JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Review on Antimicrobial Potential of *Andrographis paniculata* Plant

Correspondence Authors: Deepak Kumar Bakoriya, Syed Shahab Ahmad, Aashish Vishwakarma
Department of UTD, Biotechnology, Sri Satya Sai University of Technology & Medical Sciences, Sehore (M.P.)

Abstract:

Infectious disease (ID) is one of the top-most serious threats to human health globally, further aggravated by antimicrobial resistance and lack of novel immunization options. IDs have long been treated with andrographis paniculata (Burm. f.) Wall. Ex Nees and its metabolites. Derived from A. paniculata, androgenolide has the ability to control host immunity and decrease the virulence factors of invading microorganisms. A. paniculata therapy is safe and effective for treating acute respiratory tract infections, such as sinusitis and the common cold, according to controlled clinical investigations. Thus, A. paniculata primarily Andrographolide might be a great option for the development of antibacterial drugs. This study assessed the antibacterial therapeutic potency of A. paniculata and its metabolites critically in light of the plant's significance, medicinal qualities, and important role as antimicrobial agents.

Keywords: antimicrobial agent; clinical trial; ethnopharmacology; infectious disease; medicinal plant; metabolites; natural products

Introduction

Infectious disease (ID) is a serious global health problem that leads to a high mortality rate worldwide every year [Bloom *et al.*, 1990]. The world has recently witnessed a most formidable threat in recent human history, COVID-19, in the modern era of highest advancement of medical sciences. Infectious agents (i.e., invading microbes or pathogens) evolved a variety of strategies, such as modulating their cell surfaces, releasing proteins to inhibit or degrade host immune factors, or even mimicking host molecules to evade the host immunity and ensuring their own survival within a host [Paczosa *et al.*, 2016]. Pathogens, particularly bacteria, are multifaceted in their methods used to escape host immune detection. Due to having the mastery of these camouflaging and precise weaponry techniques by pathogens, developing new vaccines and innovative treatments become challenging, even in some cases almost impossible, for example, treating antibiotic-resistant strains. As a result, the death toll is increasing rapidly. For example, multidrug-resistant tuberculosis (MDR-TB) has recorded around 206,000 new cases in 2019, a 10% increase from 187,000 in 2018, and a total of 1.4 million people died from tuberculosis in 2019 [WHO, 2020]. Furthermore, antibiotics usage causes some common side effects, including

hypersensitivity and depletion of beneficial gut microorganism [Namita *et al.,* 2012 and Levy e*et al.,* 2004].

Acute upper respiratory tract infections (URTIs) are another significant cause of antibiotic resistance since physicians needlessly write antibiotic prescriptions [Thomas *et al.,* 2008 and Aabenhus*et al.,* 2017]. Even though the vast majority of URTIs are mild, self-diagnosed and self-treated, they are the most common reason for absenteeism from school or work [Poolsup *et al.,* 2004]. URTIs can be mainly caused by viruses, such as a rhinovirus, influenza virus, adenovirus, enterovirus, and respiratory syncytial virus. Bacteria like *S. pyogenes*, a Group *A. Streptococcus*, may cause roughly 15% of sudden on set pharyngitis presentations [Thomas *et al.,* 2020]. Viral pharyngitis is mainly treated based on the symptoms that appeared, where as bacterial pharyngitis can be treated with antibiotics. However, current evidence does not support the usefulness of antibiotics treatment in non-specific URTIs [Poolsup *et al.,* 2004 and Little *et al.,* 1999]. Therefore, research is urgently required to find alternatives to conventional medications for eradicating IDs. Natural products based therapy could be an excellent source of antimicrobial agents that would offer symptomatic relief since they have the high potentiality to inhibit the growth of microbes in the host-defence mechanism [Bizzell *et al.,* 2021], as well as they offer promising outcomes in the scientific investigations [DeCorte *et al.,* 2016- Fang *et al.,* 2017]. Additionally, it would reduce unnecessary antibiotic prescription; therefore, the chances of antibiotic-resistance would be reduced. Now a day, the philosophy of drug discovery has transformed into "*one drug, multi- target*" from "*one drug, one target*" [Fang *et al.,* 2017– Reddy *et al.,* 2013]. Plant-derived secondary metabolites hold the potential of multi-targeting properties as they need to undergo evolving defence mechanisms of the plant against predators like bacteria, fungi, virus, even insects and herbivores [DeCorte *et al.,* 2016, Fang *et al.,* 2017, Rodriguez *et al.,* 2017, Rodrigues *et al.,* 2016, Taylor *et al.,* 2019, Li *et al.,* 2009]. A majority of the world population relies on medicinal plants for first-line treatment due to the severe side effects of synthetic drugs [Ismail *et al.,* 2015]. More over, plants ability to cure diseases and the necessity of their study in sacred texts motivated people to use natural remedies and researchers to study their pharmacology [Hossain *et al.,* 2016, Urbi *et al.,* 2014]. Plant-based secondary metabolites commonly isolated are phenols, tannins, flavonoids, lignans, terpenes, and a wide range of alkaloids [Rodriguez *et al.,* 2017]. Since natural products are better models with ideal pharmacokinetics/ pharmacodynamics properties [Fang *et al.,* 2017], often feature biologically relevant molecular scaffolds and pharmacophore patterns that have evolved as preferred ligand-protein binding motif [Rodrigues *et al.,*] they gained tremendous importance for the development of polypharmacological drugs for IDs, cancers, and neurological disorders [Patridge *et al.,* 2016]. Furthermore, about 80% of drugs are either natural products or analogues mimicking them, and steadily increasing approval rate (after the 1990s, the average annual approval rate is 10.3) of natural product-derived drugs from the US Food and Drug Administration (FDA) have encouraged researchers and pharmaceutical industries to search the effective multitarget drugs for various ailments. Currently, a number of natural products, including morphine, quinine, reserpine, cocaine and ephedrine are now available in pure form as drug substances [DeCorte *et al.,* 2016]. Besides many pure compounds are identified by pharmaceutical scientists worldwide because of having advanced

