

Review on Evaluation of Phytochemical Analysis of Kalmegh (*Andrographis Paniculata*) Leaf Extract

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Abstract: *Andrographis paniculata* is an herbaceous plant which is commonly known as “King of Bitters” and belongs to the family Acanthaceae. *Andrographis paniculata* is one of the commonly used herbal medicines worldwide. It is an erect plant which grows mainly as under shrub in tropical moist deciduous forests. It grows erect to a height of 32-100cm in moist shady places with glabrous leaves and white flowers with rose-purple spots on the petals. This plant is the richest source of bioactive constituents. Andrographolide is generally considered an essential bioactive component of plant *A. Paniculata*. Andrographolide is colorless, crystalline in appearance and has a very bitter taste. The andrographolide are multifarious and include: analgesic, antipyretic, antiretroviral, antimalarial, anti-hyperglycaemic, hepatoprotective, immunomodulatory, protective against alcohol induced toxicity and cardio protective activity and anticancer activity. Its phyto extract can protect human against a number of diseases. Kalmegh is used both in Ayurvedic and Unani System of medicines for possess immunological, antibacterial, anti-inflammatory, antithrombotic and hepatoprotective properties. Andrographolide is an interesting pharmacophore with anticancer and immunomodulatory activities and hence has the potential to be developed as an anticancer chemotherapeutic agent as well. The *A. paniculata* treats a wide range of diseases in traditional medicinal systems, and its intended benefits must be evaluated. The present communication deals with the anti-diarrhoeal properties of the alcoholic extract, its fractions and pure compounds isolated from *A. paniculata*. In the ayurvedic system of medicine currently widely practiced in Indian, *Andrographis paniculata* is often used in combination with other herbs and health care procedures for helping patients suffering from diverse spectrums of organ pathologies and mental health problems.

Keywords: *Andrographis paniculata*, Kalmegh, Phytochemicals, Andrographolide, Neoandrographolide, hepatoprotective, diterpenoids

I. INTRODUCTION

Andrographis paniculata is a well-known medicinal plant from the Acanthaceae family. Kalmegh and 'King of Bitters' are two names for *Andrographis paniculata*. *Andrographis paniculata* has been formally included in the Indian Pharmacopoeia [1]. *Andrographis paniculata* Nees is a traditional medicinal annual herb, widely distributed in Madhya Pradesh and India. This plant is the richest source of bioactive constituents. Its phyto extract can protect human against a number of diseases. It is an erect plant which grows mainly as under shrub in tropical moist deciduous forests. It is one of the most widely used plant in Ayurvedic formulations and homeopathic system of medicine [2]. *A. paniculata* is widely used to get rid of body heat, dispel toxins from the body; prevent common cold, upper respiratory tract infections including sinusitis and fever and as an antidote against poisons of snakes and insects [3]. *A. paniculata* have been reported for a wide range of pharmacological activities, such as analgesic, anticancer, anti-diabetic, anti-fertility, anti-inflammatory, anti-malarial, anti-microbial, anti-oxidant, anti-pyretic, anti-retroviral, anti-venom, cardio protective, hepatoprotective and immunomodulatory, etc [4]. Both fresh and dried *A. paniculata* leaves, as well as the juice extract, are widely endorsed in folk medicines as a curative lead for liver disorders, bowel and colic pain, general debility, and convalescence after fever [5]. The plant shows a similar taste and appearance as that of neem, though it is small in size. The herb's curative potential is due to its mechanism of action and it acts via enzyme induction. It is widely utilized in the Ayurvedic, Siddha, and Unani systems of medicine as a home remedy to treat various diseases [6]. Recent clinical studies reveal that andrographolide in the herb demonstrates better hypoglycaemic effect, choleric and

