Review on Antibacterial Activity of *Andrographis Paniculata* on Dental Caries

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Abstract

Dental decay or dental caries, is a condition where dental bacteria produce acids that break down tooth enamel. This may cause the teeth to develop cavities or holes in them. One important bacteria, *Streptococcus Mutans*, is typically responsible for this caries. Dental caries can be caused by germs in the mouth, fluoride deficiency, regular consumption of sugary food and drink, and poor oral hygiene. In cases where medicines such as amoxicillin or, clavulanate are administered for infections that result in adverse effects such as Constipation, vomiting, nausea, and so on, to combat these side effects, the herbal plant *Andrographis paniculata* is discussed in this paper as a potential treatment for dental caries. *Andrographis paniculata*, sometimes referred to as green Chiretta, is a South Asian native and member of the Acanthaceae family of medicinal plants. Majorly utilized in traditional medicine, especially in Thai, Traditional Chinese, and Ayurvedic medicine. With its anti-inflammatory, antiviral, and antioxidant qualities, andrographolide is the primary active ingredient in *Andrographis paniculata*.

Key Words- *Andrographis Paniculata*, Ayurveda, Acanthaceae, Caries, Amoxicillin.

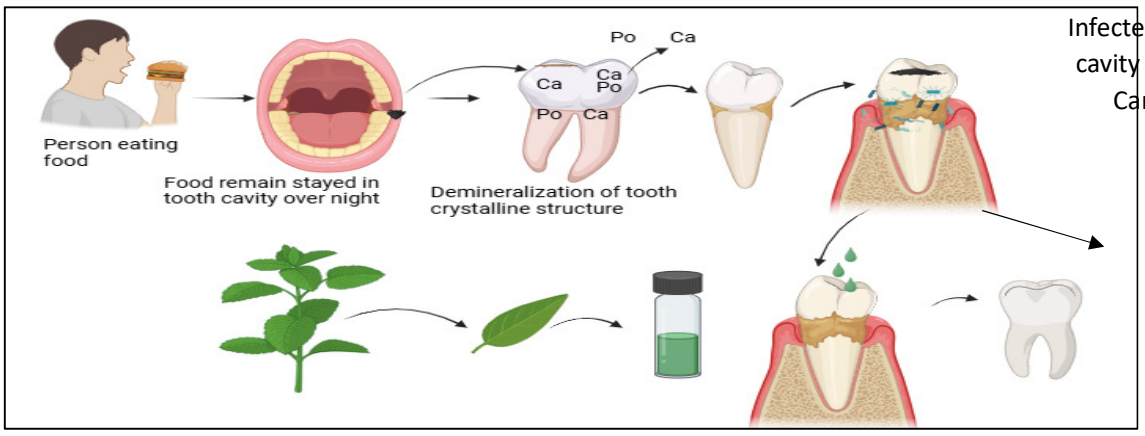


Figure: The Graphical Abstract

Introduction

Tooth decay is among the most significant and harmful illnesses on oral health in today's world, a chronic condition that exclusively affects humans. Tooth decay and oral pain are commonly associated with dental caries, which is a common and preventable disorder. It is a serious oral disease that makes it challenging for individuals of all ages to achieve and preserve optimal oral health. Bacterial fermentation of carbohydrates in food induces dental caries, which are localized acidic byproducts that cause the weakening of the hard tissues in the teeth. A chronic illness that advances slowly affects the majority of those who have it. The balance between plaque, and tooth minerals is out of balance on an ecological level [1]. In US youngsters, the frequency of untreated caries is still high (41.4 to 45.7% among 1 to 9 year old). 5M. of children wasted school hours annually due to dental health issues being predicted [2]. It is determined that oral health issues cause more than 51M. lost school hours annually [3]. Due to microorganisms illness attacks the tooth's rigid tissue directly and, a cavity is created due to an intricate procedure that occurs over time between bacteria that generate acid and fermentable carbohydrates [4]. Dental caries is a non-transmissible disease worldwide public health problem. In the 2015 literature research were held on the theme "Global Burden of Disease", it was also ranked as the most common ailment, ranking first for Perennial tooth decay, which impacts 2.3B. human beings, and 12th for caries of deciduous teeth, which affects 560M. children. The issue is required more to treat, accounting for 5 to10% of healthcare spending in affluent nations and frequently leading to hospital admissions [5,6,7].

In 1634 during the literature, the Latin term "Caries" was first introduced, meaning decay. Cariogenic bacteria that adhere to teeth and break down saccharides to produce acid, which progressively removes minerals from tooth structure, cause dental caries, a prevalent and chronic infectious disease. Carious lesions have a greater proportion and incidence of certain bacterial species that have been identified from advanced caries [8]. *Amoxicillin/clavulanic acid*, a combined *penicillin* antibiotic being given in microbial illnesses, is an allopathic treatment for dental caries, it works by inhibiting the growth of microbes but doesn't work for communicable diseases like the flu or the common cold. Any overused antibiotic may lose its ability to prevent further infections. There may be vomiting, nausea, or diarrhoea. To get past these challenges It is recommended to use effective herbal remedies. Many plants and species have been used, including *Andrographis Paniculata* (*Kalmegh*), which is significant plant's for treating dental caries. However, more research is needed to determine which specific

plant species can effectively treat dental caries without causing negative side effects [9]. Numerous clinical symptoms and indicators, including spontaneous pain, discomfort at night, transferred pain, temperature sensitivity, and percussion pain, can manifest as tooth caries progresses. These symptoms, to some degree, represent the pulp's state [10]. This review subjected to give brief information on *Andrographis paniculata* as a means of overcoming adverse effects, such as nausea and diarrhea, that are connected with the use of *Amoxicillin* and *Clavulanic acid*. There is a tremendous chance for more research here because the antibacterial activity of *Andrographis Paniculata* against dental caries is unknown.

Pathophysiology of Dental Caries

De- Mineralization and Re- Mineralization

The mechanism that causes dental caries is the demineralization of the tooth's crystalline mineral structure by organic acids generated by biofilm bacteria from the metabolism of fermentable carbohydrates, mostly sugars, in the diet. This process usually begins at its enamel surface and under it (the earliest demineralization occurs subsurface). The primary byproduct of sugar metabolism [11], lactic acid is thought to be a key component in tooth cavities. When the pH drops to the point where undersaturation at the biofilm–enamel interface takes place, acids build up in the fluid phase of the biofilm and significantly demineralize the tooth's surface layer. As minerals are lost, the enamel's surface softens, the gaps between its crystals widen, and it becomes more porous. Demineralization of the mineral under the surface, or subsurface demineralization, results from the acids' ability to penetrate deeper into the tooth. Fluoride can also stop the demineralization of the surface layer [12]. *Streptococcus mutans* (*S. Mutans*) and several Lactobacillus species which metabolize dietary sugar to make lactic acid—are the predominant pathogenic bacteria in deposits. According to epidemiology, the most common bacteria is *S. mutans*, and its prevalence affects caries risk more than sugar intake and inadequate dental care [13]. Dental decay is undoubtedly a plaque-dependent process that starts with a pellicle, an acellular proteinaceous film produced by saliva that covers all oral mineralized surfaces. This is relevant to the concept of mineralized tissue infection. Microorganisms adhere to the pellicle and create a biofilm, which grows in size and develops into dental plaque with a deep anaerobic layer near to the enamel surface and a superficial aerobic zone [14].

