THE CYTOTOXICITY AND ANTIMICROBIAL ACTIVITY OF *Andrographis paniculata* HERBAL MOUTHWASH FORMULATION

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by

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LIST OF SYMBOLS

°C	Degree celcius
cm	Centimeter
%	Percentage
kg	Kilogram
mmol	Millimolar
mm	Millimeter
µL	Microliter
nm	Nanometer
mL	Milliliter
L	Liter
µg/mL	Microgram per milliliter
µM/kg	Micromolar per kilogram
(w/w)	Weight per weight
mg/kg/day	Milligram per kilogram per day
g	Gram
g/mL	Gram per milliliter
mg/mL	Milligram per milliliter

LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrometry
AP	*Andrographis paniculata*
As	Arsenic
ATCC	American type culture collection
Av	Average
BHT	Butylated hydroxy toluene
C	Cell concentration
CC_{50}	50% cytotoxicity concentration
Cd	Cadmium
CLSI	Clinical and Laboratory Standards Institute
COVID-19	Corona virus disease 2019
DMEM	Dulbecco’s modified eagle medium
DMSO	Dimethyl sulfoxide
DPPH	1,1-diphenyl-2-picrylhydrazyl
FBS	Fetal bovine serum
FIMS	Flow injection mercury system
FTLEE	First true leaf ethanolic extract
FTIR	Fourier Transform Infrared
GC-MS	Gas chromatography-mass spectrometry
GFAS	Graphite furnace absorption spectrometry
Hg	Mercury
HPV	Human papillomavirus
HNO_3	Nitric acid
IC_{50}	Half-maximal inhibitory concentration
IQR	Interquartile range
LC_{50}	Lethal concentration 50
LD_{50}	Median lethal dose
LOD	Limit of detection
MBC	Minimum bactericidal concentration
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth

MIC	Minimum inhibitory concentration
MTCC	Microbial Type Culture Collection
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NA	Not available
Nc	Negative control
NCI	National cancer institute
ND	Not detected
NPRA	National Pharmaceutical Regulatory Agency
N_T	Number of cell population
Nv	Number of viable cells
OD	Optical density
Pb	Plumbum
PBS	Phosphate buffer saline
PDA	Potato dextrose agar
pH	Potential of hydrogen
SARS-COV-2	Severe acute respiratory syndrome coronavirus 2
SC	Sterility control
SD	Standard deviation
SPSS	Statistical Package for Social Science
T	Test
USM	Universiti Sains Malaysia
WHO	World Health Organization

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SITOTOKSISITI DAN AKTIVITI ANTIMIKROB FORMULASI PENCUCI MULUT HERBA *Andrographis paniculata*

ABSTRAK

Penyakit mulut adalah masalah kesihatan global yang disebabkan oleh patogen mulut. Dalam pencegahan penyakit mulut, pencuci mulut biasanya digunakan. Walau bagaimanapun, pencuci mulut komersial didapati toksik dan mempunyai kesan sampingan. Selain itu, antibiotik sedia ada tidak berkesan kerana penggunaan yang berlebihan dan menyebabkan kerintangan kepada patogen mulut. Oleh itu, rawatan alternatif yang lebih selamat dan berkesan dalam produk pergigian sangat diperlukan. Oleh kerana kajian tentang sitotoksisiti dan aktiviti antimikrob ubatan herba, *Andrographis paniculata* (AP) terhadap patogen mulut adalah terhad, kajian ini mencadangkan ekstrak akueus AP sebagai pencuci mulut herba untuk menentukan keberkesanannya terhadap patogen mulut seperti; *Porphyromonas gingivalis*, *Streptococcus mutans*, *S. sobrinus*, *Staphylococcus aureus*, dan *Actinomyces viscosus*. Dalam kajian ini, kesan antibakteria ekstrak akueus AP terhadap patogen mulut ditentukan oleh ujian resapan cakera dan resapan telaga, diikuti oleh kesan antibakteria lima kepekatan berbeza pencuci mulut herba AP pada 1000, 500, 250, 125 dan 62.5 mg/mL menggunakan kaedah resapan telaga dan kaedah pencairan mikro kaldu. Penilaian ketoksikan ditentukan oleh ujian kematian udang air garam dan ujian 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) ke atas sel fibroblas L929 manakala analisis logam berat menggunakan spektrometri penyerapan atom (AAS). Kajian kestabilan telah dijalankan ke atas produk akhir pencuci mulut herba AP. Keputusan menunjukkan ekstrak akueus AP dengan ketara menghalang pertumbuhan patogen mulut (p<0.05) kecuali ke atas bakteria *S.aureus* dengan zon perencatan antara 2.83 ± 0.83 mm hingga 14.37 ± 0.83.mm. Pencuci mulut herba AP mempamerkan aktiviti antibakteria dengan ketara (p<0.05) kecuali ke atas bakteria *S.aureus* antara median (IQR) of 9.50 (3.80)hingga median (IQR) of 15.00 (3.40). Kesemua kepekatan pencuci mulut menyebabkan perencatan bakteria daripada 10.56% hingga 261.09% dengan kepekatan perencat minimum (MIC) dan kepekatan membunuh bakteria minimum (MBC) masing-masing dipamerkan pada 3.90 mg/mL hingga 7.81 mg/mL dan 7.81 mg/mL hingga 62.50 mg/mL. Walau bagaimanapun, tiada MBC direkodkan ke atas *S. aureus*. Formulasi pencuci mulut adalah tidak toksik kepada udang air garam dan sel fibroblast L929 dengan nilai LC_{50} 3255.06 µg/mL dan nilai IC_{50} 43.55 mg/mL. Pencuci mulut herba AP bebas daripada pencemaran logam berat dan mengekalkan ciri-ciri dan steriliti selama tempoh penyimpanan iaitu 12 bulan. Kesimpulannya, pencuci mulut herba AP pada 7.81 mg/mL ialah formulasi pencuci mulut yang terbaik. Walau bagaimanapun, lebih banyak kajian penyelidikan perlu dilakukan ke atas pencuci mulut herba AP untuk kegunaan klinikal dan pengkomersilan produk.