hepatoprotective activity against CCL₄, as well as galactosamine and paracetamol intoxication while dehydroandrographolide is a better in vitro inhibitor against the human immunodeficiency virus (HIV). These different effects imply that qualitative and quantitative control of the herbs is of paramount importance to ensure its technique maximal therapeutic value [7]. Traditional extraction of these constituents from the *A. paniculata* has been performed by maceration, Soxhlet, reflux and ultrasonic extraction. Microwave-assisted extraction (MAE) has received increasing attention as a potential alternative to traditional solid-liquid extraction methods, mainly due to considerable savings in processing time and solvent consumption. This technique has been widespread interest in the application to the extraction of chemical constituents in contaminations and pesticides, such as polycyclic aromatic hydrocarbons (PAHs), organophosphate pesticides, polychlorinated biphenyls (PCBs) pesticides [8]. The present communication deals with the anti-diarrhoeal properties of the alcoholic extract, its fractions and pure compounds isolated from *A. paniculata*. Anti-secretory properties of four diterpenes isolated from this plant have been compared with a recently introduced opiate derivative loperamide (Imodium) which has been recommended and is being widely used as an anti-diarrhoeal drug [9]. Andrographolide, the major diterpenoid of the *Andrographis paniculata* extract has shown cytotoxic activity against KB (human epidermoid carcinoma) and P388 (lymphocytic leukaemia) cells. The methanol extract of aerial parts of *Andrographis paniculata* and some of the isolated compounds showed growth inhibitory and differentiating activity on M1 (mouse myeloid leukaemia) cells. The ethyl alcohol extract and purified diterpene andrographolides are reported to stimulate both antigen specific and non specific immune responses in mice [10]. Andrographolide is the major bioactive constituent found in *A. Paniculata*. Andrographolide is colorless, crystalline in appearance and has a very bitter taste. A γ -lactone ring is connected to a decalin ring system via an unsaturated C2 moiety. Andrographolide in comparison to other two diterpenoids, deoxyandrographolide and neoandrographolide, had more potent anti-cancer activity against human leukaemia HL-60 cells and other cancer cells [11]. In Malaysia, this plant is used in folk medicine to treat diabetes and hypertension. It was also observed that an aqueous extract of *A. paniculata* could improve glucose tolerance in normal rabbits. The water extract of *A. paniculata* was reported to inhibit lipid peroxidation in spontaneously hypertensive rats [12]. *Andrographis paniculata* was able to inhibit the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella dysenteriae* and *Escherichia coli*, it was recommended for the treatment of different diseases ranging from bacterial dysentery, gastrointestinal disorders, tonsillitis, pneumonia, pyelonephritis to abscesses [13]. The leaves of *Andrographis paniculata* Nees, known as "Fah Talai Joan" in Thai folklore, are used extensively for the treatment of various diseases such as the bowels and liver, colic, undiagnosed fever, cholagogues and anti-helminthetics etc. Anti-neoplastic constituents from Thai medicinal plants, the methanolic extract from the leaves of *A. paniculata* Nees, were found to show significant cytotoxicity against culture cell of the human epidermoid carcinoma of the nasopharynx (KB) and P388 lymphocytic leukaemia [14]. A prospective, randomized, double blind, and placebo-controlled study in patients with rheumatoid arthritis (RA) was performed. Tablets (Paractin®) made of an extract of *A. paniculata* (30% total andrographolides) were administered three times a day for 14 weeks, after a 2-week washout period to 60 patients with active RA [15]. In the ayurvedic system of medicine currently widely practiced in Indian, *Andrographis paniculata* is often used in combination with other herbs and health care procedures for helping patients suffering from diverse spectrums of organ pathologies and mental health problems. Modern Ayurvedic scholars often classify *Andrographis paniculata* as Rasayana herb useful for maintaining stomach integrity and regulating energy metabolism and immune functions [16].

1.1 Botanical Description

Andrographis paniculata grows erect to a height of 32 to 100 cm in moist shady places with glabrous leaves and white flowers with rose-purple spots on the petals. Stem is dark green 0.3 to 1.0 m in height, 2 to 6mm in diameter, quadrangular with longitudinal furrows and wings on the angle younger parts, slightly enlarged at the nodes. Leaves glabrous up to 8.0 cm long and 2.5 cm broad lanceolate, pinnate. Flowers are small, possess calyx with five sepals which are small and linear, corolla tubes are narrow, about 6mm long, bilabiate upper lip oblong, lower tips are broad, three lobed, white with violet markings. Stamens 2, inserted in the throat [17]. The flowers are small in size, borne in spreading racemes which possess botanical features of calyx 5-partite, small, linear; corolla tube narrow, about 6 mm long; limb longer than the tube. The fruit of the plant is a capsule which is erect, linear-oblong, 1–2 cm long and 2–5 mm wide, compressed, longitudinally furrowed on broad faces, acute at both ends, thin, glandular-hairy which

contains many yellow brown seeds which are very small in size and sub quadrate. Inflorescence of the plant is characterized as patent, terminal and axillary in panicle, 10– 30 mm long; bract small; pedicel short [18].

