1. Introduction and Review

1.1 Oral infections and present remedies

Oral health can be defined as being free from chronic mouth and facial pain, oral sores, periodontal (gum) disease, tooth decay, tooth loss and other diseases/disorders that affect the mouth and oral cavity. The mouth can be considered an ideal environment for the growth of microorganisms, because it is warm and moist and has a constant influx of nutrients through saliva and food intake. Oral infections are one of the most common diseases worldwide, leading to dental caries and periodontal disease.\(^1\) Dental caries and periodontal diseases are among the most important global oral health problems, although conditions such as oral and pharyngeal cancers and oral tissue lesions are also significant health concerns.\(^2\) In India, dental caries affect 60-65% of the general population.\(^3\) In addition, periodontal disease is estimated to occur in 50-90% of the population in India, depending on age.\(^4\)

Dental caries is an infectious microbial disease that results in localized dissolution and destruction of the calcified tissues of the teeth or it is the localized destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates. The acid released through microbial action would cause local pH to fall below a critical value resulting in demineralization and cavitations of tooth.\(^5\)\(^-\)\(^8\) Developed dental caries involve three factors that must be present at the same time: a susceptible tooth, high consumption of fermentable carbohydrates and mainly, the bacterial mutant streptococci.\(^5\) Different kinds of gram-positive bacteria are closely related to the formation and progression of dental caries.\(^9\) More than 750 species of bacteria dwell in the oral cavity (\(~50\%\) of which are yet to be identified) and a number of these are implicated in oral disease.\(^10\) Dental caries is a supragingival condition.\(^11\) It is a significant Dental-Public-Health dilemma and is most widespread oral disease in the world and an estimated five billion people worldwide have experienced dental caries.\(^2\) In developing countries like India, the rate of dental caries is rising and since more than 80% of the world’s children live in these countries, so that it has been considered to be a major public health problem.

Periodontal disease is one of the world’s most prevalent chronic diseases, which has been considered as a possible risk factor in some systemic diseases; periodontal diseases seriously threaten people’s quality of life. Periodontitis, a destructive gum disease, may progress irreversibly in breaking down supporting periodontal structures,
results in loss of tooth and about 20% population of the world are affected by these diseases.\textsuperscript{1-2} An etiology of chronic periodontal disease remains unknown, although gram-negative anaerobic bacteria have been implicated in the disease.\textsuperscript{12-13} It is a sub-gingival condition that has been linked and afforded a varied environment for the colonization of gram negative facultative or obligate anaerobes like \textit{Porphyromonas gingivalis}, \textit{Bacteroides} species, \textit{Capnocytophaga} species, \textit{Actinobacillus actinomycetemcomitans} and anaerobic gram-negative bacteria such as \textit{Porphyromonas gingivalis}, \textit{Actinobacillus} species, \textit{Prevotella} species and \textit{Fusobacterium} species.\textsuperscript{10,14-15}

A dental abscess also termed as a dentoalveolar abscess, tooth abscess or root abscess, is a localized collection of pus associated with a tooth. The most common type of dental abscess is a periapical abscess and the second most common is a periodontal abscess.\textsuperscript{16} The acute dental abscess usually occurred secondary to dental caries, trauma or failed root treatment. Historically, the potential for a dental abscess to spread, causing severe sepsis and death has been known since antiquity, although the role of bacteria in this process was not recognized until the turn of the 20\textsuperscript{th} century.\textsuperscript{17} To date, only ‘chlorhexidine’ and ‘listerine’ (a combination of “essential oils”) have gained the approval of the American dental association council on dental therapeutics. Several methods, such as topical or systemic use of fluorides, fissure sealants, and dietary control, have been developed to prevent dental caries in humans. Some antiseptics as well as antibacterial agents like triclosan, chlorhexidine and amine based fluorides are also used to treat caries. Various compounds have been studied for their antibacterial activity against cariogenic bacteria.\textsuperscript{9} The third generation cephalosporins such as ceftriaxone and fourth generation cephalosporins like cefepime, exhibited good activity. The quinolones, glycopeptides and rifampicin confirmed a good activity against oral \textit{streptococci}.\textsuperscript{18} There have been numerous reports on the use of traditional plants and natural products for the treatment of oral diseases.\textsuperscript{19-21} A large numbers of plant species are known for thousands of years to prevent or cure oral diseases.\textsuperscript{15}