THE CYTOTOXICITY AND ANTIMICROBIAL ACTIVITY OF *Andrographis paniculata* HERBAL MOUTHWASH FORMULATION

ABSTRACT

Oral disease is a global health problem caused by oral pathogens. To prevent the oral disease, mouthwash is commonly used. However, commercial mouthwash contained alcohol and other chemicals found to be toxic with unfavourable side effects. Besides, existence drugs seemed to be ineffective due to excessive use and multidrug resistance against the oral pathogens. Thus, there is a demand for a safer and effective alternative treatment in dental product. As the studies on cytotoxicity and antimicrobial activities of medicinal herb, *Andrographis paniculata* (AP) against oral pathogens are limited, this study proposed AP aqueous extract as herbal mouthwash to determine its effectiveness against selected oral pathogens; *Porphyromonas gingivalis, Streptococcus mutans, S. sobrinus, Staphylococcus aureus*, and *Actinomyces viscosus*. In this study, antibacterial effect of AP aqueous extract against the tested oral pathogens was determined by disc diffusion and well diffusion assays, followed by antibacterial effect of five concentrations of AP herbal mouthwash at 1000, 500, 250, 125 and 62.5 mg/mL using well diffusion and broth microdilution method. Assessment of toxicity was determined by brine shrimp lethality bioassay and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay onto L929 fibroblast cells while the analysis of heavy metal was analysed using atomic absorption spectrometry (AAS). Stability study was carried out on the final product of AP herbal mouthwash. Results revealed that AP aqueous extract significantly inhibited the growth of all oral pathogens (p<0.05) except for *S. aureus* with inhibition zones ranging from 2.83 ± 0.83 mm to 14.37 ± 0.83.mm. Similar to AP aqueous extract, AP herbal mouthwash exhibited significant antibacterial activities onto all tested pathogens except for *S. aureus* (p<0.05) with inhibition ranging from median (IQR) of 9.50 (3.80) to median (IQR) of 15.00 (3.40). All mouthwash concentrations caused bacteria inhibition from 10.56% to 261.09% with minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) at 3.90 mg/mL to 7.81 mg/mL and 7.81 mg/mL to 62.50 mg/mL respectively. However, there was no MBC detected for *S. aureus* at these concentrations. Formulation of mouthwash was non-toxic to brine shrimp and L929 fibroblast cell line with LC_{50} value 3255.06 µg/mL and IC_{50} value 43.55 mg/mL respectively. AP herbal mouthwash was free from heavy metal contamination and maintained its characteristics and sterility for 12 months storage. In conclusion, AP herbal mouthwash at 7.81 mg/mL was found to be the best mouthwash formulation. However, more research works need to be carried out for its clinical use for further product commercialization.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Oral cavity is naturally inhabited by a wide number of microorganism species living together as normal microbiota. Among the population, a range of pathogenic bacteria was found in the oral cavity associated with oral infections are such as *Streptococcus mutans*, *Staphylococcus aureus* (Faden, 2018), *S. sobrinus*, *Porphyromonas gingivalis* (Alharthi *et al*., 2020) and *Actinomyces viscosus* (Abdollahzadeh *et al*., 2011).

S. mutans is the main oral bacteria linked to tooth decay. Other than this species, *S. sobrinus*, *S. sanguinis* and *S. salivarius* are the cause for dental plaque. The process of dental decay occurs when the normal living *S. mutans* in the mouth is in contact with sucrose or sugar-containing products. This condition causes the overgrowth of *S. mutans* in the oral cavity and lowers the pH inside the mouth. This acidity condition causes tooth decay as it slowly destroys tooth enamel (Motallaei *et al*., 2021).

P. gingivalis is not commonly present in a healthy mouth. It is an inflammatory-causing bacterium which affecting the periodontal tissue and the alveolar bone supporting the teeth. Periodontitis is a serious and progressive disease that can cause significant dental pain and can eventually lead to tooth loss. Thus, it is not a disease to be taken lightly (Stephens *et al*., 2018).

S. aureus has been isolated from a wide range of infective oral conditions, such as angular cheilitis and parotitis. More recently, a clinical condition known as staphylococcal mucositis has emerged as a clinical problem in many debilitated elderly patients. Higher carriage rates of *S. aureus*, in patients prone to joint infections raises the possibility of the oral cavity serving as a potential source for bacteraemic spread to compromised joint spaces (Deppe *et al*., 2019).

Mouthwash is a supplementary cleansing tool other than toothbrush and toothpaste. It is used for dental hygiene practice to freshen the breath and whiten the teeth. The swishing mechanism makes it very effective to remove food debris hidden between the teeth. Besides, mouthwash able to kill bacteria in the mouth. Nowadays, mouthwash formulated from natural source is an improvement of the dental products. It delivers the same function as other commercial mouthwash but with some additional medicinal purposes such as.anti-inflammatory and anti-plaque properties (Harshitha & Preethi *et al*., 2020).

The excessive use of antibiotics has led to an accelerated increase in antibiotic resistance among microorganisms, and thus presents a significant challenge to the development of novel antimicrobial. According to World Health Organization, herbal medicine has been practiced by 80% of the world's population since earlier centuries for the treatment of various diseases (Samouh *et al*., 2019). The use of plants as therapeutic agents remains a central component of the traditional medicinal system. Recently, literatures have showed a surged in interest to discover such compounds with therapeutic properties. Many medicinal plant extracts have been documented for their therapeutic and pharmacological properties (Palombo, 2011).

One of the potential herbs to aid in the exploration of new antimicrobial for oral disease is *Andrographis paniculata* (AP). AP is an ancient medicinal herb with an extensive ethnobotanical history in Asia. Local people called it as 'Hempedu Bumi', which means 'Bile of Earth', or literally 'King of Bitters'. AP has been used successfully in a variety of Asian cultures. The plant is well described in both modern and ancient Chinese medicinal literature, and it is prominently listed in the *Indian Pharmacopoeia* (Niranjan *et al*., 2010).