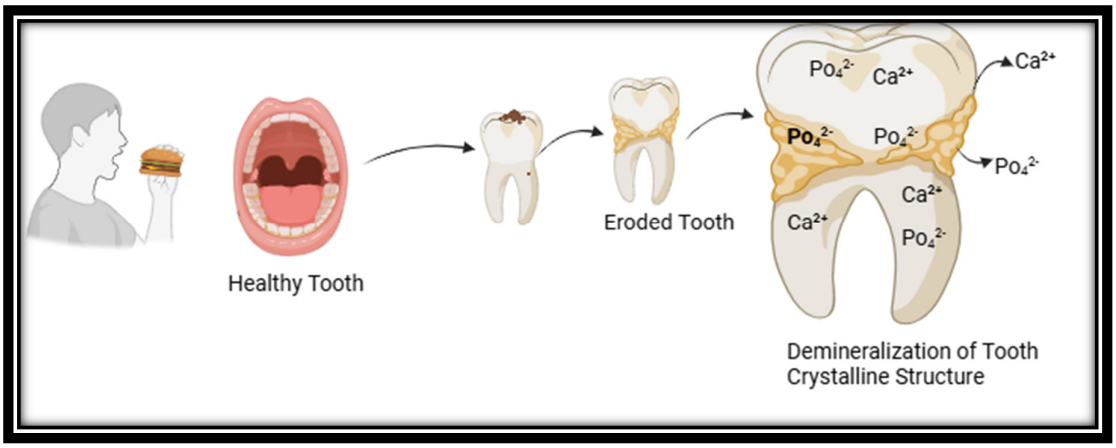


Figure 1: The graphical form of the pathophysiology of Dental Caries

Prevalence of Dental Caries

According to certain research, the prevalence rate among 13–19-year-olds in poor nations like India is 36.7%, whereas in Saudi Arabia, the prevalence among 6–8-year-olds is as high as 83% [15,16]. Globally, 486M. children have primary tooth decay and around 2.4B. adults have permanent dental decay [17]. India's dentist population ratios range from 1:20,000 to more, depending on the state [18]. Only 10% of the 30,570 dentists that are employed in India each year work with patients in rural regions [19]. Although most studies have focused on dental caries in children under the age of 18, there was an age-specific prevalence of 62% in patients over the age of 18 and 52% in those between the ages of 3 and 18 ($P < 0.0001$). The mean prevalence was 49% in the 5–12 age group and gradually rose from 60% to 84% in the 15–74 age group [20]. According to a 2019 UN prediction, one in six people will be 65 or older by 2039, doubling the number of individuals in the elderly demographic [21]. A countrywide survey carried out in the United States between 2001 and 2012 found that, even though dental caries have been declining, around 37% of children aged 2 to 8 and 60% of teenagers aged 12 to 19 had dental caries in their primary teeth [22].

About *Andrographis Paniculata*

Many people consider *Andrographis paniculata*, also called *Kalmegha* in Sanskrit, to be a helpful plant because of its many medical uses. Found in abundance across India belongs to the *Acanthaceae* family and is referred to as the "Bitter of King." [23] Herpes, fever, gastrointestinal issues, upper pneumonia, and other infectious and chronic illnesses are only a few of the conditions for which *Andrographis paniculata* has a substantial therapeutic promise [24]. The primary location of the presence is Bengal [25]. In Bengal, it is referred to

as a "*Kalmegha*" and the "king of bitter"; another name for it is *Bhuineem*. Ent-labdane is the main bioactive component of diterpenoid lactone. In South Asia, the Kalmegh is the primary traditional drug. In *England*, it is utilized as a *Quinine* replacement [26]. Mostly found in the leaves and roots, andrographolide is used for a variety of medicinal purposes in the *Ayurvedic* and *Unani* systems. It is primarily utilized for newborns. The Indian Pharmacopeia (IP) address states that it is a powerful combination that is utilized in over 26 Ayurvedic formulations [27]. *Andrographis Paniculata* is used for its Immunostimulants [28], cardiovascular [29], anticancer [30], anti-microbial [31,32], Free radical scavengers [33], antiviral [34], and Hypo-glycemia [35] properties. Below Table 1 and Table 2 depict the taxonomical classification and morphology of the *Andrographis Paniculata* plant.

Table.1 The Taxonomy Classification of *Andrographis Paniculata*

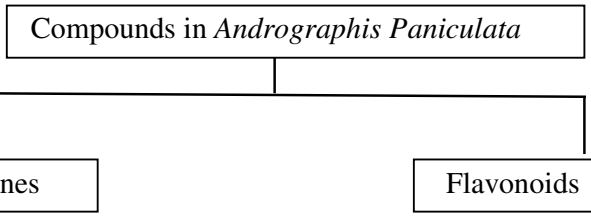
S.no	Scientific classification	Name
1.	Domain	Eukaryote [36]
2.	Kingdom	Plantae
3.	Sub- kingdom	Tracheophytes
4.	Divisions	Angiosperms
5.	Super divisions	Spermatophyta
6.	Class	Dicotyledons [37]
7.	Subclass	Gamopalatae
8.	Series	Bicarpellate
9.	Order	Lamiales
10.	Family	Acanthaceae
11.	Sub-family	Acanthoideae
12.	Tribe	Justiciae
13.	Sub-tribe	Andrographideae
14.	Genus	*Andrographis*
15.	Species	*Paniculata* [38]

Table. 2 The Morphology of Plants		
S. no	Traits	Characteristics
1.	Plant Height	30-110 cm [39]
2.	Stem Length Diameter Shape	Dark Green [40] 30-100 cm 2-6 mm Quadrangular, longitudinal furrow, wings on the angles, and slightly swollen nodes.
3.	Leaves Length Width Arrangement Shape	Glabrous 2-12 cm 1-3 cm Lanceolate Pinnate, acute apex, entire margin
4.	Flower Size	The petals are white with purple spots [41]. Axillary and terminal racemes or are small, laxly spreading racemes or panicles.
5.	Seed Size Colour Shape	Linear oblong capsules with acute ends. 1.9 cm length, 0.3 cm width. Yellowish brown Sub-quadrate
6.	Flowering and fruiting	December to April [42]