technology that eases and fasten the characterization and structural elucidation of isolated metabolites. Capitalizing on these findings are crucial for medical advancement to overcome unavoidable circumstances happed by synthetic drugs. One attractive medicinal plant and its metabolites that have gained considerable and progressive interest for decades are *Andrographis paniculata* (Burm. f.). This annual plant belongs to the *Acanthaceae* family and is commonly known as"*King of the bitters*" or"*Kalmegh*". It is native to India and Sri Lanka and widely found in Southern and Southeastern Asia, including Bangladesh, China, Hong Kong, Indonesia, Malaysia, Myanmar, Philippines, and Thailand [Kumar *et al.*, 2012– Hossain *et al.*, 2014]. Usually the aerial parts, roots or leaves of *A. paniculata* are used separately. These plant parts are used traditionally as powder, infusion, or decoction form either alone or in combination with other medicinal plants for the treatment of leprosy, gonorrhoea, respiratory tract infections, scabies, boils, skin eruptions, chronic and seasonal fevers, griping, irregular bowel habits, loss of appetite, alopecia, general debility, diabetes, jaundice, dyspepsia, hemopathy, cough, oedema, liver complaints, dysentery, malaria, enteritis, helminthiasis, herpes, peptic ulcer, skin infections (topical use), and snake-bites (topical use) [Kumar *et al.*, 2012, Akbar *et al.*, 2011, Hossain *et al.*, 2014, Anju *et al.*, 2012].

1. *Andrographis paniculata*—"The King of Bitter"

1.1 Botanical Data of A. paniculata

A. Paniculata is an economically important herb of the *Andrographis* genus. It is an erect and branched and annual flowering herb. This herb grows well in hedgerows throughout the plane lands, hill slopes, waste ground, farms, moist habitat, seashores, and roadsides [Hossain *et al.*, 2016]. It can also be grown in the garden as well. For their excellent development, moist and shady places, forests, and wastelands are preferable. It is a salt-sensitive plant [Hossain *et al.*, 2016]. Therefore its growth is limited under stress conditions, particularly salinity stress that drastically affects plant growth and crop productivity [Hossain *et al.*, 2020].