AP is an annual herb with a bitter aftertaste. The genus *Andrographis* consists of 28 species distributed in tropical Asia. Only a few species are medicinal, of which *A. paniculata* is the most popular. Unlike other species of the genus, AP is commonly scattered in most places in Malaysia, including the grasslands and hilly areas up to 500 m, which accounts for its wide distribution (Nyeem *et al*., 2017).

AP is used by Chinese and Ayurvedic practitioners mainly for treating dysentery and liver pain (Akilandeswari *et al*., 2019). The therapeutic value of this plant is due to its enzyme induction mechanism. The plant is used externally for skin diseases, snakebites, and swellings. Decoction of the plant is used for the cure of torpid liver, jaundice, dermatological diseases, dyspepsia, febrifuge, and anthelmintic (Sidha *et al*., 2021). Besides, AP has anti-typhoidal activity against *Salmonella typhi* and antifungal against *Helminthosporium sativum*. Aqueous extract of the AP leaves showed antibiotic properties against *S. aureus* and *Pseudomonas aeruginosa* (Hossain *et al*., 2021).

Herbs with antibacterial properties are still not fully explored in Malaysia. Those containing antibacterial drugs ingredients are often effective for long term oral infection treatment. However, some has led to antibiotic resistance. The ability of *S. aureus* to develop resistance to additional antibiotics, including trimethoprim-sulphamethoxazole and tetracyclines, via the many pathways of resistance, is well recognised (Mancuso *et al*., 2021). WHO listed antimicrobial resistance as one of the top ten dangers to world health in 2019 because of its impacts on human’s health.

With the current craze for alternative medicine, there is a need for the development of new mouthwash from natural products. Due to presence of antimicrobial activity of AP, this plant will be tested on selected oral pathogens that has been associated with oral diseases to determine the antibacterial activity against *S. mutans*, *S. sobrinus*, *S. aureus*, *A. viscosus*, and *P. gingivalis.*

This study is aimed at formulating a new type of herbal mouthwash derived from AP, as well as determination of its antimicrobial properties, toxicity, and stability. In this study, the antimicrobial properties of AP herbal mouthwash will be investigated against selected microorganisms associated with oral infections.

1.2 Herbal medicine

Herbal medicine practice has existed for a long time ago even before the emergent of modern medicine. In the beginning of the human existence, herbal plants have become primary food resources by primitive man. Along with the revolution, people started to distinguish herbal species based on their pharmacological effects and medicinal purposes. It is estimated that more than 80, 000 of herbal species have been recognized and used worldwide as medicinal plants (Jashmidi-kia *et al*., 2018). Among of these species, about 1, 300 plant species have been found and historically used in Malaysia (Alsarhan *et al*., 2014).

According to the data from WHO, more than half of the world population which is about 80% are taking herbal medicine as basic healthcare (Samouh *et al*., 2019). The organization has also acknowledged herbal medicine as an alternative to modern medicine. In the present day, the use of herbal medicine in prevention and cure of diseases are gaining wide interest especially in the field of pharmaceutical, cosmetics and food supplements (Shakya, 2020). Growing factors of implementing the use of herbal medicine vary according to the socio-demography and cultural differences (Ismail *et al*., 2021). The knowledge is passed down from generation to generation as a part of cultural belief and tradition. In fact, people who have grown up in suburban areas possess more knowledge regarding herbal medicine compared to those living in the city. The factor contributing to this might be that rural residents are highly dependent on herbal medicine as they are closer to nature especially in under-developed countries (Yen Yen *et al*., 2020). Furthermore, the rural health centre lack of health facilities contributes to a lack of interest in modern medicine.

On top of that, professional medical care requires a high cost and is time-consuming (Komolafe *et al*., 2021). The biggest driving force behind the use of herbal medicine is the belief that they are safer when compared to modern medicine. They believe that herbal medicine is natural and has fewer side effects than modern drugs (Alexander, 2017). Although herbal medicine has been widely used for thousands of years, most herbal medicine lacks current data on their scientific proof and clinical evaluation (Zhou *et al*., 2019). This is due to the nature of herbal medicine practice, which depends heavily on direct clinical experience, the precise clinical data for most herbal medicine are still limited compared to modern medicine.

Besides, herbal medicine provides alternative option, especially for patients with minor illnesses such as common cold, fever, stomachache, sore throat and muscle pain. In such cases, herbal medicine is considered as a convenient, reliable, fast, and low in cost alternative. Apart from that, people prefer herbal medicine for minor illness because it has a multi-component, multi-target and multi-pathway mechanism (Burgos *et al*., 2020).

With the growing interest in herbal medicine, more research has been done and it has proven the potential of herbal medicine in treating mild to more serious illnesses such as diabetes, high blood pressure and cancer.

1.2.1 Medicinal properties of herbal medicine

Herbal medicine is considered as health system that originated from ancient China since 5 000 years ago (Zhou *et al*., 2019). Herbal medicine is made up of thousands of components and phytochemicals subjected to their medicinal properties. There are several different compounds in herbal plants, some of which are highly complex. It has been found that metabolites play such a vital part in providing pharmacological properties in plants. Plant metabolites include organic compounds that can be categorized into primary metabolites and secondary metabolites. Glucose, sugar, polysaccharide, collagen, lipids, and nucleic acid are the primary metabolites known to be beneficial for human growth and development (Shakya, 2020).

Plants synthesize secondary metabolites, including alkaloids, flavonoids, saponins, terpenoids, hormones, glycosides, tannins, volatile oils, and vitamins. Plants are therapeutically effective because of these secondary metabolites. These compounds act as herbicides and play an important role in protecting the plant from microbial infection and insect invasion (Singh *et al.*, 2017) . According to the literature, medicinal plants are considered as multi-target, demonstrated anti-inflammatory (Przeor, 2022), antimicrobial (Puttipan *et al.*, 2018), antidiabetic (Eff *et al*., 2020), antioxidant (Mussard *et al*., 2019), and anticancer (Aboalola *et al*., 2020) properties by various mechanisms.