Phytochemistry of *Andrographis Paniculata*

Andrographolide, the primary active ingredient in *Andrographis paniculata*, is present throughout the entire plant. The properties of andrographolide include being an Ent-labdane diterpene lactone compound that is bitter and colourless. Andrograpanin, Andrographiside, andrographolide, bis-andrographolide, iso-andrographolide, neo-andrographolide, 14-deoxy-11oxoandrographolide, 14-deoxy-11, 12-di-de-hydro-andro-grapholide, and 14-deoxy

andrographolide are a few of the chemical components of *Andrographis paniculata* [43,44]. The most noticeable component with the highest amount from the leaves is andrographolide (diterpenoids), which is also obtained from the aerial portions or the entire plant. It readily separates into a crystalline crystal from the crude extracts [45,46]. From ethanol and methanol extract, more than 20 diterpenes, 10 flavonoids, xanthones, noriridoides, and other substances have been identified. The list of diterpenes and flavonoids is shown in the graphic below [47,48] i.e.



- Andrographolide
- 14-deoxy andrographolide
- Neo-andrographolide
- 14-deoxy-11, 12 di-de-hydro-andrographolide
- 14-deoxy-14, 15 di-de-hydro-Andrographolide
- Iso-andrographolide
- Andrograpanin
- 14- acetyl-andrographolide

-7-O- Methyl-wogonin
-Apigenin
- Onysilin
-3,4, Di-Caffe-Oylquinic

The Medicinal Plants and Extracts Used to Treat Dental Caries

The list of medicinal plants that are used in dentistry to treat specific microorganisms is shown in Table No. 3

Table No.3 The list of Medicinal plants used in dental caries.

S. no	Medicinal Plants	Microorganisms
1.	Glycyrrhiza glabra [49], Cinnamomum burmannii [50], Cocos nucifera [51], Liquorice Root [52], Sterculia lychnophora Hance [53], Pongamia Pinnata [54], Acacia Catechu [55], Bauhinia Forficata [56], Bauhinia Forficata [57].	*Streptococcus mutans*
2.	Erythrina variegate, Helichrysum italicum, Thymus vulgaris, Rhus corriaria, Rhus corriaria, Melissa officinalis, Magnolia grandiflora, Melissa officinalis,	*Streptococcus Sanguis*

	Harungana madagascariensis.	
3.	Erythrina variegata, Polygonum cuspidatum, Mikania lavigata, Harungana madagascariensis, Mentha arvensis, Helichrysum italicum.	*Streptococcus sobrinus*
4.	Caesalpinia Martius	*Lactobacillus casei*
5.	Hamamelis virginiana, Harungana madagascariensis	*Actinomyces odontolitycus*
6.	Hamamelis virginiana, Harungana madagascariensis	*Preveotella spp.*
7.	Harungana madagascariensis	*Fusobacterium*
8.	Harungana madagascariensis	*Propioni bacterium*
9.	Pistacia lentiscus	*Porphyromonas gingivalis*
10.	Tanacetum vulgare, Thuja plicata, Ziziphus joazeiro, Syzygium aromaticum	*Streptococcus aureus*
11.	Mikania glomerata [58]	*S.cricetus.*

Pharmacological Activity of *Andrographis Paniculata*

Antibacterial Activity of *Andrographis Paniculata*

Using the agar well diffusion method, the effects of *Andrographis paniculata* methanol leaf and stem bark extract on *Bacillus subtilis*, *Streptococcus Aureus*, *Enterococcus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* were investigated. As per the results, it shows that *Andrographis paniculata* extract's antibacterial activity is concentration-dependent and more effective against Gram-negative bacteria than Gram-positive ones. Significant antibacterial activity was demonstrated by *Andrographis paniculata* extracts at different four concentrations: 100, 75, 50, and 25 mg/ml [59]. At 500 mg/ml, 750 mg/ml, and 1000 mg/ml concentrations, antibacterial discs derived from Andrographis paniculata leaf aqueous extracts did not show any zone of inhibition even after 48 hours of incubation.

The leaves of *Andrographis paniculata* were extracted with ethanol, and the results varied depending on the concentration. While plant extracts at doses of 1000 mg per millilitre generated very less intense effects, plant extracts at 500 mg and 750 mg per milliliter did not

exhibit any zone of inhibition against the test organisms. A plant leaf methanolic extract charged at 750 mg/ml in the disc showed a zone of inhibition of 6.16 ± 0.089 and 7.08 ± 0.083 mm against strains of Staphylococcus aureus and Escherichia coli, respectively, following quintuplicate testing [60]. Utilizing the agar well diffusion method, *Andrographis paniculata* leaves, roots, stem, and complete plant extracts were tested for their antibacterial qualities against gram-positive (*Bacillus subtilis* and *Streptococcus aureus*) and gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus vulgaris*) bacteria using methanol and water extracts. They concluded that the entire plant and leaf methanolic extract had stronger antibacterial action against gram-positive bacteria. Conversely, no antibacterial activity was demonstrated by the root and stem's aqueous extract. The leaves' ethanolic extract shown encouraging antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* using the disc diffusion technique [61]. Methanol, ethanol, and aqueous extracts of *Andrographis paniculata* and *Piper betle* were first tested for their antibacterial activity against *Escherichia coli* UST- collection of microbial stain [UST-CMS 1030], *Pseudomonas aeruginosa* UST- collection of microbial stain [UST-CMS 10013], and *Staphylococcus aureus* UST- collection of microbial stain [USTCMS 1097] using the disk diffusion method. The sole methanol extract from *Piper betle* that demonstrated an inhibitory zone against *Escherichia coli* UST collection of microbial stain [UST-CMS1030] 1030 was found. The inhibitory zone against *Escherichia coli* UST collection of microbial stain [UST-CMS1030] was not detectable in the other plant extracts. When compared to the *P. betle* methanol extract, the amoxicillin exhibited a larger zone of inhibition, with an average of 19 mm. Only one of the six extracts exhibited antibacterial activity against *Pseudomonas aeruginosa* UST collection of microbial stain USTCMS 10013, as shown by the disk diffusion experiment. The average inhibitory zone of *Piper betle* methanol extract was 7.66 ± 0.6 mm; however, *Pseudomonas aeruginosa* USTCMS 10013 was resistant to *amoxicillin*. The investigation's findings, which revealed significant antibacterial activity against *Pseudomonas aeruginosa*, differed from those of the *Piper betle* ethanol and methanol extracts. Their findings also run counter to the findings of the current investigation regarding *Andrographis paniculata* extracts, since they discovered that the plant's water extract had antibacterial activity against *Pseudomonas aeruginosa* [62].