This plant, under cultivation, can reach up to a height of 30 to 110 cm. Its stem is dark green, 30–110 cm in length, 2 to 6 mm in diameter, quadrangular with longitudinal furrows and wings at angles of the young parts, slightly enlarged at the nodes. The leaves are dark green, glabrous, 2–12 cm long and 1–3 cm broad, opposite, decussate, lanceolate, entire margin, and venation pinnate; the petiole is very short. The flowers are small; consist of five linear particle calyxes, narrow tube, and about 6 mm long white corolla with rose-purple spots on the petals. December to April is the flowering and fruiting period. The fruits are small 2-celled odourless erected capsules, 1–2 cm long, 2–5 mm wide, linear-oblong, acute at both ends and compressed. The leaf taste is intensely bitter, and seeds are numerous, sub-quadrate and yellowish-brown [Hossain *et al.*, 2016]. The detailed botanical description has been reviewed somewhere else. Different extracts and their secondary metabolites, particularly andrographolide, are one of the extensively studied natural products. The therapeutically active extracts are prepared, or metabolites isolated from aerial parts, leaves, roots, whole plants or callus [Arifullah *et al.*, 2013].

1.2 Recent Progress in Publication of A. paniculata

The measurement of scientific interest in a particular topic can be revealed from its trend of

publications. Due to having tremendous medicinal importance, the relentless interest in this plant and its versatile molecules has resulted in overwhelming publications over the past ten years. The publication numbers amounted to 3279 (as of 15 February 2021). In other words, we can say, daily, almost one publication, of which about 14% publications were about the antimicrobial study of either *A. paniculata* extracts or its metabolites, especially andrographolide. This bibliometric data was extracted from the Scopus database using the query of the term "*Andrographis paniculata*" OR "andrographolide" in titles, abstracts, and keywords. This number might be increased if data can be combined from different databases like PubMed, Web of Science and so on.

2. Invasive Microbes Used in the Antimicrobial Study of *A. paniculata.*

There are about 1400 known species of human pathogens. Although this seems like a large number, they are less than 1% of the total number of microbial species on the planet earth [Editorial *et al.*, 2011]. Even though this is less than 1% of total microorganisms, a harmless microbe can sometimes be harmful under a specific condition like an immunocompromised patient. Exploring proper medications for these spontaneous behavioural changing microbes is a continuous effort of the scientific community. *A. paniculata* extracts and their bioactive molecules were investigated against a wide variety of pathogens, including several antibiotic-resistant species, for example, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella* spp., *Salmonella* spp., *Candida* spp., *Streptococcus pneumoniae*. We have identified a total of 59 invasive microbes that have been used to investigate the antimicrobial efficacy of *A. paniculata* extracts and/or their isolated pure compounds. The categorized microbes included 33 bacterial, four viral, 12 fungal and 10 parasite species. The details antimicrobial effectiveness of different extracts has been discussed in the later section. The list of tested microbes, their types, mode of transmission, and a disease caused, and infecting organisms are presented in the Supplementary.

3. Antimicrobial Secondary Metabolites of *A. paniculata.*

A. Paniculata contains therapeutically active secondary metabolites that include lactones, diterpenes, flavonoids, quinic acid, xanthones, noriridoids, and other compounds. In our previous study [Hossain *et al.*, 2014], we reported more than 55 ent-labdane diterpenoids, 30 flavonoids, eight quinic acid derivatives, four xanthones, and five rare noriridoids in *A. paniculata*; however, in this study, our extensive review revealed at least 142 secondary metabolites that already isolated from *A. paniculata* using different plant parts and fractionations of organic solvents (i.e., acetone, butanol, chloroform, ethanol, methanol, and hexane) or water and chromatographic analysis like thin layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), liquid chromatography, micellar electrokinetic capillary chromatography (MECC), high-speed counter-current chromatography (HSCCC), high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), Silica Gel Chromatography (SGC), and flow injection spectrophotometry (FIS) [Aminah *et al.*, 2021]. Among the chromatographic methods, HPLC and TLC are more commonly used. This might be due to easy accessibility and accuracy. The Andrographolide (1), neoandrographolide (2) and isoandrographolide (3) are the most abundant lead bioactive compounds that can be isolated from any part of *A. paniculata* for example, aerial apart, leaves, whole plant, and even roots. However, these present in high amounts in

leaves. The yield reached the maximum level while the plant materials collected between 110–130 days of cultivation [Dua *etn al.,* 2004]. Compounds 4 and 5 are the next most abundant, followed by 6, 7, 14, 15. The least abundant are compounds 17–20, which are only available in roots [ChemFinder *et al.,* 2020].