Ginger (*Zingiber officinalis*) is widely used for its anti-inflammatory properties, which is proven by its inhibition on prostaglandin synthesis (Przeor, 2022). Ginger contains phytochemical components that mimic anti-inflammatory drugs. Another example of common herb, *Centella asiatica* has been traditionally used in the treatment of skin disorder and skin inflammation. *C. asiatica* extracts have been shown to positively influence wound healing by improving collagen synthesis and microcirculatory function (*Arribas-López E et al*., 2022).

Moreover, *Z. officinalis* is used in the cancer-therapy as it displayed chemo-preventive effects against skin and breast cancer (Przeor, 2022). Other than that, *Hyoscyamus albus* which belong to the family *Solanacae* was also found to have an anticancer activity (Yahia *et al*., 2020). *Hyoscyamus* species are known to have a pseudo tropine-derived group of alkaloids which have a strong inhibitory activity on ß-glycosidases which is important for cell recognition and can possibly be used in the treatment of some types of cancer (Aboalola *et al*., 2020).

Numerous medicinal plants, including the *Mentha* genus, contain high levels of antioxidants including phenolic compounds, ascorbic acid and carotenoids that can delay or inhibit the oxidation of different molecules (Tafrihi *et al*., 2021). Other than that, AP is commonly known as "Hempedu Bumi" by locals has been used traditionally in treating various diseases. AP contributes antioxidant properties by its ability to scavenge the generation of reactive oxygen species. AP extract was found to inhibit the formation of superoxide (32%), hydroxyl radicals (80%), lipid peroxidation (80%), and nitric oxide (42.8%) in an in vitro model (Mussard *et al*., 2019).

C. asiatica also has antidiabetic property and this has been proven in a study of Alloxan induced diabetic rats, where the plant components improve insulin synthesis by restoring damaged pancreatic cells (Eff *et al*., 2020). The fruits, seeds and callus of *Momordica charantia* contain some insulin-like proteins which are homologous to human insulin, and it produced consistent hypoglycemic effect when tested on rats, gerbils, langurs and human beings. In India and China, *M. charantia* was believed to be a treatment for diabetes mellitus for thousands of years (Tran *et al*., 2020).

Caesalpinia sappan has been reported for its antimicrobial activity against oral pathogens including gram positive and negative strains and its synergism when combines with antibiotics (Puttipan *et al*., 2018). The inhibition activity showing potential treatment of preventing future oral diseases such as dental caries, gingivitis and periodontitis (Puttipan *et al.*, 2018). Significant antibacterial activity of hexane, chloroform, n-butanol, and aqueous fractions of 50% ethyl alcohol-treated extracts of AP exhibited antibacterial activity against *E. coli.* It was reported that the co-presence of andrographolide and arabinogalactan proteins in the ethanol extract was further acknowledged for its enhanced antibacterial activity (Hossain *et al*., 2021).

1.2.2 Safety of herbal medicine

The usage of herbal medicine in the community is increasing year by year globally. Almost 80% of the population consumes herbal products and the number has been increased to 10-20% annually (Samouh *et al*., 2019). They believe herbal medicine is more natural and safer compared to modern medicine. With the increasing usage of herbal products, many studies regarding the safety and efficacy of herbal medicine has been conducted in order to reduce the safety risks and for the continuous development of herbal medicine.

Although herbal medicine has been practised since ancient and is widely used, most herbal medicine does not possess enough clinical data regarding their safety and efficacy. When it comes to herbal medicine, the major concern is the toxicity of the product. Some can be toxic when consumed together with prescribed medication (Suriyo *et al*., 2021). Along with that, the synergistic effect may take place when the phytochemical compounds are mixed with other ingredients in herbal products, or in some cases, patients use modern medicine and herbal medicine simultaneously without proper guidance by the doctors.

With a lack of pharmacology knowledge, one may experience side effects from drug-herb interaction when consuming them together. Hence, it is always advisable to understand that herbal medicine is not always completely safe and effective, especially when used incorrectly. Besides, herbal poisoning is always associated with self-prescribed patients (Karimi *et al*., 2015). In most countries, herbal medicine can be purchased easily over the counter and there is no guarantee that the customers will follow the safety instruction on the label.

According to the literature, toxicity of herbal medicine is frequently occurred when it is taken as dietary supplement for a long period of time (Mussard *et al*., 2020). For example, *Glycyrrhiza spp*. (liquorice) which are often used in Chinese herbal medicine as a treatment of ulcers, heartburn or indigestion, contains mineral corticoid, which can cause adverse reactions such as oedema, hypertension and electrolytes imbalance when consumed in a large amount excessively (Yang & Kang, 2020).

In addition, contamination of dust, microbes, parasites, insects, and heavy metals are commonly detected in herbal product (Welz *et al*., 2018). Furthermore, mass spectrometry revealed the presence of heavy metals such as arsenic, lead, and cadmium. According to the WHO, maximum limit for As in medicinal herb is 1 mg/kg. The herbal is considered as toxic if it contains arsenic levels more than 1 mg/kg above the safe ingestion guidelines (Dinu *et al*., 2021). Contamination of herbal product can lead to the most serious adverse effects including multi-organ failure, poisoning, and death.

On top of that, most of the herbal products produced by the local manufacturer do not undergo proper research process. The effectiveness and safety remain unknown due to lack of lab testing and clinical trials. Many people are unaware that plant active compounds can degrade when improperly stored. Long exposure to heat and light may cause content degradation. As a result, the efficacy of the products is not guaranteed (Yang *et al*., 2018). Inconsistency of herbal medicine quality due to the non-uniform chemical compositions raises a significant challenge for developing quality standardized herbal product.

Due to the nature of herbal medicine practice, which is the formulation is usually tailored to the patient's symptom, thus it is difficult to design a proper methodology for clinical trials studies. The safety remains unknown due to the lack of clinical trials and not many clinical trial studies met the standard of single or double randomized controlled trial. According to a study by Zhang *et al*., (2012), only 2 out of 2000 included clinical trials reported has met the methodological quality.

There is still inadequate scientific evidence to support the basic knowledge of herbal medicine when compared to modern medicine. Hence, more works need to be done to study the safety and efficacy of herbal medicine.