Anti-inflammation Action

Among the main causes of bacterial illnesses, pathogenic bacteria are the main culprits. It typically triggers an inflammatory response in living things and associated illnesses [63].

Andrographolide has strong bioactivities against this kind of illness. By altering the nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) signalling pathways and reducing pro-inflammatory expression at the protein and mRNA levels, andrographolide chemical components prevent lipopolysaccharide (LPS)-induced inflammation in RAW264.7 cells [64].

Furthermore, andrographolide decreases by preventing periodontal ligament fibroblasts' activation of NF-κB and signal transducer and activator of transcription 3 (STAT3) [65]. BABL/c mice were utilized to test the effects of *Andrographis paniculata* at dosages ranging from 0.78 to 6.25 mg/kg on inflammation brought on by soybean oil. By decreasing the blood levels of proinflammatory cytokines tumour necrosis factor (TNF)-α and IL12-p40, as well as the synthesis of macrophage inflammatory protein (MIP-2) mRNA, they discovered that *Andrographis paniculata* successfully reduced the mouse model [66].

Anti- Diabetic Effect

At a certain dosage, the pure extract and andrographolide dramatically reduced blood glucose, triglycerides, and LDL [67]. Compared to petroleum ether and chloroform, the ethyl-derived alcohol and aqueous extracts demonstrated a notable decrease in systemic sugar levels. In comparison to the control group, the Ethanolic extract of aerial portions significantly decreased the level of glucose in empty stomach blood in rats with diabetes treated with *Streptozotocin* (*STZ*), but it did not affect normal rats [68]. When andrographolide's anti-diabetic action was studied in rats with STZ diabetes, the results indicated that it might enhance glucose consumption and lower plasma glucose in rats with insulin-deficient diabetes [69].

Anti-Fungal & Anti-Oxidant

For decades, traditional remedies have utilized crude extracts of *Andrographis paniculata* to treat fungus-related infections. At 3% (v/v) concentration, the ethanol crude extract of the whole plant displayed significant antifungal effectiveness against *Aspergillus oryzae*, showing 60% inhibition, *Aspergillus Niger*, showing <60% inhibition, and *Penicillium saprophytic,* showing <40% inhibition [70]. Using the radical scavenging technique 2, 2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) experiment, the aqueous extract demonstrated strong antioxidant properties because of its flavonoid and phenolic acid content. Various fractions extracted from a hydro-alcoholic extract have been shown to exhibit antioxidant activity. The increased phenolic content of both aqueous and t-butanol extracts resulted in enhanced antioxidant activity compared to other extracts and fractions [71].

Extraction Method of *Andrographis Paniculata*

To increase extraction yield and achieve high extraction selectivity, parameter adjustment is essential. However, altering the extraction procedure is necessary to boost extraction efficiency. The different extraction methods included in *Andrographis Paniculata* is

Maceration

Maceration is a straightforward stable state extraction technique that requires little experimental setup. It involves putting whole or powdered medicinal materials and pouring the solvent into a sealed container letting it at room temperature for at least three days, and stirring until the chemical dissolves [72,73]. After seven days of room temperature cultivation with periodic stirring and 100 millilitre's of methanol, just 1.8% weight percent of AG was produced from 10 grams of *Andrographis paniculata* [74].

Soxhlet Apparatus Extraction

Ethanol and methanol were used as solvents in the Soxhlet extraction process. The Soxhlet equipment was used to extract the dry powder of *Andrographis paniculata*. At a steady 60°C, the extraction procedure was carried out for six hours [75]. A rotary evaporator was used to evaporate the *Andrographis paniculata* crude extract. Following solvent evaporation, the extract's weight was determined and stored in an airtight container [76].

Ultrasonic Assisted Extraction of *Andrographis Paniculata*

Fisher FB705 sonic dismembrator, an A½ inch ultrasonic probe sonicator operating at 40 kHz, was utilized for the extraction of *Andrographis paniculata*. The apparatus was configured with an amplitude range of 10 A to 100 A. 50 millilitre of solvent (solid-to-liquid ratio of 1:17) were combined with three grams of powdered *Andrographis paniculata* in a 100-centimeter beaker. 3 mm of the ultrasonic probe's horn was immersed below the solution's surface. Following the ultrasonic irradiation process, vacuum filtration was used to filter the mixture. An oven was used to dry the filtrate at 50 ± 2 °C after it was concentrated using rotary evaporators [77].

Marketed Formulation of *Andrographis Paniculata*

Around 5000 papers from Elsevier (2382) and Scopus (2623), and the findings were limited to standard dose forms. Regarding the search, we came across a variety of solid dosage forms, including andrographolide pills and capsules as well as extracts from *Andrographis paniculata.* There is, however, some research on marketed medicines and liquid dosage forms that use the extract from *Andrographis paniculata* with andrographolide. The same is true for semi-solid dosage forms like gels, creams, or patches; however, studies on gaseous dosage

forms like aerosols are essentially non-existent [47]. Solid dosage forms are the most often recommended and favoured by the majority of medical professionals in the pharmaceutical business because of their precise dosing, exceptional stability, and high accuracy [78,79]. In October 2015, the Therapeutic Goods Administration (TGA) regulatory body approved 59 types of solid dosage forms of *Andrographis paniculata* [80,81]. Looking at Table 5.1 details of the dosage form is

Table 4 The Marketed Products of *Andrographis Paniculata*

S.no	Dosage Form	Country	Brand name	Ref. No
1.	Solid	China	Chuan Xin Lian Tablets, Xiaoyan Lidan Tablets, KanJang tablets	[82]
2.	Solid	China, India	film-coated tablets, sugar-coated tablets, hard and soft capsules, enteric-coated capsules, and granules,	[83]
3.	Liquid	India	Kalmegh Syrup (500 mL), Homeopathic Mother Tinctures Kalmegh Q (30 mL, 100 mL), Vasuliv syrup, HAPDCO Kalmegh's Drop, and Hahnemann Laboratory's Kalmegh Drop (15 mL)	[84]
4.	Liquid	Globally	Andrographis Tincture, 1 fl oz (30 mL), and Chuan Xin Lian, Andrographis paniculata Tincture, Nanhua Qianmu Andrographis paniculata liquid injection and Chuanxinlian injections	[85]
5.	Semi-Solid		Fiog Kang Wang Ke Ji Andrographis paniculata Cream 10 g (skincare set) and Bianca Rosa Andrographis 3% Cream (2 oz) 50 mL, AP gel, AP ointments, AP Cream	[86],[87], [88],[89]

Future Perspective

Research in several areas is required to determine whether *Andrographis paniculata* can be used to treat dental caries. It is a promising challenge for the future to assess the anti-bacterial activity, anti-swelling properties, and effectiveness of *Andrographis paniculata* extracts against oral microbes, such as *Streptococcus Mutans*, that cause dental caries. By carrying out these studies, a thorough grasp of the possible advantages and restrictions of *Andrographis paniculata*

in treating dental caries will be possible, and it may also open up the opportunity for its incorporation into contemporary dental care procedures.