4. Antimicrobial Activity

4.1 *Antibacterial Effects.*

For centuries, the traditional use of *A. paniculata* in treating several IDs caused by bacteria encouraged researchers to study its anti-bacterial properties and how it fights against invasive microbes. Our review found about 33 different types of bacteria that were inhibited by different types of extracts.

***A. paniculata* Extracts as Antibacterial Agents.**

Researchers used different types of extracts of *A. paniculata* to explore their poten- tiality against numerous invasive microbes, including antibiotic-resistant strains, such as methicillin-resistant *S.aureus* (MRSA), vancomycin-resistant *E. faecalis* (VRE), carbapenem- resistant *Actinobacillus baumannii*, β-Lactamase-negative, ampicillin-resistant (BLNAR) *Haemophilus influenzae*, *P. aeruginosa*. A summary of the antibacterial activity of the different types of *A. paniculata* extracts is shown in. The aqueous extract of *A. paniculata* showed significant antibacterial activity, which was further linked to the presence of andrographolides and arabinogalactan proteins [Fabricant *et al., 2001*]. The role of andrographolide and neoandrographolide in treating bacillary dysentery caused by *Shigella S*p. was reported in several studies as well [Farnsworth *et al.,* 1985]. In an experiment, *A. panic- ulata* was used to treat 1,611 cases of bacterial dysentery and 955 cases of diarrhoea [Parveen *et al.,* 2019]. The efficacy of *A. paniculata* extracts for dysentery was proved from laboratory stool test with 82.5% and 91.3% [Zaidan *et al.,* 2005].

Solated Compound as Antibacterial Agent: Mechanisms of Action a substantial number of evaluations proved the efficacy of different extracts of *A. paniculata* against many severe pathogenic microbes. Besides this, 13 pure secondary metabolites of *A. paniculata* were also reported to have significant antibacterial effects. These are compounds 1, 3–5, and 7–16. These compounds have been used to evaluate antibacterial potency against a wide range of bacteria. Overall, Gram-positive bacteria were more susceptible to compound 1 than Gram-negative bacteria due to the presence of the outer membrane and the polarity nature of the compound [Zou *et al.,* 2010]. Depending on the bacterial species, the mode of actions of compound 1 differs by a large extent. *S. aureus* was largely susceptible (MIC is 0.1 mg/mL) to compound 1 among the tested microbes. Healthcare-associated infections are prevalently (10.7%) caused by *S. aureus*, a major bacterial human pathogen that can form biofilm [Taylor *et al.,* 2020]. It causes a wide variety of clinical manifestations, including pneumonia, mastitis, osteomyelitis, endocarditis, skin infections, abscesses, food poisoning, toxic shock syndrome, and sepsis, and treatment remains challenging due to the emergence of multi-drug resistant strains such as MRSA [Wang, *et al.,* 2010]. Compound 1 act on bacteria themselves as well as plays an important role in the regu lation of host immunity by regulating macrophage phenotypic polarization and Antigen specific antibody production [Zhang *et al.,* 2017]. When *S. aureus* infected the lungs, it significantly promotes NF-κB p65 phosphorylation and increases TNF-α and IL-6 production. Compound 1 can downregulate them

sufficiently but retain the immune cells at the level that can kill bacteria without serious immune damage. In comparison to penicillin, compound 1 showed better management of bacterial infection and persistent host immunity. Since compound 1 works on immune regulation, there are fewer chances of drug resistance. Therefore, compound 1 would further reduce the problems associated with antibiotic resistance, one of the current severe health crises. In another study, Banerjee et al. [Zou *et al.,* 2010] reported that compound 1 could strongly inhibit DNA synthesis (approximately 31%) and consequently RNA (about 26% inhibition) and protein (around 36% inhibition) synthesis in *S. aureus*. This result was similar to that of antibiotic ciprofloxacin (25% incorporation). However, cell wall biosynthesis was not hampered [Hentzer *et al.,* 2003]. Secondary metabolites showing antimicrobial can work on a specific target site. Compound 1 can affect the quorum sensing system (QSS), a communication system between bacteria; thereby, it is an effective antibacterial target. This system enhances the production of biofilm by bacteria, such as *P. aeruginosa*. The antibacterial drug effect in this system resulting in regulates the production of bacterial efflux pumps and virulence factors [Sakuragi *et al.,* 2007]. Compound 1 effects on the QSS, especially Las and Rhl systems, resulting in reduced production of compositions of extracellular polymeric substance (EPS), such as carbohydrate, nucleotide, and amino acid polymers, as well as inhibiting virulence factors [Ma *et al.,* 2012, Wu *et al.,* 2008]. In addition, compound 1 could restore the antibiotic sensitivity in *P. aeruginosa* by reducing expression of *mexAB-oprM* efflux pump and inhibit bacterial adhesion, such as *E. coli* and *S. epidermidis,* to the epithelial cells of lungs; therefore, significantly reduced respiratory colonization and level of *fimA*, *papC*, and *TSH* [Bjarnsholt *et al.,* 2013].

