal properties. Many of its medicinal properties have been scientific validated; however, its safety and toxicity studies need a detailed analysis. Though a number of studies have proved the hepatoprotective activity of AP, very few such products have been developed. The increase in the andrographolide content of AP still needs to be handled using more economical methods.

Table 2. List of Patents related to Andrographis paniculata

Title	Publication number	Publication type	Publication year/Filing year	Inventors	Original assignee
Andrographis paniculata extract.	US8557308 B2	Grant	2013	Jifeng Duan, Zhiming Ma, Xiaoqiang Yan,Weihan Zhang, Tao Wang, Yu Cai	Nutrition Science Partners Limited
Crude extracts from andrographis paniculata.	USRE42718 E1	Grant	2011	Xiaoqiang Yan, Tao Wang, Zhiming Ma,Weihan Zhang, Jifeng Duan, Yu Cai	Hutchison Medipharma Enterprises Limited
Andrographis Paniculata Compositions and Methods for Treatment of Addictions	US20140072662 A1	Application	2013	Roberto Ciccocioppo	Omeros Corporation
Compositions comprising Andrographis paniculata extracts combined with Ginkgo biloba extracts complexed with phospholipids, and their use	US9192636 B2	Grant	2015	Ezio Bombardelli, Andrea Giori	Indena S.P.A.

A hepatoprotective herbal composition	WO2011074001 A2	Application	2011	Shiv Prakash Ratnam, Kilambi Pundarikakshudu	Shiv Prakash Ratnam, Kilambi Pundarikakshudu
An improved process for the isolation of andrographolides from andrographis paniculata"	543/DEL/2003	Application	2009	Dharam Chand Jain, Shiwani Singh,Bali Ram Tyagi, Sudeep Tandon	Council of Scientific & Industrial Research
Method for separating and purifying deoxyandrographolide	CN20091185163	Unexamined application	2009	Wencheng Zhang , Renjuan Zheng , Haitao	Wencheng Zhang , Renjuan Zheng , Haitao Zhang , Ling Liu , Ruixia Wang
Compositions for the treatment of chronic degenerative inflammatory conditions	US7993683 B2	Grant	2011	Ezio Bombardelli, Paolo Morazzoni	Indena S.P.A.
Composition For Enhancing Immunity and Reducing Inflammation Related	US20080254110 A1	Application	2008	Marvin Heuer, Ken Clement, Chaudhuri Shan,Megan Thomas	Marvin Heuer, Ken Clement, Chaudhuri Shan,Megan Thomas
Composition of labdane diterpenes extracted from andrographis paniculata, useful for the treatment of autoimmune diseases, and alzheimer disease by activation of ppr-gamma receptors	WO2005074953 A1	Application	2005	Orozco Juan Luis Hancke, Aguilera Rafael Agustin Burgos	Universidad Austral De Chile, Körber, Martin
A process for manufacturing an herbal composition for curing diabetes and herbal composition made thereof	WO2008015699 A2	Application	2008	Babulal Bhawarlal Jain	Babulal Bhawarlal Jain
Andrographis Extract Formulations	US20070202164 A1	Application	2007	Yuqing Wang, Li Wang, Xun Zhang	Hutchison Medipharma Enterprises Limited

1.http://www.swansonvitamins.com/swanson-premium-full-spectrum-andrographis-paniculata-400-mg-60-caps

2.http://www.planetaryherbals.com/products/GP1746/

3. http://www.iherb.com/nature-s-way-andrographis-standardized-60-vcaps/1855

4.https://www.healthmug.com/product/dr-reckeweg-kalmegh-andrographis-paniculata-1x-q-20ml/813585216

5.https://www.healthmug.com/product/willmar-schwabe-india-andrographis-paniculata-kalmegh-1x-q-30ml/1326219116

6.http://botanics.asia/andrographis_paniculata_capsules

7http://ayurvedicjuices.com/wpcproduct/kalme gh-_andrographis-paniculata/

8.http://www.dxn2u.com/products/health_androg.php

9.http://www.banlab.com/hearb/Kalmegh_Andrographis_paniculata/export

10.http://www.regenerativenutrition.com/news.asp?news=102

11.http://kr.pipingrock.com/andrographis/andrographis-paniculata-extract-400-mg-10120

12. http://www.herbalist-alchemist.com/item/Andrographis--AND-ON-SALE-14

Figure 4. Healthcare and well-being products developed from *Andrographis paniculata*

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Table 3. Commercially available health and well-being products of *Andrographis panicula.*

S No.	Company name	Product type	Price
1	Swanson Health Products	Herbal immune support capsules	$3.49
2	Planetary Herbals	Winter Herb, 400 mg tablets	$10.25
3	Nature's Way	Immune support, 60 capsules	$13.49
4	Dr. Reckeweg	Kalmegh (Andrographis Paniculata) 1X (Q) (20 ml)	INR 251
5	Willmar Schwabe India	Andrographis Paniculata (Kalmegh) 1X (Q) (30 ml)	INR76
6	SBL	Kalmegh (Andrographis Paniculata) 1X (Q) (30 ml)	INR 56
7	Botanics Asia	Immunity boosting 100% Pure Andrographis Capsules	$9.99
8	Ayurvedic juices	Immunity boosting, antipyretic juice	
9	DXN	ANDROG capsules	
10	Vadik Herbs	Kalmegh (Andrographis Paniculata), 100 ct, sugar balance and immune boosting capsules	$19.73
11	BAN Labs	Livex Plus syrup (Andrographis paniculata), hepatoprotective , regenerative	
12	Regenerative nutrition	Andrographis paniculata capsules to treat infections of respiratory tract, skin and intestines	£14.61
13	Piping Rock	Andrographis Paniculata Extract (10% andrographolides) 400mg, 60 capsules	$4.49
14	Herbalist & Alchemist	Andrographis (AND), immune boosting_	$22.75

MORE ECONOMICAL METHODS

Rhizosphere microbe interventions can offer an economical solution.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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