Conclusion

Lastly, it is common knowledge among clinicians, researchers, and the general public that Amoxicillin antibiotics can have adverse outcomes. Bacterial infections have become more pathogenic as a result of the development and spread of antibiotic-resistant components. Due to its ability to treat the underlying cause of the condition, lack of adverse effects, and affordability for patients, Ayurvedic drugs can be used in conjunction with *Andrographis Paniculata* as a therapeutic option.

Author Contribution

The original draft was written by Saloni Saw, and the paper was overseen by Sandip Tiwari. Khushboo Gupta conducted a formal analysis of the work, while Pranjul Shrivastava completed the visualization of the entire paper and Prince Kumar Jha offered the conceptualization.

Reference

[1] Wilson M, Wilson PJ, Wilson M, Wilson PJ. Tooth decay. Close Encounters of the Microbial Kind: Everything You Need to Know About Common Infections. Springer Nature Link. 273-91 [2021] https://doi.org/10.1007/978-3-030-56978-5.

[2] Roth GJ. Global burden of disease collaborative network. global burden of disease study 2017 (GBD 2017) results. Seattle, United States: Institute for health metrics and evaluation (IHME), 2018. The Lancet. 2018;392:1736-88.

[3] Ribeiro, A. A., & Paster, B. J. Dental caries and their microbiomes in children: what do we do now? Journal of Oral Microbiology.15**,**1-13[2023] https://doi.org/10.1080/20002297.2023.2198433.

[4] Selwitz RH, Ismail AI, Pitts NB. Dental caries. The Lancet.369,51-9 [2007] 10.1016/S0140-6736(07)60031-2.

[5] World Health Organization. Sugars and dental caries. World Health Organization; 2017.

[6] Cvikl B, Haubenberger-Praml G, Drabo P, Hagmann M, Gruber R, Moritz A, et al. Migration background is associated with caries in Viennese school children, even if parents have received a higher education. BMC oral health.14,1-6[2014] https://doi.org/10.1186/1472-6831-14-51.

[7] da Fonseca MA, Avenetti D.Social determinants of pediatric oral health. Dental Clinics. 61, 519–32 [2017] 10.1016/j.cden.2017.02.002.

[8] .Rathee M, Sapra A. Dental Caries. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024. https://www.ncbi.nlm.nih.gov/books/NBK551699/.

[9] https://www.webmd.com/drugs/2/drug-1922-6240/amoxicillin-potassium-clavulanate-oral/amoxicillin-clavulanic-acid-oral/details.

[10] Sun C, Xie Y, Hu X, Fu J, Zhou J, Wu L. Relationship between clinical symptoms and the microbiota in advanced caries. Journal of Endodontics. 46,763-70 [2020] https://doi.org/10.1016/j.joen.2020.02.011.

[11] Takahashi N. Microbial ecosystem in the oral cavity: metabolic diversity in an ecological niche and its relationship with oral diseases. International Congress Series.1284,103-112 [2005] https://doi.org/10.1016/j.ics.2005.06.071.

[12] Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, Tagami J, Twetman S, Tsakos G, Ismail A, Dental caries, *Nat Rev Dis Primers*, 3, 1-16 [2017] https://doi.org/10.1038/nrdp.2017.30.

[13] Babaeekhou L, Mehrizi AA, Ghane M. Streptococcus mutans, sugar consumption, and oral hygiene: Which one has more effect on decayed, missing, and filled teeth (DMFT) score in Iranian adults?. Dental Research Journal.17,134-41 [2020].

[14] Chinnadurai S, Chilukuri B, Mahendran B, Mantharam V, Selvakumar B, Sankaralingam R. Clinical profile of osteonecrosis in systemic lupus erythematosus–Experience from a tertiary care centre in South India. Journal of Family Medicine and Primary Care. 9, 4363-7 [2020] 10.4103/jfmpc.jfmpc_1234_19.

[15] Alhabdan YA, Albeshr AG, Yenugadhati N, Jradi H. Prevalence of dental caries and associated factors among primary school children: A population-based cross-sectional study in Riyadh, Saudi Arabia. Environ Health Prev Med. 23,1-16 [2018] https://doi.org/10.1186/s12199-018-0750-z.

[16] Punitha VC, Amudhan A, Sivaprakasam P, Rathanaprabu V. Role of dietary habits and diet in caries occurrence and severity among urban adolescent school children. J Pharm Bioallied,7, 296-300 [2015] 10.4103/0975-7406.155963.

[17] Sharma V, Gupta N, Arora V, Gupta P, Mehta N. Caries experience in permanent dentition among 11–14 years old school children in Panchkula district (Haryana) India. Int J Sci Study. 3, 112-5 [2015] 10.17354/ijss/2015/167 .

[18] Padminee K, Anusha R, Lakshmi K, Madan Kumar PD. Geographic disproportions in dental workforce distribution and its impact on oral disease burden: An Indian perspective. SRM J Res Dent Sci, 11, 76-81 [2020] 10.4103/srmjrds.srmjrds_79_19 .

[19] Chandu VC, Pachava S, Viswanath V. Strategies for improving accessibility to oral health care services in rural India: An insight. Int J Oral Health Med Res, 4, 44-6 [2017] ISSN 2395-7387.

[20] Janakiram C, Antony B, Joseph J, Ramanarayanan V. Prevalence of dental caries in India among the WHO index age groups: A meta-analysis. J Clin Diagn Res, 12, 8-13 [2018] 10.7860/JCDR/2018/32669.11956.

[21] World Population Ageing 2019: 2019 Revision|Multimedia Library—United Nations Department of Economics and Social Affairs [Internet] Un.org. 2020.

[22] Wang Y, Xing L, Yu H, Zhao L. Prevalence of dental caries in children and adolescents with type 1 diabetes: a systematic review and meta-analysis. BMC oral health. 19,1-9 [2019] https://doi.org/10.1186/s12903-019-0903-5.