4.2 Antiviral Effects

Since the last three decades, researchers have extensively studied the antiviral properties of *A. paniculata*. Although antiviral activity against a limited number of viruses viz. dengue virus serotype 1 [DENV-1] [Sule *et al.,* 2012], herpes simplex virus type 1 [HSV-1], influenza A virus, HIV [Chen *et al.,* 2009], hepatitis B and Hepatitis C [Singha *et al.,* 2003] has been reported, their findings are very encouraging and significant considering the role of these viruses on human morbidity and mortality worldwide. It is noteworthy that the formation of syncytia in co-culture of HIV-1 infected MOLT cell lines was significantly inhibited by the methanol extracts of *A. paniculata* [Yao *et al.,* 1992]. The aqueous bark extracts of *A. paniculata* was investigated for HIV-1 protease inhibition activity, and this result supports an earlier report by Yao, et al. [St-Pierre *et al.,* 2010]. They reported positive results, but the extracts were less effective (29.6% and 26.3% inhibition at 250 µg/mL and 25 µg/ml, respectively) against HIV-1 protease. Methanol extracts of *A. paniculata* showed antiviral effects against dengue virus (DENV) serotype-1 in vitro assay. After treating with the extracts, the viability of DENV-1 infected Vero E6 cells was 1134.65% with maximum non-toxic dose (0.050 mg/ml), and the percentage of inhibition was 75%. Panraksa, et al. [Panraksa *et al.,* 2017] evaluated andrographolide's antiviral activity against DENV serotype-2 in HepG2 and HeLa cell lines and DENV serotype-4 in one HepG2 cell line. They found a significant reduction of cellular infection and virus output levels in both cell lines, HepG2 (EC_{50} = 21.304 µM) and HeLa (EC_{50} = 22.739 µM) for DENV 2. The antiviral activity of andrographolide

was confined to a post-infection stage [Panraksa *et al.*, 2017]. Andrographolide was more potent to inhibit DENV compared to the chikungunya virus (CHIKV). The CHICKV EC_{50} (77 µm) was about 3.5 fold higher than the DENV, comparable to two different DENV serotypes. In addition, andrographolide affected CHIKV replication. Both cases in HepG2 and HeLa cell lines did not show any toxicity sign after treating with andrographolide at a maximum concentration of 100 µm for 24 h [Panraksa *et al.*, 2017]. Reddy, et al. [Chang *et al.*, 2017] have investigated several pure metabolites of *A. paniculata*, including bis-andrographolide ether, andrographolide, 14-deoxy-11, 12-didehydroandrographolide, andrograpanin, 14-deoxyandrographolide, 5-hydroxy-7,8-dimethoxyflavanone and 5-Hydroxy-7,8-dimethoxy flavone against HIV. Among these compounds, only andro- grapholide and 14-deoxy-11, 12-didehydroandrographolide have demonstrated significant anti-HIV properties with (EC_{50} = 49.0 mg/mL) and (EC_{50} = 56.8 mg/mL).

4.3 Antifungal Effects

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* (<60% inhibition) and *Penicillium* sp. (<40% inhibition) at 3% (*v*/*v*) concentration [Rehman *et al.*, 1999]. The hexane and methanol root extracts were evaluated for their antifungal activity against. *A. Niger* and *Penicillium chrysogenum*. Two concentrations (100 gm/ml and 200 mg/ml) of each extract were studied, which involved determining the inhibition zone diameter for a specific time. It was found that both extracts exhibited significant inhibition, 13 mm and 12 mm at 200 mg/ml concentration against *A. Niger* and *Penicillium chrysogenum*, respectively. However, these inhibitions were less than the standard fluconazole, 17 mm and 16 mm at 100 µg/ml concentration, respectively [Sule *et al.*, 2011].