1.2 Botanical Description of *Andrographis paniculata*:

Traits	Values/ Characteristics
Plant height	32-100 cm
Stem	Dark green
Length	32-100cm
Diameter	2-6mm
Shape	quadrangular with longitudinal furrows and wings on the angle younger parts, slightly enlarged at the nodes
Leaves	Glabrous
Length	8.0 cm
Width	2.5cm
Arrangement	Lanceolate, pinnate
Flowers	White with rose-purple spots on the petals
Size	Small, in lax spreading auxiliary and terminal racemes or panicles
Seed capsules	linear-oblong, acute at both ends
Size	1.9 cm × 0.3 cm
Shape	Sub quadrate, numerous

Table 1- Botanical Description of *Andrographis paniculata*

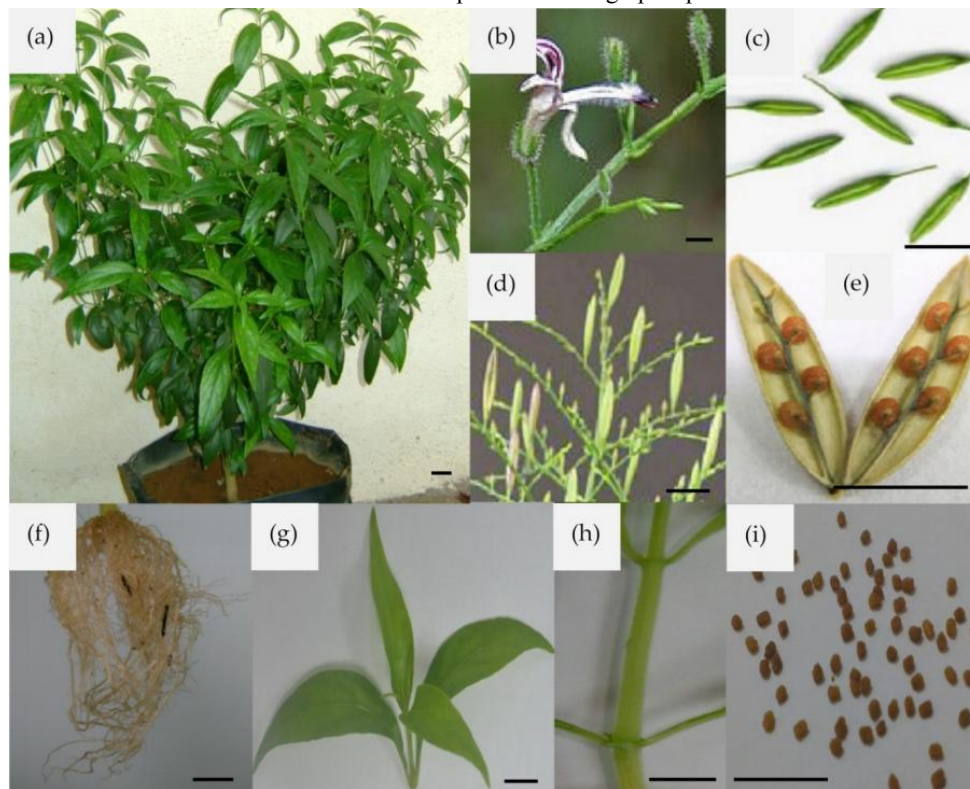


Fig- 1-*Andrographis paniculata* and its different parts. (a) Aerial part, (b) flower, (c) pod stage with panicles: mature capsule, (d) fruit, (e) opened capsule, (f) roots (g) leaves: opposite arrangement, (h) stem, and (I) seed.



Fig-2- Andrographis paniculata plant

II. TAXONOMICAL CLASSIFICATION

Sr. No	Classification	Scientific name and Common name
1	Kingdom	Plantae, Plants
2	Subkingdom	Tracheobionta, Vascular plants
3	Super division	Spermatophyta, Seed plants
4	Division	Angiosperma
5	Class	Dicotyledonae
6	Subclass	Gamopetalae
7	Series	Bicarpellatae
8	Order	Personales
9	Tribe	Justicieae
10	Family	Acanthaceae
11	Genus	Andrographis
12	Species	A.paniculate (Burm.f) Nees

Table 2 - Taxonomical Classification of Andrographis Paniculata

Phytochemistry

The phytochemical screening of the leaf extract of *Andrographis paniculata* Nees was done quantitatively and qualitatively [19]. A number of diterpenoids and diterpenoid glycosides of similar carbon skeleton have been isolated from *Andrographis*; mainly the most bitter compounds among them are andrographolide, neoandrographolide, deoxyandrographolide. Other such phytochemicals amassed by the plant are 14-deoxyandro-grapholide, 14-deoxy-11, 12-didehydroandrographolide, andrographiside, deoxyandrographiside, homoandrographolide, andrographan, andrographon, andro-graphosterin and stigmasterol. The leaves of *Andrographis* contain the highest amount of

andrographolide (2.39%), the most medicinally active phytochemical in the plant, while the seeds contain the lowest [20]. The most important metabolites are terpenoids that accounts for a large proportion of its components and therapeutic properties. The other compound includes flavonoids (flavones), xanthenes, polyphenols, macro and trace elements [21]. The most prominent compound with the highest quantity from the leaves is andrographolide (diterpenoids). This compound has also been extracted from the aerial parts or whole plant and is easily isolated from the crude extracts into a solid crystal [22].