\textbf{1.2 Herbal remedies for oral infections}

Herbal medicine represents one of the most important fields of traditional medicine. Natural products have been used for thousands of years in folk medicine for several purposes. In India, plant wealth is greatly exploited for its therapeutic potential and
medicinal efficacy to cure various oral ailments since time immemorial. Therefore, the search for alternative products continues, however, plant-derived compounds (phytochemicals) have been attracting much interest as natural alternatives to synthetic compounds.\textsuperscript{22-24} Plant products are currently gaining attention for treatment of various ailments. Thereby people today are diverting to the natural system of medicines and natural products like herbal tooth pastes, gels, tablets, chewing gum, mouthwash etc. The world health organization (WHO) recognized that medicinal plants played an important role in the health care of about 80 percent of the world population in developing countries and depends largely on traditional medicine.\textsuperscript{25} So, there is a great scope for new drug discoveries based on traditional plant uses.\textsuperscript{26-28}

Progressively more attention has been focused to natural antibacterial substances as useful antimicrobials.\textsuperscript{29} These plant-derived substances, mainly polyphenols, including extracts of miswak,\textsuperscript{30} green tea,\textsuperscript{31-32} twigs of plants like babul, neem, clove oil\textsuperscript{33-34} and tea tree oil\textsuperscript{35} have previously been incorporated into products such as mouth rinses to enhance their antimicrobial properties. Literature survey reveal several reports on herbal formulations against dental diseases viz., development of guava extract chewable tablets for anticariogenic activity against \textit{Streptococcus mutans}.\textsuperscript{36} Thombre \textit{et al.},\textsuperscript{37} had attempted formulation of chewable tablet using polyherbals viz., \textit{Mangifera indica} (fruit), \textit{Azadirachta indica} (bark), \textit{Caryophyllus aromaticus} (fruit), \textit{Terminalia chebula} (bark), \textit{Emblica officinalis} (fruit), \textit{Terminalia belerica} (fruit), \textit{Cinnamomum zeylanicum} (bark), \textit{Quercus infectoria} (fruit).

The antimicrobial potential of \textit{Spilanthes acmella} gel against various microorganisms (\textit{E. coli}, \textit{Streptococcus}, \textit{Bacillus cereus}, \textit{Lactobacilli}) was responsible for causing tooth decay.\textsuperscript{38} The polyherbal formulations of hydro-alcoholic extracts of \textit{Achyranthes aspera}, \textit{Syzygium aromaticum}, \textit{Piper nigrum}, \textit{Mimusops elengi}, \textit{Azadirachta indica}, \textit{Curcuma longa}, \textit{Zingiber officinale}, \textit{Salvadora persica}, \textit{Acacia nilotica}, \textit{Zanthzylum armatum} and \textit{Sapindus mucorosaias} were the effective herbal remedies for maintaining oral hygiene, since they possess potent antimicrobial activity against bacteria (\textit{Staphylococcus aureus}; \textit{Bacillus subtilis}; \textit{Micrococcus luteus}; \textit{Pseudomonas aeruginosa}; \textit{Escherichia coli}; \textit{Streptococcus mutans}) and yeast (\textit{Candida albicans}) strains which are a major cause of dental infections.\textsuperscript{39} Amongst all reviewed herbal formulations herbal gel and herbal mouthwashes were found to be most widely accepted for the treatment of various dental diseases. Therefore, the global markets
are turning to plants as a potential and realistic source of ingredients for health care products.27

1.3 Extraction methods and choice of solvents
Plant materials are composed of heterogeneous mixtures of constituents, some of which are pharmacologically active and others pharmacologically inactive and considered inert. Among the varied plant constituents sugars, starches, mucilages, proteins, albumins, pectins, cellulose, gums, inorganic salts, fixed and volatile oils, resins, tannins, colouring materials and a number of very active constituents such as alkaloids and glycosides. Extracts can be defined as preparations of crude drugs which contain all the constituents which are soluble in the solvent used in making the extract. Prior to any isolation and purification work, natural products have to be extracted from the biomass.

In many instances, the active constituents of a plant drug are of the same general chemical type, have similar solubility characteristics and can be simultaneously extracted with a single solvent or a mixture of solvents. Extraction concentrates the active constituents of a crude drug and removes from it the extraneous matter. Hydro alcoholic mixtures are perhaps the most versatile and most widely employed menstruum. A hydro alcoholic menstruum generally provides inherent protection against microbial contamination and helps to prevent the separation of extracted material on standing.