Sule, et al. [Misra *et al.*, 1992] first reported on the isolation of antifungal bioactive compounds from dichloromethane (DCM) and methanol extracts of *A. paniculata* whole plant. All the isolated bioactive, 3-O-β-D-glucosyl-14-deoxyandrographolide, 14-deoxyandrographolide and 14- deoxy-11, 12-didehydroandrographolide, showed significant antifungal activity against *Microsporum canis*, *A. Niger*, and *C. albicans*. MIC values for all antifungal compounds ranged from 50 to 150 µg/ml, and minimum fungicidal concentration (MFC) values ranged from 50 to 200 µg/mL. Among the isolated antifungal substances, 14-deoxyandrographolide exerted the lowest MIC (50 µg/ml) and MFC (50 µg/mL) against *M. canis*, which indicates the most potent antifungal activity. It is noted that no anti-fungal activity was reported against *T. mentagrophytes* and *T. rubrum* at 250 µg/ml. Even though different extracts of *A. paniculata* reported to have potential anti-fungal activity, the mode of actions yet under report.

4.4 Anti-Parasitic Effects

A.Paniculata and its bioactive compounds have been tested and found extensive anti-parasitic activity against various parasites, such as *Ascaris lumbricoidis*, *Plasmodium falciparum*, *P. berghei*, *Trypanosoma cruzi*, etc. The extract and fractions reduced parasitaemia level in *Mastomys natalensis* while used in a dose-dependent manner [Dua *et al.*, 1999]. Misra, et al. [Dua *et al.*, 1999] has also studied the anti-malarial

activity of the four diterpenes-andrographolide, neoan- drographolide, and deoxyandrographolide and andrographolide isolated from *A. paniculata* and revealed that neoandrographolide (2.5 mg/kg BW) exhibit the highest activity when administered by gastric lavage than other diterpenes.

The fractions of *A. paniculata* also possessed significant anti-malarial activity [Chen *et al.*, 2014]. The methanol extract was found to have complete inhibition at a concentration of 2.5 mg/ml by 48 h. Chloroform extract also achieved the same effect by 24 h at only 0.05 mg/ml concentration [Mishra *et al.*, 2011]. The isolated andrographolide also exhibited substantial anti-malarial activity against the MRC-pf-303 strain of *P. falciparum* and particularly inhibited the parasites at the ring stage [Sachdeva *et al.*, 2011]. Consequently, the methanol fractions soluble in chloroform were evaluated as significant inhibition of parasitaemia (74%) at the concentration of 1 mg/ml. additionally, andrographolide showed the highest (53.9%) inhibition of parasitaemia level [Kaleysa *et al.*, 1975].

It has been found that the alcohol rhizome extract possessed significant in vitro activity against *A. lumbricoides* [Dutta *et al.*, 2004]. Two reviews were also reported *A. paniculata* rhizome exhibited extensive activity against *A. lumbricoides*. Dutta and Sukul [Spasov *et al.*, 2004] have stud- ied in vitro anti-filarial activity of leaves decoctions of *A. paniculata* against *Dipetalonema reconditum* and found the decoctions kill microfilaria in 40 min. In vivo study revealed that more than 85% of microfilaria in the blood reduced after three subcutaneous injections of the extract into infected dogs at 0.06 ml/kg BW.

5. Controlled Clinical Trials of *A. paniculata* Treatment: a Systematic Evaluation

A. paniculata and its bioactive compounds have been used to treat patients with uncomplicated upper respiratory tract infections (URTIs), including common cold, rhinitis, nasopharyngitis, pharyngitis, and pharyngotonsillitis. The surrounding bacteria and viruses are the usual source of infection for the URTIs. In the treatment of URTIs, pills (made by whole powdered plant and water) and tablets (water extract of the plant) have been used, and the cure rates are 88% and 61%, respectively. The therapeutic effectiveness differed mainly due to the preparation method and duration of treatment [Spasov *et al.*, 2004]. Several randomized, double-blind, placebo-controlled trials proved the efficacy of *A. paniculata* standardized extracts and/or andrographolide. Some other bioactive compounds treat various infectious diseases associated with cold symptoms and infections caused by virus- like influenza [Gabrielian *et al.*, 2002– Mkrtchyan *et al.*, 2005]. Our systemic investigation revealed a total of 41 individual clinical trials after removing the duplicates from three different databases. After critical evaluation, 23 individual trials (n = 2760) were finally selected for this study which was distributed in 13 different countries. These included 11 controlled clinical trials of the treatment of uncomplicated URTIs (eight studies) [Mkrtchyan *et al.*, 2005– Caceres *et al.*, 1997, Mkrtchyan *et al.*, 2005], viral infections (three studies) influenza (two studies) and HIV (1 studies), and one trial focused on the immunity enhancement [Caceres *et al.*, 1999] that prevent the occurrence of the common cold in a rural school-going healthy student.