1.3 Oral infection

Oral cavity is predominantly colonized by over 750 species of living bacteria (Saleem *et al*., 2016). In the subgingival crevice alone, 400–500 oral bacteria have been discovered. The remaining are found around the oral spaces, such as the tongue, tooth surface, buccal mucosa, tonsils, soft and hard palate, and lip vestibule (Krishna *et al*., 2017). These bacteria play an important role in maintaining good microbiome inside the oral cavity while there are a few harmful species causing infections. Unlike other diseases, oral infections are not caused by only a single bacterium (Krishnan *et al*., 2017).

Oral diseases are among the most prevalent diseases globally and have serious health and economic burdens, greatly reducing quality of life for those affected (Marco *et al*., 2019). Despite being mostly preventable, oral diseases nevertheless have a high prevalence, which is a result of pervasive social and economic inequality and insufficient support for prevention and treatment, especially in low- and middle-income countries. Dental caries, gingivitis, periodontal disease, thrush, hand, foot, and mouth disease, herpangina, and canker sores are the seven most prevalent oral diseases. Although the exact aetiology of canker sores is unknown, *Helicobacter pylori*, the same bacterium that causes peptic ulcers, may be to blame (Luong *et al*., 2022).

Dental caries as shown in Figure 1.1 is the most prevalent oral disease among children and adults. Many cases reported early dental caries lesion are left untreated because they are always asymptomatic especially in non-developing countries. *S. mutans* and *S. sobrinus* are the specific bacterial agent associated with dental caries infection (Al-Khalifa *et al*., 2021). Besides, there are some other bacteria species such as *Actinomyces* spp. that are also contributed to the disease at the initial stage (Ferrazzano *et al*., 2013).

Another common bacterial infection is periodontal disease which is defined as an infection of the gum tissue. The infection leads to pus formation (dental abscess) or inflammation as shown in Figure 1.1 (a). Severe stage of infection can cause breathing problems and further infection at the upper part of the mouth. In some cases, it can potentially spread up to the brain. Periodontitis is a chronic inflammation induced by gingivitis, a non-destructive form of periodontal disease. It is a disease caused by gram negative anaerobic bacteria, such as *P. gingivalis* (Paulraj & Ramamurthy, 2019).

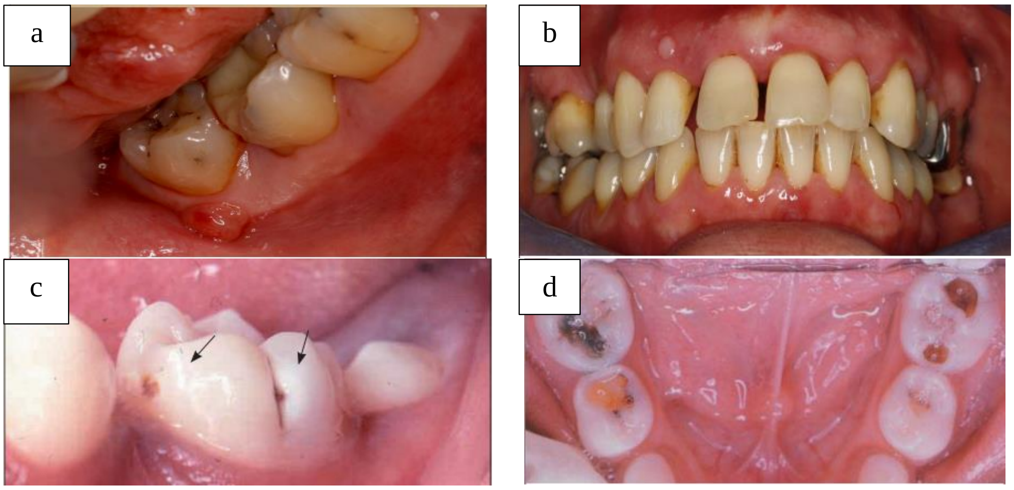

Figure 1.1 (a) Periodontal abscess, (b) Chronic periodontitis, (c) Early childhood caries, (d) Dental caries with cavity (Stephens *et al*., 2018)

However, periodontal bacteria can be a cofactor to the viral infections such as the transmission of Human papillomaviruses (HPV). Oral HPV infections have been linked to sexual behaviour, shows that they are transmitted horizontally, mouth to mouth. The infected area can be very painful which are presented with cold sores and appears as multiple fluid vesicle sacs. From the study (Syrjänen, 2018), infection at oral mucosa was reported as the primary factor of the transmission of HPV in adults to the new born.

Oral fungal infection is commonly associated with *Candida albicans* that causes oral thrush. It is a very common problem and estimated 30–60% of healthy adults having this problem. The infection appears as whitish spots that can spread and cover the area within the mouth including cheek and palate (Hellstein & Marek, 2019). Figure 1.2 shown oral cavity infected with candidiasis.