[23] Paul T, Basu S, Saha NC. Anticancer effect of Andrographis paniculata by suppression of tumor-altered hypoxia signaling cascade in mouse melanoma cells. Journal of Cancer Research and Practice. 6, 117-23 [2019] 10.4103/JCRP.JCRP_9_19.

[24] Lakhmale SP, Washimkar VB, Yewatkar N. A REVIEW ON KALMEGHA ANDROGRAPHIS PANICULATA (BURM. F.) NEES. Health. 65 [2023] 10.5281/zenodo.8153408.

[25] Ala'a A, Canatan H, Ezeamuzie CI. In vitro and in vivo anti-inflammatory effects of andrographolide. International immunopharmacology. 9, 313-8 [2009] https://doi.org/10.1016/j.intimp.2008.12.002.

[26] Kumar G, Singh D, Tali JA, Dheer D, Shankar R. Andrographolide: Chemical modification and its effect on biological activities. Bioorganic chemistry. 95 [2020] https://doi.org/10.1016/j.bioorg.2019.103511.

[27] Sareer O, Ahmad S, Umar S. Andrographis paniculata: a critical appraisal of extraction, isolation, and quantification of andrographolide and other active constituents. Natural product research. 28, 2081-101 [2014] https://doi.org/10.1080/14786419.2014.924004.

[28] Rajanna M, Bharathi B, Shivakumar BR, Deepak M, Prabakaran D, Vijayabhaskar T, Arun B. Immunomodulatory effects of Andrographis paniculata extract in healthy adults–An open-label study. Journal of Ayurveda and integrative medicine.12, 529-34 [2021] https://doi.org/10.1016/j.jaim.2021.06.004.

[29] Adiguna SB, Panggabean JA, Swasono RT, Rahmawati SI, Izzati F, Bayu A, Putra MY, Formisano C, Giuseppina C. Evaluations of andrographolide-rich fractions of Andrographis paniculata with enhanced potential antioxidant, anticancer, antihypertensive, and anti-inflammatory activities. Plants. 12, 1220 [2023], https://doi.org/10.3390/plants12061220.

[30] Suriyo T, Chotirat S, Rangkadilok N, Pholphana N, Satayavivad J. Interactive effects of Andrographis paniculata extracts and cancer chemotherapeutic 5-Fluorouracil on cytochrome P450s expression in human hepatocellular carcinoma HepG2 cells. Journal of Herbal Medicine. 26 [2021] https://doi.org/10.1016/j.hermed.2021.100421.

[31] Banerjee M, Parai D, Chattopadhyay S, Mukherjee SK. Andrographolide: antibacterial activity against common bacteria of human health concern and possible

mechanism of action. Folia microbiologica. 62, 237-44 [2017] https://doi.org/10.1007/s12223-017-0496-9.

[32] Murugan K, Selvanayaki K, Al-Sohaibani S. Antibiofilm activity of Andrographis paniculata against cystic fibrosis clinical isolate Pseudomonas aeruginosa. World Journal of Microbiology and Biotechnology. 27,1661-8 [2011] https://doi.org/10.1007/s11274-010-0620-3.

[33] Krithika R, Verma RJ, Shrivastav PS. Antioxidative and cytoprotective effects of andrographolide against CCl4-induced hepatotoxicity in HepG2 cells. Human & experimental toxicology. 32, 530-43 [2013] https://doi.org/10.1177/0960327112459530.

[34] Kaushik S, Dar L, Kaushik S, Yadav JP. Identification and characterization of new potent inhibitors of dengue virus NS5 proteinase from Andrographis paniculata supercritical extracts on in animal cell culture and in silico approaches. Journal of Ethnopharmacol.267, 1-44 [2021] https://doi.org/10.1016/j.jep.2020.113541.

[35] Imani SF, Khairani AA, Arbianti R, Utami TS, Hermansyah H. The effect of fermentation time and sonication temperatures on extraction process of bitter leaves (Andrographis Paniculata) against antidiabetic activity through α-Glucosidase enzyme inhibition test. InAIP Conference Proceedings. 2255,030047-1-7 [2020] https://doi.org/10.1063/5.0015260.

[36] S. K. Mishra, N. S. Sangwan, and R. S. Sangwan, “Andrographis paniculata (Kalmegh): a review,” Pharmacognosy Reviews, 1, 283–298 2007 http://www.phcogrev.com.

[37] USDA (United States Department of Agriculture), RS (Agricultural Research Service), NGRP (National Genetic Resources Program), and GRIN (Germplasm Resources Information Network), “GRIN Taxonomy for Plants,” April 2014, http://www .ars-grin.gov/cgi-bin/npgs/html/taxon.pl?414228.

[38] Tropicos.org, “Andrographis paniculata (Burm. f.) Wall. ex Nees,” 2014, http://www.tropicos.org/Name/100007.

[39] Hossain MS. *The effect of salinity stress on the morpho-physiology and protein profile of Andrographis paniculata* (Doctoral dissertation, Kulliyyah of Science, International Islamic University Malaysia) [2016].

[40] Jarukamjorn K, Nemoto N. Pharmacological aspects of Andrographis paniculata on health and its major diterpenoid constituent andrographolide. Journal of health science. 54, 370-81 [2008] https://doi.org/10.1248/jhs.54.370.

[41] Anju D, Jugnu G, Kavita S, Arun N, Sandeep D. A review on medicinal prospectives of Andrographis paniculata Nees. Journal of pharmaceutical and scientific innovation.1,1-4 [2012] http://jpsionline.com/admin/php/uploads/3_pdf.pdf.

[42] Boopathi CA. Andrographis spp.: a source of bitter compounds for medicinal use. Ancient science of life.19, 164-8 [2000].

[43] Hossain MS, Urbi Z, Sule A, Rahman KH. Andrographis paniculata (Burm. f.) Wall. ex Nees: a review of ethnobotany, phytochemistry, and pharmacology. The Scientific World Journal. 2014, 1-28 [2014] https://doi.org/10.1155/2014/274905.

[44] Intharuksa A, Arunotayanun W, Yooin W, Sirisa-Ard P. A comprehensive review of Andrographis paniculata (Burm. f.) Nees and its constituents as potential lead compounds for COVID-19 drug discovery. Molecules. 27,4479 [2022] https://doi.org/10.1186/1749-8546-5-17.

[45] Chao WW, Lin BF. Isolation and identification of bioactive compounds in Andrographis paniculata (Chuanxinlian). Chinese medicine. 5, 1-5 [2010] https://doi.org/10.1186/1749-8546-5-17.

[46] Okhuarobo, A.; Ehizogie Falodun, J.; Erharuyi, O.; Imieje, V.; Falodun, A.; Langer, P. Harnessing the medicinal properties of Andrographis paniculata for diseases and beyond: A review of its phytochemistry and pharmacology. Asian Pac. J. Trop. Dis. 4, 213–222 [2014] https://doi.org/10.1016/S2222-1808(14)60509-0.