6. Evaluation of A. paniculata Efficacy against Infections.

Eleven randomized, and 1 non-randomized clinical trials (n = 2008) were met inclusion criteria. The compliance rate of this study was 95.97%. Of these, nine were double-blind, of which eight were

placebo-controlled. Others were either simple randomized control or randomized parallel-group or randomized controlled open-label. Melchior, et al. [Caceres *et al.*, 1997] and Kulichenko, et al. included one pilot trial and one main trial in one article. Therefore, we split their findings and presented them separately in. A comparative, randomized, double-blind study had been done on 152 Thai patients with pharyngotonsillitis to investigate the efficacy of *A. paniculata* extracts using either paracetamol (3.9 g/day) or encapsulated *A. paniculata* dried leaves for low dose group (LDG) (3 g/day) or high dose group (HDG) (6 g/day) for seven days. There was no significant difference in efficacy of relieving fever (p = 0.16) and sore throat (p = 0.49) among three groups on day 7. Most of Paracetamol and HDG stopped taking medication on day three because their symptoms had disappeared. However, LDG patients discontinued taking medications due to persistent adverse side effects or worsening symptoms. The incident of mild or self-limiting side effects (i.e., nausea, vomiting, abdominal discomfort, dizziness, drowsiness, and malaise) was not statistically significant among the three groups (p = 0.8), and patients were almost equally satisfied with paracetamol and a high dose of *A. paniculata* treatment. The findings indicated that daily 6 g of *A. paniculata* dried leaves extracts that contain at least 6% of andrographolide can be replaced by paracetamol treatment as a standard treatment for the patients with symptoms of pharyngotonsillitis. In a placebo-controlled study conducted by Caceres, Hancke, Burgos and Wikman [Caceres *et al.*, 1999], Kan Jang tablets, a standardized *A. paniculata* extract, had been administrated to 107 healthy students in a rural school at a dose of two tablets (200 mg) per day for three months to evaluate the efficacy of Kan Jang to prevent common colds. The common colds were successfully prevented; 2.1-fold higher prevention in the Kan Jang group compared to the placebo group.

7. Evaluation of Safety of A. paniculata Treatment

The incidents of the adverse effect of treatment with the natural product are sporadic. To our best knowledge, there was no such severe adverse event reported for *A. paniculata* treatment to the drug safety body. Our critical observations revealed that *A. paniculata* treatment showed a mild adverse event in some cases. Out of 14 reports, six reports stated (n = 579) that they did not observe any adverse effects (or report not pro-vided). These studies cover only 1991–2010. Our systematic investigation did not reveal any clinical studies conducted from 2010 to now. However, several clinical studies conducted using *A. paniculata* extracts or pure compounds on the healthy volunteer to check semen quality [Chen *et al.*, 2012], pharmacokinetics and tolerance ability [Burgos *et al.*, 2009], ulcerative colitis [Burgos], arthritis, fatigue, Familial Mediterranean Fever [Ang *et al.*, 2021], hyper triglyceridemia and type 2 diabetes mellitus. Saxena, et al. (n = 223) reported mild adverse effect (2.73%): vomiting (1 case), epistaxis (1 case), Urticaria (1 case) and diarrhoea (3 case). Except for vomiting (patient in the treatment group) and urticaria, all other effects stopped spontaneously without any medication. Minimal and self-limiting side effects (n = 152) (i.e., nausea, vomiting, abdomi- nal discomfort, dizziness, drowsiness, and malaise) were found about 20% in treatment (LDG & HDG) and paracetamol groups (9–11 cases) [Ang *et al.*, 2021]. These are pervasive mild effects that usually recovered shortly without any medications. Three cases out of 200 subjects were also reported mild side effects: increase in nasal discharge and epigastric pain (1), nose blocked (1), and severe headache (1) for the treatment of *A. paniculata* fixed combination Kan Jang in