The principal methods of drug extraction are maceration and percolation. The method of extraction selected for a given drug depends on several factors, including the nature of the crude drug, its adaptability to each of the various extraction methods and the interest in obtaining complete or nearly complete extraction of the drug. Frequently, a combination of maceration and percolation is actually employed in the extraction of a crude drug. The drug is macerated first to soften the plant tissues and to dissolve much of the active constituents and percolation separates the extractive from the marc.40-41

1.3.1 Maceration
The properly comminute drug is permitted to soak in the menstruum until the cellular structure is softened and penetrated by the menstruum and soluble constituents are dissolved. Maceration is usually conducted at a temperature of 15 °C to 20 °C for three days or until the soluble matter is dissolved.
1.3.2 Infusion
Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs.

1.3.3 Digestion
This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable. The solvent efficiency of the menstruum is thereby increased.

1.3.4 Decoction
In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat stable constituents.

1.3.5 Percolation
The comminute drug is extracted off its soluble constituents by the slow passage of a suitable solvent through a column of the drug. The drug is packed in a special extraction apparatus termed as a percolator, with the collected extractive called the percolate. Percolators for drug extraction vary greatly as to their shape, capacities, composition and above all, their utility.

1.3.6 Hot continuous extraction (Soxhlet)
The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a smaller quantity of solvent. This affects tremendous economy in terms of time, energy and consequently financial inputs.

1.3.7 Choice of solvents
An ideal solvent for a certain pharmacologically active constituent should,

- be highly selective for the compound to be extracted.
- have a high capacity for extraction in terms of coefficient of saturation of the compound in the medium.
- not react with the extracted compound or with other compounds in the plant material.
- have a low price and be harmless to man and to the environment.
- be completely volatile.

Aliphatic alcohols with up to three carbon atoms, or mixtures of the alcohols with water, are the solvents with the greatest extractive power for almost all natural
substances of low molecular weight like alkaloids, saponins and flavonoids. Ethanol is usually mixed with water to induce swelling of the plant particles and to increase the porosity of the cell walls, which facilitates the diffusion of extracted substances from inside the cells to the surrounding solvent. The most common solvents used in extraction include water, ethanol, methanol, acetone, n-hexane, n-butanol, ethyl acetate, etc.

1.4 Microorganisms from caries and abscess

Cultural analysis remains the backbone of clinical practice and the findings of a number of prospective and retrospective studies give a valuable insight into the bacteria which are often present. The ideal clinical sample from an acute dental abscess is an aspirate through intact mucosa disinfected by an appropriate antiseptic mouthwash or swab like chlorhexidine, although some researchers have sampled purulent exudates from within infected canals. This will reduce contamination from the normal oral flora. Previous studies using swabs of purulent material have demonstrated poor recovery of strict anaerobes and low mean numbers of isolates per sample (range 1.0–1.6). A number of gram positive bacteria have been closely related to the formation and progression of dental caries. Organisms such as *Streptococcus mutans*, *Staphylococcus aureus*, *Streptococcus mitis*, etc. were the primary cariogenic bacteria involved. A number of studies have found that children with high caries activity are more likely to carry both *S. mutans* and *S. sobrinus*. A wide range of *Lactobacillus* species can be isolated from carious lesions. Several studies have found considerable numbers of *Bifidobacterium* in caries lesions and show that their numbers correlate with other caries associated bacteria. A recent study detected three hundred different species and has revealed further complexity and introduced new caries-associated species such as *Pseudoramibacter alactolyticus*. In a pioneering report examining twenty three species, the strong association with caries for *S. mutans* was observed; *S. sobrinus* was not implicated though several other species were, including *Actinomyces gerencseriae* and a *Bifidobacterium*. It has been reported that the microorganisms that colonize the periodontal abscesses are primarily gram negative anaerobic rods. Although they are not found in all cases of periodontal abscesses, high frequencies of *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Campylobacter rectus* and *Capnocytophaga*
species have been reported. \(^52\) *Streptococcus viridans* is the most common isolate in the exudates of periodontal abscesses. Spirochetes have been found as the predominant cell types in periodontal abscesses when assessed by darkfield microscopy. Strains of *Peptostreptococcus, Streptococcus milleri (S. anginosus and S. intermedius), Bacteroides capillosus, Veillonella, B. fragilis* and *Eikenella corrodens* have also been isolated. The culture studies of periodontal abscesses have revealed a high prevalence of *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Campylobacter rectus* and *Prevotella melaninogenica*.\(^{53-55}\) *Bacillus pumilus, Bacillus thuringiensis, Pseudomonas aeruginosa, Enterococcus faecalis* and *Acinetobacter junii* were well associated with earlier reported literature in dental diseases.\(^{56-58}\)

### 1.5 Antimicrobial activity: conventional and advanced methods

In general, antimicrobial activity of any substance can be investigated by various screening methods. Both *in vivo* and *in vitro* methods are widely used for screening of compounds for antimicrobial activity. Amongst them, *in vitro* methods are extensively used for the preliminary evaluation of antibacterial and antifungal activities. Further, *in vivo* studies are employed on animal models of the human condition necessary to elucidate the mechanisms of antimicrobial action and to develop drugs that can control infection caused by pathogenic microbes. During the last decades, several experimental procedures were developed for antimicrobial susceptibility testing (AST) by CLSI (clinical and laboratory standards institute) that created standards to perform ASTs. These methods are extensively being used to determine the molecular potency against microbes.