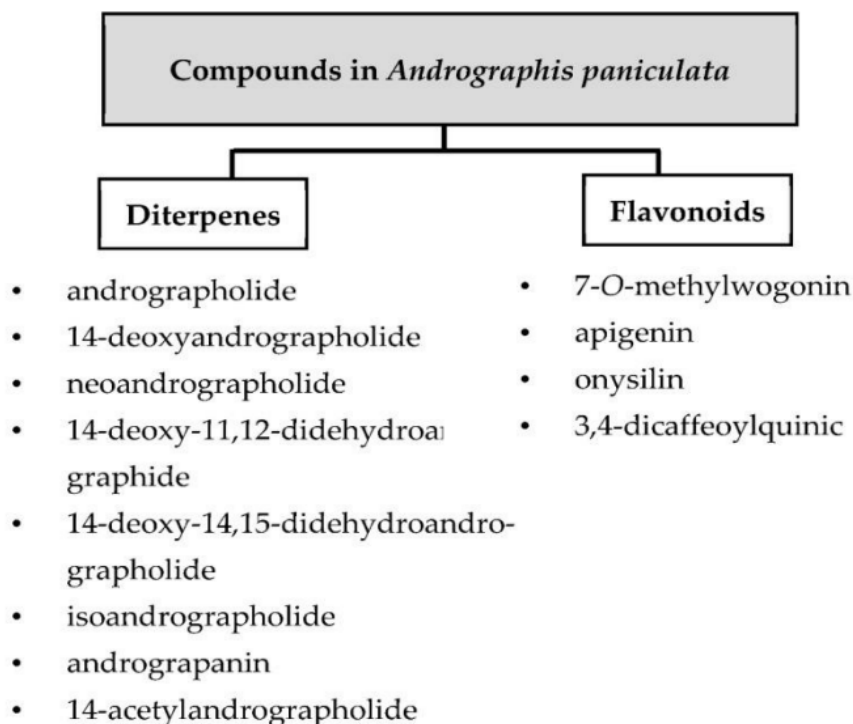


Fig-3-List of compounds isolated from A. Paniculata

Andrographolide:

Andrographolide is the most well known diterpenoids isolated from A. paniculata, both in quantity and frequency known of the diterpenoids isolated from A. paniculata. Deoxyandrographolide and neo-andrographolide are two winning diterpenoids that have been bounded by and wide from the flying parts of A. Paniculata [23]. Andrographolide is known to contribute to the most of the pharmacological properties of this plant. Chemically known as 3-(2-(Decahydro-6-hydroxy-5-(hydroxymethyl)- 5,8a-dimethyl-2-methylenenaphthyl) ethylidene) dihydro-4-hydroxyfuran-2(3H)-one. Clinical properties of the andrographolide are multifarious and include: analgesic, antipyretic, antiretroviral, anti-proliferative, antimalarial, antithrombotic, anti-hyperglycaemic, anti-urolithiasis, hepatoprotective, immunomodulatory, protective against alcohol induced toxicity and cardio protective activity and anticancer activity [24]. The main active compound of A. paniculata is andrographolide, which is found in the whole plant, leaves, stem, and roots. The characteristics of andrographolide are a colourless and bitter ent-labdane diterpene lactone substance. Andrographolide can be extracted from all parts of the plant but it is most highly concentrated in the leaves [25]. The molecular formula of andrographolide is $C_{20}H_{30}O_5$. Andrographolide can be easily dissolved in methanol, ethanol, pyridine, acetic acid and acetone, but slightly dissolved in ether and water. The melting point of this compound is 228°C – 230°C and the ultraviolet spectrum in ethanol, λ_{max} is 223nm. The analysis of andrographolide can be done by thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and crystallisation [26].