[47] Raman S, Murugaiyah V, Parumasivam T. Andrographis paniculata dosage forms and advances in nanoparticulate delivery systems: An overview. Molecules. 27, 1-17 [2022] https://doi.org/10.3390/molecules27196164.

[48] Samy, R.P.; Thwin, M.M.; Gopalakrishnakone, P. Phytochemistry, pharmacology and clinical use of Andrographis paniculata. Nat. Product Commun. 2, 607-618 [2007] https://doi.org/10.1177/1934578X0700200519.

[49] Jafari-Sales A, Bolouri P. Evaluation of the antimicrobial effects of Glycyrrhiza glabra l. on some gram positive and gram-negative pathogenic bacteria in laboratory conditions. Jorjani Biomedicine Journal.6, 78-84 [2018] 10.29252/jorjanibiomedj.6.4.78.

[50] Alshahrani AM, Gregory RL. In vitro Cariostatic effects of cinnamon water extract on nicotine-induced Streptococcus mutans biofilm. BMC complementary medicine and therapies.20, 1-9 [2020] https://doi.org/10.1186/s12906-020-2840-x.

[51] Adwan G, Salameh Y, Adwan K, Barakat A. Assessment of antifungal activity of herbal and conventional toothpastes against clinical isolates of Candida albicans. Asian

Pacific Journal of Tropical Biomedicine. 2, 375-9 [2012] https://doi.org/10.1016/S2221-1691(12)60059-8.

[52] Malvania EA, Sharma AS, Sheth SA, Rathod S, Chovatia NR, Kachwala MS. In vitro analysis of licorice (glycyrrhiza glabra) root extract activity on streptococcus mutans in comparison to chlorhexidine and fluoride mouthwash. J Contemp Dent Pract. 20, 1389-94 [2019] 10.5005/jp-journals-10024-2724.

[53] Alloha IB, Aziz NA, Faisal GG, Abllah Z, Arzmi MH. Effects of Eurycoma Longifolia Jack (Tongkat Ali) alcoholic root extract against oral pathogens. Pharmacognosy Journal.11, 1299-1302 [2019] 10.5530/pj.2019.11.201.

[54] Yang Y, Park BI, Hwang EH, You YO. Composition analysis and inhibitory effect of Sterculia lychnophora against biofilm formation by Streptococcus mutans. Evidence-Based Complementary and Alternative Medicine. 2016, 9 [2016] https://doi.org/10.1155/2016/8163150.

[55] Chatterjee T, Das SM. Antimicrobial efficacy of some medicinal plant extract against Streptococcus mutans causing dental caries. J Med Plants. 5,315-7 [2017];5:315-7.

[56] Ferreira-Filho JC, Marre AT, de Sá Almeida JS, Lobo LD, Farah A, Romanos MT, Maia LC, Valença AM, Fonseca-Gonçalves A. Therapeutic potential of bauhinia forficata link in dental biofilm treatment. Journal of medicinal food. 23,998-1005 [2020] https://doi.org/10.1089/jmf.2019.0277.

[57] Ferreira-Filho JC, Marre AT, Almeida JS, Lobo LA, Farah A, Valença AM, Fonseca-Gonçalves A. Treatment of dental biofilm with a tincture of Bauhinia forficata leaves: An ex-vivo study. Natural product research. 33, 3432-5 [2019] https://doi.org/10.1080/14786419.2018.1477146.

[58] Pandey p, raja w. A comparative study on antibacterial activity of andrographis paniculata different bark extracts against some human pathogenic bacteria's. World Journal of Pharmaceutical Research. 12, 933-94110.20959/wjpr202312-28936.

[59] Kabra P, Loomba K, Kabra SK, Majumdar DS, Kumar N. Medicinal plants in the treatment of dental caries. Asian J Oral Health Allied Sci. 2012 Jan;2(1):12-6.

[60] Garnayak N, Senapti SK, Patra RC, Panda SK, Jena GR, Mishra SR. In vitro antibacterial activity of Andrographis paniculata aqueous, methanolic & ethanolic leaf extracts. The Pharma Innovation. 12, 573-577 [2023].

[61] Mehta S, Sharma AK, Singh RK. Pharmacological activities and molecular mechanisms of pure and crude extract of Andrographis paniculata: An update. Phytomedicine Plus. 1, 1-19 [2021] https://doi.org/10.1016/j.phyplu.2021.100085.

[62] Mejos CB, Poblete JG, Sarino PJ, Cruzada MF. Antibacterial Activity of Andrographis paniculata and Piper betle and their Interactive Effects with Amoxicillin Against Selected Respiratory Pathogens. bioRxiv. 1-29 [2023] : https://doi.org/10.1101/2023.04.05.535803.

[63] Hall JE, Hall ME. Guyton and Hall Textbook of Medical Physiology E-Book: Guyton and Hall Textbook of Medical Physiology E-Book. Elsevier Health Sciences; 2020.

[64] Li Y, He S, Tang J, Ding N, Chu X, Cheng L, Ding X, Liang T, Feng S, Rahman SU, Wang X. Andrographolide inhibits inflammatory cytokines secretion in LPS-stimulated RAW264. 7 cells through suppression of NF-κB/MAPK signaling pathway. Evidence-Based Complementary and Alternative Medicine. 2017, 9 [2017] https://doi.org/10.1155/2017/8248142.

[65] Ambili R, Janam P, Babu PS, Prasad M, Vinod D, Kumar PA, Kumary TV, Nair SA, Pillai MR. An ex vivo evaluation of the efficacy of andrographolide in modulating differential expression of transcription factors and target genes in periodontal cells and its potential role in treating periodontal diseases. Journal of ethnopharmacology. 196, 160-7 [2017] https://doi.org/10.1016/j.jep.2016.12.029.

[66] Wen-Wan C, Kuo YH, Hsieh SL, Bi-Fong L. Inhibitory Effects of Ethyl Acetate Extract of Andrographis paniculata on NF-κB Trans-Activation Activity and LPS-Induced Acute Inflammation in Mice. Evidence-Based Complementary and Alternative Medicine. 2011,9 [2011] 10.1093/ecam/nep120.

[67] Grover M. Green Chiryata (Andrographis paniculata): A potential blood purifier. International Research Journal of Pharmacy and Medical Sciences (IRJPMS), Volume 4, 1-7 [2021], ISSN: 2581-3277.