URTIs and sinusitis [Melchior *et al.,* 2000]. For viral infections, the treatment group (Kan Jang) experienced dry cough, rhinitis, and pain in the throat (22 cases out of 540). Control group received antiviral agent amantadine which showed significantly ($p < 0.01$) higher (67.8% cases) influenza complications compared to treatment group (30.1% cases). For main trials, influenza complications were found in 31.43% of *A. paniculata* treated patients and 70.97% of standard medicine (amantadine) treated patients ($p < 0.01$). One HIV positive experience an anaphylactic reaction in phase-I clinical trials. All but one (92%) reported at least one adverse event during the study. About 75% reported an adverse event by the healthy volunteer. Treatment of uncomplicated acute URTI patient experienced unpleasant sensations in the chest and intensified headache (1 case out of 180) [Caceres *et al.,* 1997], and for common cold and sinusitis, two patients out of 50 experienced urticaria.

8. Methodology

The information related to this article was systematically collected from worldwide accepted scientific databases including PubMed (http://www.ncbi.nlm.nih.gov/pubmed (accessed on 27 March 2021)), ScienceDirect (http://www.sciencedirect.com/ (accessed on 27 March 2021)), Scopus (http://www.scopus.com/ (accessed on 27 March 2021)), Web of Science (https://apps.webofknowledge.com/ (accessed on 27 March 2021)), Springer Nature (http://link.springer.com/ (accessed on 27 March 2021)), Wiley Online Library (http://onlinelibrary.wiley.com/ (accessed on 27 March 2021)), and advanced search in Google Scholar (http://scholar.google.com.my/ (accessed on 27 March 2021)), as well as recognized books, abstract, and thesis/dissertation using the keywords "*Andrographis pan- iculata*", "antimicrobial", "anti-bacterial" "antiviral" "antifungal". In the aforementioned databases, other relevant papers from the list of references of all available articles were searched. For searching the controlled clinical trials, the following keywords: "antipara- sitic", "clinical trials", "controlled clinical trials", and "randomized clinical trials" were used in PubMed, Scopus, and web of science. Controlled clinical trials were systematically screened and selected for further evaluation of their outcomes in this study. The studies were selected for this review (clinical section) if they were controlled clinical trials dealing with *A. paniculata* to treat infections incredibly uncomplicated upper respiratory tract infec- tion. Used of *A. paniculata* for other health conditions and healthy volunteers were also selected in this study. English language only restriction was imposed.

Conclusions and Recommendations

Human invasive microbes are growing resistant to the available antibiotics for many reasons. As *A. paniculata* works on immune regulation, there are fewer chances of drug- resistance occurrence. Even though *A. paniculata* has a potential antimicrobial activity, the detailed study regarding the mode of actions, effects concerning the available antimicrobial agents and specific administration route as well as schedule remain to be explored. The active constituents of *A. paniculata* could be a potential source of antimicrobial agent, and exploring the therapeutic potentiality of them based on the clinical implications is worthwhile. We have explored substantial antimicrobial agents in *A. paniculata*; however, very little is still known about their molecular mechanisms in response to microbes or host-infected

cells. A majority of metabolites are not investigated to identify the molecular target to understand the drug-target-disease network. In addition, our checklist of secondary metabolites of *A. paniculata* can be used for further exploration of their effectiveness because about 44% of metabolites are yet to be evaluated. In some cases, pure compounds from medicinal plants, such as aristolochic acids, possess severe side effects like kidney failure and urinary tract cancers [Ang *et al.*, 2021]. Therefore, moving forward, the further requires us to take a more comprehensive approach to harness the true potential of *A. paniculata* for IDs fully. Different disease conditions have diverse responses to the drugs; therefore, to obtain a complete picture of the drug-target-disease network, elucidating each secondary metabolite's mechanism is crucial. Andrographolide has the potential to target multiple sites since it has shown significant efficacy against different disease conditions. Therefore, this natural product could be considered as a potential candidate for polypharmacology. We would obtain the full advantage of using andrographolide for therapeutic Supplementary.

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