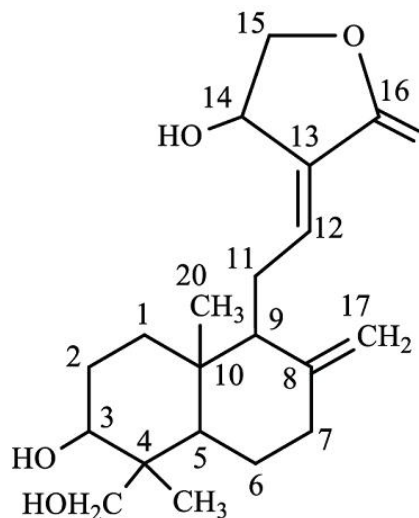


Fig- structure of Andrographolide

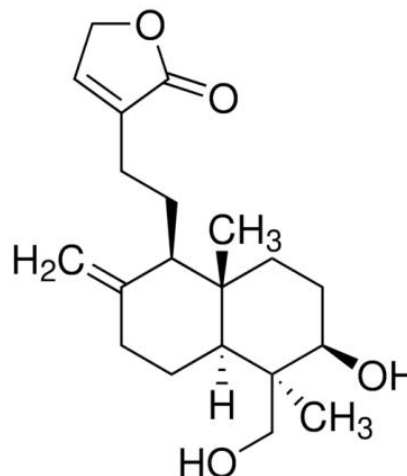


Fig- Structure of 14-deoxyandrographolide

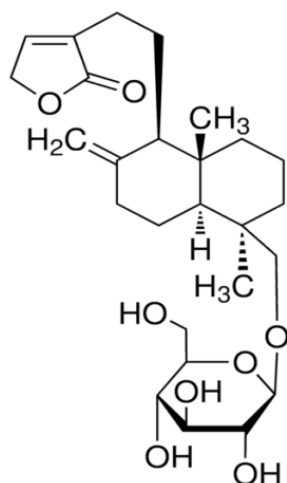


Fig-structure of Neoandrographolide

Didehydroandrographolide

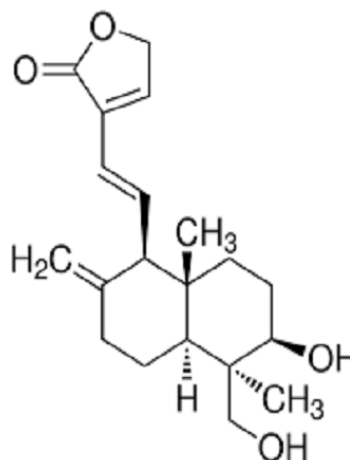


Fig- Structure of 14-deoxy-11, 12

Pharmacological effects:

A. paniculata is a well-known phytochemical from the Indian System of Medicine that has been used from ages to treat a variety of illnesses [27].

Anti-inflammatory effect:

Andrographis paniculata extracts and their phytoconstituents reported to exhibit anti-inflammatory activity by inhibiting nitric oxide and prostaglandin production [28]. It is reported that inflammation caused by histamine, dimethyl benzene and adrenaline was significantly reduced by dehydroandrographolide followed by neoandrographolide and andrographolide [29]. In vivo inflammatory progresses are mediated by the involvement of pro-inflammatory mediators, including tumor necrosis factor α (TNF- α), interleukin 6 (IL-6), IL-12, IFN- γ , NO and macrophage inflammatory protein-2 (MIP2) [30]. Intragastric administration of deoxyandrographolide, andrographolide, neoandrographolide or 11,12 didehydrodeoxyandrographolide to mice inhibited the increase in cutaneous or peritoneal capillary permeability induced by xylene or acetic acid, and reduced acute exudation in Selye granulocysts treated with croton oil. 11, 12-Didehydrodeoxy andrographolide had the most potent anti-inflammatory activity in vivo [31].

Hepatoprotective effect:

Andrographis paniculata (Kalmegh) is used extensively in the Indian traditional system of medicine as a hepatoprotective and hepatostimulative agent. The aqueous extract of the leaves of this plant has traditionally been used for treatment of various liver disorders and jaundice [32]. Administration of Andrographis paniculata prevented hexachlorocyclohexane induced increase in the activities of γ -glutamyl transpeptidase, glutathione-S-transferase and lipid peroxidation in mouse liver, an indication of potential antioxidant and hepatoprotective effects of Andrographis paniculata. Andrographolide also showed significant hepatoprotective effect against various types of liver damage induced by galactosamine or paracetamol, and had a higher capability than a classical antioxidant silymarin in preventing a decrease of bile production induced by paracetamol [33]. Antihepatotoxic action of andrographolide was reported against Plasmodium berghei K173-induced hepatic damage of mastomys natalensis. Andrographolide was suggested to play an effective role as potent stimulator of gall bladder function by producing a noteworthy increase in bile flow, bile salt and bile acid in conscious rats and anesthetized guinea pigs [34]. The A. paniculata aqueous extract and andrographolide ameliorated the dysfunction in the brain associated with nicotine toxicity. Arabinogalactan, another aqueous component of the A. paniculata, Tris-buffer extract and andrographolide minimized the toxicity in pre-treated mice [35].