The following three methods have been shown to consistently provide reproducible and repeatable results when followed correctly; \(^{59-60}\) diffusion, broth dilution and agar dilution.

#### 1.5.1 Diffusion methods

Diffusion methods involve two important techniques, *viz.* Stokes method and Kirby-Bauer method. These methods are typically used for antimicrobial susceptibility testing, which are being well recommended by the national committee for clinical laboratory standards (NCCLS).

#### 1.5.1.1. Stokes method

In this method a known quantity of bacteria is grown on agar plates in the presence of...
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61 If the bacteria were susceptible to a particular antimicrobial agent, an area of the clearing surrounding the wafer was formed where bacteria were not capable of growing (called a zone of inhibition). Also, the rates of antimicrobial diffusion are determined and these values are used to estimate the bacterial sensitivity to that particular antimicrobial agent. In general, larger zones correlate with smaller concentrations of test compounds for a specific microorganism. This information can be used to choose appropriate antimicrobials to combat a particular infection.

1.5.1.2 Kirby method

The Kirby-Bauer test, recognized as the disk-diffusion method, is the most extensively used AST in determining the precise antibiotics used to treat the exact infection. Disk diffusion refers to the diffusion of an antimicrobial agent of a specified concentration from disks, tablets or strips, into the solid culture medium that has been seeded with the selected inoculum isolated in a pure culture. Disk diffusion is based on the determination of an inhibition zone proportional to the bacterial susceptibility to the antimicrobial present in the disk. The diffusion of the antimicrobial agent into the seeded culture media resulted in a gradient of the antimicrobial. When the concentration of the antimicrobial became so diluted that it could no longer inhibit the growth of the test bacterium, the zone of inhibition was demarcated. The diameter of this zone of inhibition around the antimicrobial disk was related to minimum inhibitory concentration (MIC) for that particular bacterium/antimicrobial combination; the zone of inhibition correlated inversely with the MIC of the test bacterium. Generally, the larger the zone of inhibition, the lower the concentration of antimicrobial required to inhibit the growth of the organism. However, this depended on the concentration of antibiotic in the disk and its diffusibility.

1.5.2 Dilution methods

Dilution technique principally included MIC method, which could be further classified as broth dilution and agar dilution methods.

1.5.2.1 Minimum inhibitory concentration (MIC) method

The MIC method has usually been used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganisms completely. The aim of the broth and agar dilution methods was to determine the lowest concentration of the assayed
antimicrobial that inhibits the visible growth of the bacterium being tested (MIC, usually expressed in µg/mL or mg/L). However, MIC does not always represent an absolute value. The ‘true’ MIC is a point between the lowest test concentration that inhibits the growth of the bacterium and the next lower test concentration. Therefore, MIC determinations performed using a dilution series may be considered to have an inherent variation of one dilution.

1.5.2.1.1 Broth dilution method

The broth dilution method is a simple technique for testing a small number of isolates, even single isolate. It involved serial dilution of the antimicrobial agent in a liquid medium, which was then inoculated with a standardized number of organisms and incubated for a prescribed time. The lowest concentration of antibiotic preventing appearance of turbidity was considered to be the minimal inhibitory concentration. It had the added advantage that the same tubes could be taken for minimum bactericidal concentration (MBC) tests also.

1.5.2.1.2 Agar dilution method

In this method, the compounds under screening were diluted on log_2 dilution intervals where each petri dish contained fifty percent of the concentration of given compound in the previous dilution. The diluted solution was incorporated into the agar medium and mixed by gentle rotation and poured into a petri dish. A control plate without any antimicrobial agent incorporated into the medium was also used along with each compound tested, to check for growth of the test and control strains. Readings were recorded after the petri dishes were incubated. The main advantage of the method was that it was possible to test several organisms on the same plate. Even though a variety of methods exist, the goal of in vitro antimicrobial susceptibility testing was to provide a reliable predictor of how an organism was likely to respond to antimicrobial therapy in the infected host. This type of information aided the clinician in selecting the appropriate antimicrobial agent, useful in developing antimicrobial use policy and provided data for epidemiological surveillance. The selection of a particular AST method was based on many factors such as validation data, cost, practicality, flexibility, reproducibility, accuracy and individual preference.