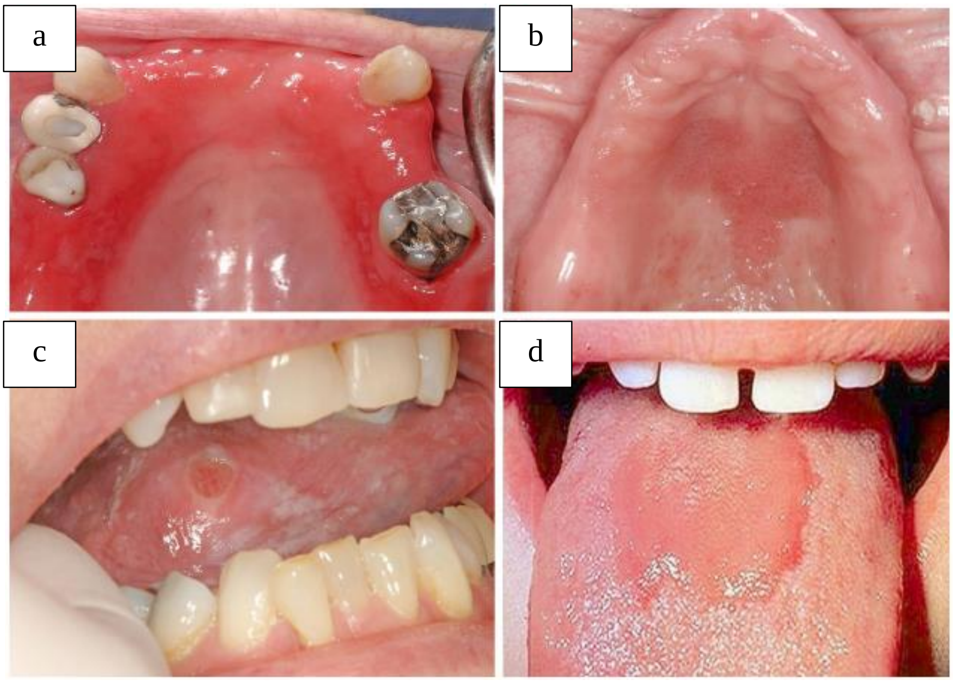

Figure 1.2 (a) Chronic atrophic candidiasis. (b) Inflammatory papillary hyperplasia (IPH). (c) Hyperplastic candidosis with traumatic ulcer and frictional changes. (d) Median rhomboid glossitis (Hellstein & Marek, 2019)

1.3.1 Oral treatment

Dental caries, pulpal necrosis, dental trauma, and periodontal diseases can all cause dental infections with serious consequences for both soft and hard tissues in the oral cavity. Pain and swelling in the oral area are common symptoms of dental infections. These infections should be treated as soon as possible because they can have serious and irreversible consequences. Surgical interventions, endodontic therapy, and antibiotic prescriptions could all be used to treat dental infections (Ahmadi *et al*., 2021).

In dentistry, antibiotics are commonly used to treat odontogenic infections, non-odontogenic infections, local infections, focal infections, and prophylaxis. Patients with immunocompromised conditions, infective endocarditis, metabolic disorders, and patients with prosthetic joints are all given antibiotic prophylaxis (Ahmadi *et al*., 2021). Antibiotics should be prescribed after the infectious sources have been eliminate. Antibiotics should be taken for two to three days after surgical treatments. Antibiotic therapy for longer periods of time was not found to be significantly beneficial and is not recommended (Martins *et al*., 2017).

In non-allergic patients, amoxicillin is commonly used as the first line of treatment. It is the most commonly used antibiotic. Amoxicillin's therapeutic dosage is 500 mg every 8 hours or 1000 mg every 12 hours (Akhavan *et al*., 2020). Dentists prefer to prescribe amoxicillin and metronidazole or co-amoxiclav to control dental infections. Moreover, clindamycin is an alternative drug in penicillin allergic patients (Kumar *et al*., 2014).

Other than that, dental hygiene practice should be applied. The first stage to oral problem is the accumulation of dental plaque on the teeth surface. Without proper dental hygiene practice, the plaque will thicken attributing to serious oral diseases. Oral treatments such as fluoride toothpaste and dental floss are commonly handy to cleanse the formation of dental biofilm on the teeth surface. Toothpaste is defined as cleansing gel works by removing dental plaque deposition. Most of the cleaning process is done by the mechanical movement of toothbrush with the aid from the toothpaste. Basically, toothpaste releases active ingredient such as fluoride to protect the teeth and prevent from tooth and gum diseases (Stephens *et al*., 2018).

However, brushing alone does not remove plaque effectively as compared to the combination use with dental floss. Flossing with the thin thread can reach the part between the teeth that is hardly to reach by the conventional toothbrush (Pandey *et al*., 2020).

Besides, mouthwash is commonly used as an adjunctive to oral treatment. It is not only a liquid used to rinse the oral cavity from pathogenic bacteria but with the therapeutic benefits to freshen the breath. Generally, mouthwash is formulated with flavouring and essential oil. Mouthwash with good active ingredients possess antimicrobial properties and help in the maintenance of healthy mouth. Other than that, mouthwash is a good addition to the cleansing routine as it is convenient to use. It can reach spaces in mouth that are hard to reach, and it also get into the cervices in between the teeth. Chlorhexidine mouthwash is always given to patient as it is considered as a gold standard in the clinical setting (Cieplik *et al*., 2019).

In dentistry, chlorhexidine is used therapeutically and prophylactically. Chlorhexidine is a bisbiguanide antiseptic with antimicrobial properties and is available in the form of mouthwash (Raszewski *et al*., 2019). The safe dosage of chlorhexidine treatment is 18 to 20 mg per application. Chlorhexidine mouthwash with 0.1% to 0.2% concentration demonstrates excellent anti-plaque properties. However, some of the most frequent side effects of chlorhexidine mouthwash also included xerostomia, hypogeusia, and a discolouration of tongue; as well as calculus and extrinsic tooth staining in long-term use (Poppolo Deus & Ouanounou, 2022).

1.3.2 Side effect of oral treatment

Oral treatment has extensively grown with multiple research and development since their early introduction. Prescription antibiotics may cause side effects such as hypersensitivity reactions, dermatological and allergic disorders. Furthermore, overuse of antibiotics can lead to a variety of serious side effects, including bacterial resistance, gastric and haematological issues, and bacterial microbiota diversion. Furthermore, this could lead to oral bacterial resistance, which is a growing concern in dentistry and medicine (Ahmadi *et al*., 2021).

Nowadays, the formulation is becoming much more complex. This, however, can be challenging and risky to the health of consumer. Despite the efficacy of oral treatment in treating oral diseases, there are some flaws to the formulation. Chlorhexidine, xylitol, fluoride and triclosan are popularly used as active ingredients in dental product. However, there is a growing concern about their safety such as multi-organ toxicity (Jayasinghe & Jayawardena, 2019).

High alcohol content in the mouthwash formulation left burning sensation. The inclusion of alcohol in mouthwash has limited its use to certain category of people. The burning sensation of alcohol leaves uncomfortable experience especially to children, patient with oral mucositis and those who does not prefer strong taste. The percentage used in commercial mouthwash should be between 18-26% which is under permitted concentration (Mangulkar, 2020). Alcohol is used in oral treatment formulation as solvent and taste enhancer. It allows the whole formulation dissolved together without phase separation. However, alcohol brings more side effects such as risk of cancer when using in the long run with high percentage (Polizzi *et al*., 2019).