Antioxidant effect:

The antioxidant activity of A. paniculata was observed due to the presence of 14- deoxyandrographolide which were isolated from the plant. The antioxidant properties of A. Paniculata and might reduce the oxidative stress[36]. Andrographolide and aqueous extract of A. paniculata herbs were screened for anti-oxidant activity on nicotine induced oxidative stress in the liver, kidney, heart, lungs and spleen of male wistar rats[37]. The Andrographis paniculata methanol extract significantly lowered MDA levels and raised the total antioxidant status in urine samples 24 hours after oral administration [38]. The antioxidant activities of Andrographis paniculata extract was determined by both in vitro as well as in vivo methods [39].

Anti-diabetic effect:

Anti-diabetic agent of Andrographis paniculata was found in extract, in fractional compound (i.e. andrographolide or its analogue AL-1: andrographolide-lipoic acid), and in combination with synthetic drug or other herbal. Therefore, Andrographis paniculata is potentially developed as an alternative anti-diabetic agent [40]. Ethanol extract of A. paniculata increase the glucose secretion from the β cells of islets of langerhens and increase the surface uptake of glucose from the adipose tissue hence inhibit the glucose absorption [41]. Andrographolide is an active bioactive synthetic constituent in A. paniculata that has anti-diabetic potential. The anti-hyperglycaemic action was studied in STZdiabetic rats, and the result proposed that andrographolide can increase glucose utilization to lower plasma glucose in diabetic rats lacking insulin [42]. Type 2 diabetes (T2DM) is closely related to intestinal barrier dysfunction. Andrographolide has been shown to improve glycemic control by enhancing intestinal barrier function and increasing microbial diversity of Akkermansia muciniphila in vitro [43]. The hot water and ethanol extracts of Andrographis paniculata exhibited a significant hypoglycaemic (blood glucose lowering) activity in both glucose-loaded and alloxan-induced diabetic rats. Oral administration of glucose (1.5 g/kg body weight) increased the blood sugar level while the intraperitoneal (ip) administration of alloxan (40 mg/kg body weight) enhanced the blood sugar level much higher than that of the glucose-loaded rats [44].

Cardiovascular effect:

A. paniculata has demonstrated an increase of blood-clotting time; hence, preand posttreatments of the extract of A. paniculata after surgery significantly prevent the constriction of blood vessels, thus decreasing the risk of the subsequent closing of blood vessels after angioplasty procedures[45]. Aqueous extract of A. paniculata produced a dose-dependent fall in systolic blood pressure of both spontaneously hypertensive rats (SHRs) and normotensive Wistar-Kyoto rats [46]. The dichloromethane (DCM) extract of Andrographis paniculata Nees was tested for cardiovascular activity. The extract significantly reduced coronary perfusion pressure by up to 74.5 ± 3.0 mm Hg at a 3 mg dose and also reduced heart rate by up to 49.5 ± 11.4 beats/minute at this dose [47].

Anticancer effect:

A. paniculata shows excellent anticancer activities against different cancer cell lines, it is alternatives medicines for cancer would replace side effect causing chemotherapeutic agent. Anticancer activity of water, ethanol and acetone extracts of *A. paniculata* leaves against HT-29 cancer cell lines shows 50% inhibition at 200 µg/ml concentration [48]. The methanolic extract of *Andrographis paniculata* was fractionated into dichloromethane, petroleum ether and aqueous extracts and screened for bioactivity. Results indicate that the dichloromethane fraction of the methanolic extract retains the active compounds contributing for both the anticancer and immunostimulatory activity. Andrographolide showed anticancer activity on diverse cancer cells representing different types of human cancers [49]. Immunostimulatory activity of andrographolide is evidenced by increased proliferation of lymphocytes and production of interleukin-2. Andrographolide also enhanced the tumor necrosis factor-alpha production and CD marker expression, resulting in increased cytotoxic activity of lymphocytes against cancer cells, which may contribute for its indirect anticancer activity [50].

Antiviral effect:

The leaves ethanolic extract was analyzed for its antiviral activity by taking A459 cell lines transfected with simian retrovirus. The antiviral activity was evaluated by observing the leaf extract activity on viral load inhibition in the A459 cell lines transfected with simian retrovirus by RT-PCR analysis [51]. Andrographolide, isolated from ethanol extracts of whole plant of AP, showed a great promise in the treatment of HIV infections. The hot aqueous aerial parts extract of AP was reported for its significant antiviral activity to reduce the percentage of HIV antigen-positive H9 cells [52]. Andrographolide, neoandrographolide and 14-deoxy11, 12- didehydroandrographolide are reported to be viricidal against herpes simplex virus 1 (HSV-1) without having any significant cytotoxicity at viricidal concentrations [53].