[68] Kumar S, Singh B, Bajpai V. Andrographis paniculata (Burm. f.) Nees: Traditional uses, phytochemistry, pharmacological properties and quality control/quality assurance. Journal of ethnopharmacology.275 [2021] https://doi.org/10.1016/j.jep.2021.114054.

[69] Bhaisare S, Pathak S, Ajankar VV. Physiological activities of the king of bitters (Andrographis paniculata): a review. Cureus. 15, 1-6 [2023] 10.7759/cureus.43515.

[70] Wanchaitanawong P, Chaungwanit P, Poovarodom N, Nitisinprasert S. In vitro antifungal activity of Thai herb and spice extracts against food spoilage fungi. Agriculture and Natural Resources. 2005 Sep 30;39(3):400-5.

[71] Kumar S, Singh B, Bajpai V. Andrographis paniculata (Burm. f.) Nees: Traditional uses, phytochemistry, pharmacological properties and quality control/quality assurance.

Journal of ethnopharmacology. 2021 Jul 15;275:114054, doi-https://doi.org/10.1016/j.jep.2021.114054.

[72] Mesmar J, Abdallah R, Badran A, Maresca M, Shaito A, BaydounE. Ziziphus nummularia: A Comprehensive Reviewof ItsPhytochemical Constituents and Pharmacological Properties. Molecules. 27, 1-24 [2022] https://doi.org/10.3390/molecules27134240.

[73] Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: A comprehensive review. Chinese medicine. 13, 1-26 [2018] https://doi.org/10.1186/s13020-018-0177-x.

[74] Liang D, Zhang WM, Liang X, Tian HY, Zhang XM, Li X, Gao WY. A review on the extraction and separation of andrographolide from Andrographis paniculata: Extraction selectivity, current challenges and strategies. Trad. Med. Res. 8, 37-42 [2023] https://doi.org/10.53388/TMR20230107001.

[75] Umadevi U, Kamalam M. Phytochemical and antioxidant studies on an important indigenous medicinal plant-Andrographis paniculata (Burm. F) nees. International Journal of Pharmaceutical Sciences and Research. 5, 5240-5244 [2014] http://ijpsr.com/bft-article/phytochemical-and-antioxidant-studies-on-an-important-indigenous-medicinal-plant-andrographis-paniculata-burm-f-nees/?view=fulltext.

[76] Bahari R, Kasim KF, Nasaruddin NN. Extraction of Andrographis paniculata as material for developing soap and sanitizer. InIOP Conference Series: Earth and Environmental Science. 765, 1-13 [2021] 10.1088/1755-1315/765/1/012059.

[77] Koay YS, Hasham R, Bohari SP, Hamid MA. Probe ultrasonic-assisted extraction of andrographolide-rich extract from Andrographis paniculata: Processing parameters optimization using response surface methodology. Trends in Sciences. 19, 1-13 [2022] https://doi.org/10.48048/tis.2022.385.

[78] Kalaria, D.R.; Parker, K.; Reynolds, G.K.; Laru, J. An industrial approach towards solid dosage development for first-in-human studies: Application of predictive science and Lean principles. Drug Discov. Today. 25, 505-518 [2020] https://doi.org/10.1016/j.drudis.2019.12.012.

[79] Arshad MS, Zafar S, Yousef B, Alyassin Y, Ali R, AlAsiri A, Chang MW, Ahmad Z, Elkordy AA, Faheem A, Pitt K. A review of emerging technologies enabling improved solid oral dosage form manufacturing and processing. Advanced drug delivery reviews. 178, [2021] https://doi.org/10.1016/j.addr.2021.113840.

[80] EMA. European Medicines Agency. Available online: http://www.ema.europa.eu/contact (accessed on 29 June 2022).

[81] Solomon Jeeva, J.J. Andrographis paniculata: A review of its traditional uses, phytochemistry, and pharmacology. Med. Aromatics Plants. 3, 1-6 [2014] 0.4172/2167-0412.1000169.

[82] Zhao, J.; Yang, G.; Liu, H.; Wang, D.; Song, X.; Chen, Y. Determination of andrographolide, deoxyandrographolide and neoandrographolide in the Chinese herb Andrographis paniculata by micellar electrokinetic capillary chromatography. Phytochem. Anal. 13, 222-227 [2002], 13, 222–227 https://doi.org/10.1002/pca.644.

[83] Gabrielian, E.S.; Shukarian, A.K.; Goukasova, G.I.; Chandanian, G.L.; Panossian, A.G.; Wikman, G.; Wagner, H. A double blind, placebo-controlled study of Andrographis paniculata fixed combination Kan Jang in the treatment of acute upper respiratory tract infections including sinusitis. Phytomedicine. 9, 589–597 [2002] https://doi.org/10.1078/094471102321616391.

[84] Hossain, S.; Urbi, Z.; Karuniawati, H.; Mohiuddin, R.B.; Qrimida, A.M.; Allzrag, A.M.M.; Ming, L.C.; Pagano, E.; Capasso, R. Andrographis paniculata (Burm. f.) Wall. Ex Nees: An updated review of phytochemistry, antimicrobial pharmacology, and clinical safety and efficacy. Life. 11, 1-39 [2021] https://doi.org/10.3390/life11040348.

[85] Widyawaruyanti, A.; Asrory, M.; Ekasari, W.; Setiawan, D.; Radjaram, A.; Tumewu, L.; Hafid, A.F. In vivo antimalarial activity of Andrographis paniculata tablets. Procedia Chem. 13, 101–104 [2013] https://doi.org/10.1016/j.proche.2014.12.012.

[86] Thawornrungroj, S.; Kuphasuk, Y.; Petmitr, S.; Srisatjaluk, R.; Kitkumthorn, N. The application of Andrographis paniculata gel as an adjunct in the treatment of chronic periodontitis: Clinical and microbiological effects. Naresuan Univ. J. Sci. Technol. NUJST. 19, 38–49 [2011].

[87] TuanKub, T.N.; Ab Manaf, N.A.; Che Ibrahim, A.S. Comparison of well diffusion, disc diffusion and broth dilution methods for antimicrobial activity of Andrographis paniculata herbal gel against acne-associated pathogen. Malays. J. Microbiol. 17, 90–96 [2017] 10.21161/mjm.200722.

[88] Samuel, A.J.; Mulla, N. Formulation and evaluation of herbal topical gel containing leaves extract of Andrographis paniculata. J. Drug Deliv. Ther. 2020, 10, 48–51

[89] Olutayo, A.A.; Babalola, C.O.; Femi-Oyewo, M.N.; Balogun, G.Y. Antimicrobial activity and stability of Andrographis paniculata cream containing Shea Butter. Nig. J. Pharm. Res. 15, 9–18 [2019] e-ISSN 2635-3555.