Although chlorhexidine mouthwash is found to be the most effective oral treatment against oral pathogen to prevent oral infection, it does lead to hypogeusia, a condition where a person experienced lack of taste or reduction in taste sensation. Besides, chlorhexidine mouthwash also caused an increase in calculus formation (Van Swaaij *et al*., 2020). Other side effects are burning sensation, hypersensitivity and mucosal lesion. Nevertheless, the major side effect of chlorhexidine mouthwash is tooth staining.

1.3.3 Herbal medicine of oral treatment

Herbal medicine has been traditionally used by folks as oral treatment due to its therapeutic activities and promising result in treating oral disease. Until now, herbal medicine is widely applied in dental treatment as an alternative to the conventional treatment. For example, *Aloe barbadensis* or commonly known as Aloe vera is used as oral gel to treat recurrent oral ulcer. Other than that, aloe vera gel has been utilized in treating oral mucositis in chemotherapy induced patient. Promising result has been reported about therapeutic effect of aloe vera gel to prevent oral mucositis in cancer patient without no serious complication compared to oral treatment, bendazamine (Karbasizade *et al*., 2021).

According to religious belief, tulsi which is an aromatic plant, *Ocimum sanctum* has been used by old folks as the traditional remedy for dental hygiene practice. Tulsi is a tiny shrub in the *Lamiaceae* mint family. It bears purple flowers and short leaves with a pungent odour. The antibacterial activity of the tulsi herbal mouthwash against the oral microorganisms examined was significant. *S. mutans* and related strains show good efficacy, whereas *Lactobacillus acidophilus* showed moderate efficacy (Rakshanaa & Lakshmi, 2017).

In Ayurvedic oral practice, *Azadirachta indica*, known as Neem contains active ingredients such as azadirachtins. According to a study by Agrawal *et al*., (2020) *A.indica* leaf aqueous extract has chemo preventive effect onto oral squamous carcinoma cell by inducing apoptosis and promoting autophagy. The plant has been cultivated for over two millennials due to its health properties. In comparison to regular chlorhexidine mouth rinses, *A. indica* mouth rinses have demonstrated positive results in controlling periodontal infections. *Fusobacterium nuceatum* strains were suppressed by both aqueous and ethanolic extracts (Sneha *et al*., 2021).

Besides, licorice root, ginger and garlic are among commonly used herbal medicine in oral treatment due to the presence of bioactive compound such as glycyrrhizin, gingerol and allicin. These bioactive compounds have been shown to display antimicrobial activity against cariogenic bacteria (Chen *et al*., 2020). According to the research of Malvania *et al*. (2019), a licorice extract produced a significantly higher inhibitory effect on oral pathogens when compared to sodium fluoride. Another action of anti-caries compound is the inhibition of glucosyltransferase which plays a key role in the synthesis of water-insoluble glucan to prevent the formation of cariogenic biofilms. Glycyrrhizin inhibits the adherence of *S. mutans* by affecting the activity of glucosyltransferase (Patel *et al*., 2020). The summary of herbal medicine that is commonly used for oral treatment shown in Table 1.1.

The hunt for herbs with antibacterial activities is progressing, and medicinal plants have long been regarded a viable alternative to manufactured drugs.

Table 1.1 Summary of herbal medicine that is commonly used for oral treatment

No	Plants	Extract & Bioactive Compound	Target Organisms	Biological activity
1	*Acacia arabica*	ethanol, acetone, and water extract	strong biofilm-forming strains isolated from patients	anti-biofilm, antimicrobial
2	*Tamarix aphylla L.*			
3	*Melia azedarach L.*			
4	*Bauhinia forficata*	tincture	*Streptococcus* spp. salivary samples from healthy volunteers	anti-biofilm, antimicrobial
5	*Bauhinia forficata*	phenolic acids, chlorogenic acids	*S. mutans* (ATCC 25175) *Streptococcus sanguinis* (ATCC 10556) *Candida albicans* (ATCC 22972) *Fusubacterium nucleatum* (ATCC 25586) *Lactobacillus casei* (ATCC 393) *Prevotella nigrescens* (ATCC 33563) *Bifidobacterium dentium* (ATCC 27534)	antimicrobial, anti-demineralizing
6	*Curcuma xanthrorrhiza*	ethanol extract, xanthorrhizol	*C. albicans*	anti-biofilm, antimicrobial
7	*Cymbopogon citratus*	lemon grass essential oil	*Streptococcus agalactiae*, *Staphylococcus epidermidisand*, *Lactobacillus fermentum*	antimicrobial, anti-biofilm
8	*Pongamia pinnata*	methanolic extract	*S. mutans* (MTCC 497) *S. mutans* (MTCC 890)	antimicrobial
9	*Acacia catechu*	methanolic extract		
10	Clove	eugenol, oleic acid, lipids	microorganisms collected from extracted teeth	antimicrobial
11	Ginger-garlic paste	gingerol, allicin		
12	Tea tree	catechins		
13	*Camellia japonica*	phenolic compound, flavonoid	*S. mutans* (ATCC 25175) *C. albicans* (NUM961)	antimicrobial, anti-biofilm, anti-GTase
14	*Thuja orientalis*			
15	*Quercus infecteria*	tannins, cardiac glycosides, sterioids, terpenoids, alkaloids	*L. casei*	antimicrobial
16	*Sterculia lychnophora Hance*	organic acids, glycosides,	*S. mutans* (ATCC 25175)	antimicrobial, cariogenic inhibition

Table 1.1 Continued

17	Cinnamon bark	methanol extract, cinnamaldehyde	*C. albicans* (ATCC 2091)	antimicrobial
18	*Cinnamomum burmannii*	water extract	*S. mutans* (UA159)	antimicrobial, anti-biofilm
19	Licorice Root	glycyrrhizin	*S. mutans* (ATCC 25175)	antimicrobial
20	*Eurycoma longifolia jack*	ethanol extract, canthin-6-one alkaloids, β-carboline alkaloids, quassinoids	*C. albicans* *S. mutans* *L. casei*	antifungal, antimicrobial

(Adapted from Chen *et al*., 2020)

1.4 *Andrographis paniculata* (AP)

A. paniculata is an annual herbaceous plant native to India, Pakistan, China, and Southeast Asia. The plant is known as Hempedu Bumi by Malaysians. In other parts of the world, the plant is known as Kalmegh (India), Chiretta (Pakistan), Chirette Verte (France), Senshinren (Japan) and Chuanxinlian (China) and is popularly recognized as the "King of Bitters" due to its extremely bitter aftertaste (Salunkhe & Patil, 2018). It grows in different geographical areas such as plain lands, hilly areas, seashores, wetlands, and dry areas (Akilandeswari *et al*., 2019). The leaves are lanceolate, acuminate at the apex and attenuate at the base, and as it matures, it becomes dark green (Figure 1.3).