Anti-obesity effect:

Andrographolide exhibits potent anti-obesity action on the expression of CCAAT/enhancer binding protein β regulated by protein kinase A (PKA)-CREB activation of cAMP response element-binding protein) in the adipogenesis of 3T3-L1 cells. The administration of *A. paniculata* extracts can also inhibit cardiovascular damage by inhibiting expression of myocardial inflammation and apoptosis-related genes in obese mice fed with a high-fat diet [54].

Antifungal effect:

A. paniculata crude extracts have been used for the treatment of fungal infections in folk medicines for centuries. The ethanol crude extract of the whole plant was reported to possess moderate antifungal activity against *A. oryzae* (60% inhibition) as well as *A. Niger* and *Penicillium* sp. The hexane and methanol root extracts were evaluated for their antifungal activity against *A. Niger* and *Penicillium chrysogenum* [55]. The methanol extract of the aerial parts of the plant *Andrographis paniculata* revealed that it exhibits mycelial growth inhibition of *Fusarium solani* and spore germination inhibition of *Alternaria solani*. Pure andrographolide present in the methanol extract also exhibited spore germination inhibition of *A. Solani* [56].

Antiulcer effect:

A. paniculata extract was found to possess antiulcerogenic activity. It reduced the development of ulcers by 31%, while the standard drug, cimetidine had an 85.4% reduction rate. Andrographolide caused a significant decrease in total stomach acidity and acid stomach juice secretion without any side effects associated with ulcer therapy [57]. Apigenin-7,4 -di-o-methyl ether produced significant dose dependent antiulcer activity in shay rats, histamineinduced ulcer in guinea pigs and in aspirininduced ulcers in rats [58].

Effects on reproductive systems / Anti-fertility effect:

Andrographis paniculata found to obsessed anti-fertility and pregnancy-terminating effects and stopped spermatogenesis in male rats. The observations suggested an anti-spermatogenic or anti-androgenic abilities as well as ovulation preventive effect of the plant [59]. *A. paniculata* leaf powder inhibited spermatogenesis, while andrographolide administration to male rats reduced sperm count and motility. *A. paniculata* extract did not affect

progesterone levels in pregnant rats and induced uterine relaxation by inhibiting calcium channels [60]. It has also been reported that intra-peritoneal injection of aerial parts of AP to female albino mice caused abortion and also it prevented implantation of embryos [61].

Antivenom effect:

Plant extracts are significantly active against the rattlesnake venom. Alcoholic extracts possess anti-haemolytic properties. Although the extracts prolong survival and delay death, they are not effective when used alone against the black mamba, cobra and other potent snake venoms. The plant extracts work by producing a significant muscarinic activity [62]. Intraperitoneal injection of an ethanol extract of the aerial parts (25 g/kg body weight) to mice poisoned with cobra venom markedly delayed the occurrence of respiratory failure and death. The same extract induced contractions in guinea-pig ileum at concentrations of 2 mg/ml. The contractions were enhanced by physostigmine and blocked by atropine, but were unchanged by antihistamines. These data suggest that extracts of the aerial parts do not modify the activity of the nicotinic receptors but produce significant muscarinic activity, which accounts for its anti-venom effects [63]. Ethanol extract of *Andrographis paniculata* increases the survival time and protection fold of the victim bitten by the Indian cobra but could not protect from death. It is found more effective when given in the larger amount i.e 2g/kg [64].

Antipyretic effect:

Kalmegh is an effective herb natural antibiotic that can be used to treat all kinds of fever, though it is best known for its use in treating dengue fever (malaria) and Viral fever affects liver badly [65]. Oral administration of andrographolide isolated from *A. paniculata* leaves, (30, 100, and 300 mg/kg) was studied for its analgesic and antipyretic effect [66]. *Andrographis paniculata* dispels heat (i.e. antipyretic) and removes toxin, which makes it a good treatment for infectious ever causing diseases. The ability of andrographolide to reduce fever has been demonstrated in several experiments. It was found that andrographolide is as effective as the same dose of aspirin in reducing fever [67]. Intragastric administration of deoxyandrographolide, andrographolide, neoandrographolide or 11, 12-didehydro-14-deoxyandrographolide (100 mg/kg body weight) to mice, rats or rabbits reduced pyrexia induced by 2, 4- dinitrophenol or endotoxins [68].

Immuno-modulatory effect:

Increases white cell phagocytosis, inhibits HIV-I replication and improves CD4+ and T-lymphocytes counts [69]. The ethanol extract and purified diterpene andrographolides of *A. paniculata* induced significant stimulation of antibody and delayed type hypersensitivity (DTH) response to sheep red blood cells (SRBC) in mice. The immunomodulatory properties of a diterpene lactone andrographolide and a standardized preparation of *A. paniculata* were investigated. Proliferation of peripheral blood lymphocytes (PBL) induced by phytohemagglutinin (PHA) was enhanced by costimulation with Andrographolide and Kan Jang [70].

Effect on Common Cold:

In several communities *Andrographis paniculata* is commonly used for the prevention and treatment of common cold. The effect of dried extract of *Andrographis paniculata* on common cold was reported. The common cold patients have used a tablet made from dried extract of *Andrographis paniculata* and significant improvement was observed [71].

Anti-diarrhoeal effect:

The Anti-diarrheal activity was studied *insitu* on *E.coli* enter toxin which produces induced secretory responses that leads to cause diarrheal syndrome in the rabbit and guinea-pig ileal loop. An ethanol, chloroform, 1-butanol extracts of the aerial parts of the plant which consists of active constituents of diterpene lactones, andrographolide, neo-andrographolide which shows the potent anti secretory activity at dose of (300mg/ml) *in vivo* against *Escherichia coli* enter toxin induced diarrhoea where as the aqueous extract of the aerial part was not active [72].

II. MATERIALS AND METHODS

Collection and preparation of sample:

Fresh leaves of *Andrographis paniculata* plant was obtained from a botanical garden. The fresh healthy leaves of *Andrographis paniculata* were air dried for three weeks and then grounded to form a fine powder.

Extraction with methanol and ethanol:

Hundred grams (100 g) powder of *Andrographis paniculata* was macerated with 600 ml of methanol for three days and washed with 400 ml of methanol during filtration. The extract was concentrated by fractional distillation to remove the methanol. Thin layer chromatography (TLC) was carried out on the extracts using ethyl acetate as eluent, while chloroform (about 2 drops) was used to make solution of the extract and the various spots were viewed using UV lamp. Similarly, 20 g powder *Andrographis paniculata* was macerated with 200 ml of ethanol for three days and washed with 200 ml during filtration. The extract was concentrated by evaporation. Thin layer chromatography was carried out in the same manner as the methanol extract. Thereafter, both methanol and ethanol extracts were spotted separately on the same plate at the same time.

Gas chromatographic-mass spectrometric analysis of extracts:

Sample solution was prepared by soaking three portions of 5 g of the dried granulated samples in ethanol and ethyl acetate for 48 hr, after which the samples were filtered to obtain a clear solution. 1 ml solution was transferred into a 2 ml vial and analyzed with GC-MS. Aqueous extract was obtained by soaking 10 g of the dried sample in 25 ml hot distilled water and kept in a still position for 48 h. Sample was filtered and the filtrate evaporated over warm water bath. The concentrate was reconstituted with 2 ml methanol and introduced into a 2 ml vial prior to analysis. GC-MS analysis on the ethanol, ethyl acetate and aqueous extracts were carried out using Agilent 7890A- 5975C GC-MS system employing the following conditions: HP5-column (30 m, 0.25 mm, 0.25 μ m), operating in electron impact mode at 70 eV; carrier gas flow (a constant) was 1 ml/min, injection volume was 0.5 μ l with split ratio of 10:1, and injector temperature was 250 $^{\circ}$ C. Ion source temperature was 280 $^{\circ}$ C and oven initial temperature was 110 $^{\circ}$ C (hold 2 mins) 110 to 200 $^{\circ}$ C at 10 $^{\circ}$ C/min.

Identification of bioactive compounds:

Elucidation of GC mass spectrum was achieved using the database.

The spectra of the unknown components were compared with the known components stored. The molecular weights and structures of bioactive compounds in the extracts were established [73].

III. CONCLUSION

A. paniculata has been extensively used as traditional medicine in India, China and Southeast Asia. The diterpenoid lactones and flavonoids are the major phytochemical constituents present in the aerial parts of *A. paniculata*. Andrographolide is the most well known diterpenoids isolated from *A. Paniculata*. Phytochemical study revealed that diterpenoid lactones which are the major phytochemical constituents and flavonoids have been isolated from the aerial parts of this specie. Diterpenoid lactones including the bitter andrographolide are pure compounds derived from this plant with most promising biological activities. *Andrographis paniculata* is a traditional remedy for fever, cold and various infections. It also employs various immunological applications in cancer, immunomodulatory activity. The plant is also beneficial in treating cardiovascular disease and in preventing liver toxicity, thus improving functions of heart and liver. It also finds immense utility in abdominal problems, body aches, respiratory disorders, snake bites. *Andrographis paniculata* is reported to decrease fertility in animals and human beings.

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