The plant usually thrives in a damp, shaded environment. Because it is a salt-sensitive plant, its growth is restricted under stress, particularly salinity stress, which has a significant impact on plant growth and crop output (Hossain *et al*., 2021). Within 90-100 days, a good yield can be obtained. The optimum time to grow the plant is during the rainy season. AP is a member of the *Acanthaceae* family and the taxonomic hierarchy is presented in Table 1.2 (Asasutjarit *et al*., 2021). The plant grows up to 30-110 cm tall, erect stem with non-woody branches. The stem is green in colour and has a leaning appearance. The leaves are dark green, glabrous, and tapering to a point at both ends, or simply being called lanceolate. The leaves margin grows from 1 to 3 cm broad and 8 cm long. The flower has small white petals with a violet mark in the middle (Joghee *et al*., 2020).

Table 1.2 Taxonomy hierarchy of *A. paniculata*

Kingdom	Plantae
Division	Angiospermae
Class	Dicotyledonae
Order	Tubittorae
Family	Acantheacae
Genus	*Andrographis*
Species	*Paniculata*

Figure 1.3 *A. paniculata* grown in its natural habitat (Mussard *et al*., 2019)

1.4.1 Chemical properties of AP

1.4.1 (a) Phytochemical properties

The extract of AP contains lactone, dipertene and bioflavonoids, quinic acid, xanthones, noriridoids, and other chemicals that have therapeutically useful secondary metabolites (Farooqi *et al*., 2020). The secondary metabolites were isolated primarily from aerial parts in the majority of cases, leaves, the entire plant, and some from roots. The quantity and quality of phytoconstituents are greatly influenced by the part used, geography, season, and time of harvesting of plant materials. The yield reached its peak when the plant materials were harvested between 110–130 days of cultivation (Hossain *et al*., 2014).

AP contains 55 ent-labdane diterpenoids, 30 flavonoids, eight quinic acid derivatives, four xanthones, five uncommon noriridoids and 142 secondary metabolites (Hossain *et al.*, 2021). The major chemical constituent in AP is andrographolide (Vetvicka & Vannucci, 2021). It is a colourless crystalline compound called diterpene lactone. Andrographolide is responsible for giving the plant a bitter taste. Diterpene lactone andrographolide and its derivatives are the most well-known bioactive chemical ingredients of AP.

Andrographolide is mostly found in the leaves but is also found in small amounts in the stems, roots, and flowering tops. Andrographolide's chemical formula and weight are $C_{20}H_{30}O_5$ and 350.4, respectively. Andrographolide has a decahydronaphthalene ring in trans conformation, a g-lactone ring in twisted conformation, a double bond bridge D_{12}, a 13-linked g-lactone ring, a decahydronaphthalene ring in E configuration, an exocyclic double bond, and three hydroxy groups in its structure (Zhang *et al*., 2021). The chemical structure of andrographolide compound is shown in Figure 1.4. Andrographolide is extracted mostly from AP, and is soluble in organic solvents such as ethanol, chloroform, ether, acetone, and dimethyl sulfoxide. High-performance liquid chromatography, thin-layer chromatography, UV absorption spectrum, liquid chromatography-mass spectrometry, and differential scanning calorimetry are commonly used to test its purity.

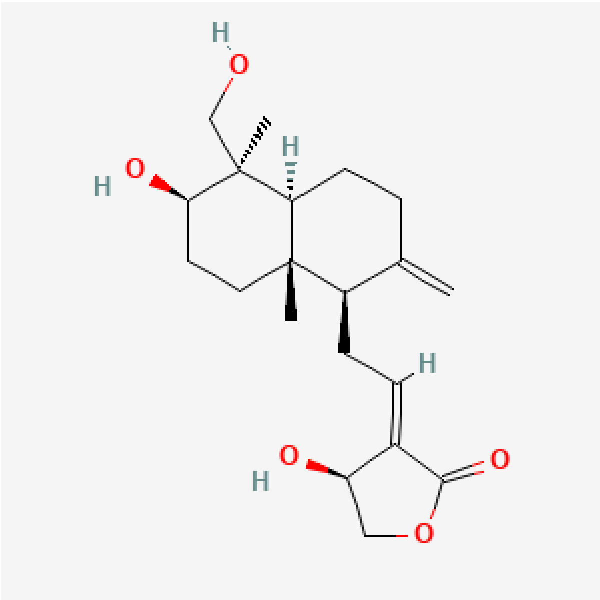

Figure 1.4 Chemical structure of andrographolide compound (Mussard *et al*., 2019)

1.4.1 (b) Phytochemical properties

Andrographolide's excellent properties in the treatment of numerous diseases related to inflammation, virus infection, bacterial dysentery, malaria, herpes, fever, laryngitis, and rheumatoid arthritis make this plant a valuable medicinal asset (Gupta *et al*., 2017).

Currently, andrographolide tablets or dropping pills, as well as water-soluble andrographolide derivative injections, are commercially available in China and used clinically to treat upper respiratory tract infections, pediatric pneumonia and bacillary dysentery (Zhang *et al*., 2021). Andrographolide and its derivatives have demonstrated many pharmacological benefits, such as anti-inflammatory, antibacterial, anticancer,