1.5.2.1.3 Advanced methods and future directions in antimicrobial susceptibility/resistance detection

Despite the traditionally used methods, microplate reader is a widely-used instrument
that allows for many samples to be simultaneously measured, as if many miniscule experiments were being performed at the same time.\textsuperscript{63} This apparatus is used in conjunction with multiwell plates, like the 96 wells plate. Regardless of the type of experiment run with the micro plate reader, standard curves were often used to determine the value of experimental samples, as well as positive and negative controls. The use of genotypic approaches for detection of antimicrobial resistance genes has been promoted as a way to increase the rapidity and accuracy of susceptibility testing.\textsuperscript{64}

Numerous DNA-based assays are being developed to detect bacterial antibiotic resistance at the genetic level. Methods that utilize the use of comparative genomics, genetic probes, microarrays, nucleic acid amplification techniques (e.g. polymerase chain reaction [PCR]) and DNA sequencing offer the promise of increased sensitivity, specificity, and speed in the detection of specific known resistance genes.\textsuperscript{64-65} Genotypic methods have been effectively applied to supplement traditional AST phenotypic methods for other organisms including methicillin-resistant \textit{staphylococci}, vancomycin-resistant \textit{enterococci}, and detection of fluoroquinolone resistance mutations.\textsuperscript{64-66} However, despite the new influx of genotypic tests, documented and agreed upon, phenotypic AST methods will still be required in the near future to detect emerging resistance mechanisms among bacterial pathogens.

1.6. Problems faced during dental caries chemotherapy

Dental caries is highly prevalent in India, which is influenced by the lack of dental awareness among the public. Even though the burden of dental caries is not so high in developing countries as compared to developed countries, the inability to pay for dental treatment has worsened the prevalence of dental illnesses in most developing countries.\textsuperscript{67} Dental treatment is usually a high expense remedy and it mainly utilizes some antiseptics as well as antibacterial agents like triclosan, chlorhexidine and amine based fluorides. But the major drawback of these products was that they possess significant toxicity and were also responsible for the staining of teeth, burning sensation on tongue, diarrhoea, vomiting, increased calculus formation.\textsuperscript{68-71} On the contrast several methods, such as topical or systemic use of fluorides, fissure sealants and dietary control, have been developed to prevent dental caries. The efficacies of these methods were not enough to eradicate dental caries in humans. Bacterial resistance to most (if not all) of the antibiotics often used to treat oral infections
(penicillins, vancomycin, erythromycin, tetracycline, cephalosporins and derivatives and metronidazole) has been documented.\textsuperscript{72-76} However, a high dose is consistent with a clinical cure has been shown to be effective and may reduce the development of resistance.\textsuperscript{77} Other antibacterial agents used in the prevention and treatment of oral diseases, including chlorhexidine, cetylpyridinium chloride, amine fluorides or products containing such agents, were reported to exhibit toxicity, cause staining of teeth or in the case of ethanol (usually found in mouthwashes) have been linked to oral cancer.\textsuperscript{78-79} The data illustrated by reports of resistance rates for amoxicillin ranged from 9 to 54\% of common isolates from acute dental abscesses.\textsuperscript{80-82}

1.7. Use of advanced technology-nanotechnology to combat the problems

One of the most recurrent problems in dental caries chemotherapy is the inability to maintain the MIC of the drug in oral mucosa. Delivering vesicles such as dentifrices or mouth rinses introduce antimicrobial agents into the oral cavity with an initial concentration. Almost instantaneously, the initial concentration started decreasing due to the dynamics of the oral cavity and eventually dropped below the MIC. Thus, there is a persistent need of alternative methods for delivering antimicrobial agents to the oral cavity.

Long retention time could be achieved by using polymeric delivery systems. A denitrifies containing both triclosan and copolymer enhanced the retention of triclosan and improved its anti-plaque efficacy when compared to triclosan alone by both \textit{in-vitro} and \textit{in-vivo} studies.\textsuperscript{83-84}

Polymeric nanoparticles have attracted much attention as delivery systems due to their ability in overcoming the physiological barriers and protecting as well as targeting the loaded substances to specific cells.\textsuperscript{85-86} A mineral-binding micellar drug delivery system was developed, which could quickly bind to the tooth surface and release encapsulated drug over a prolonged period of time. This was accomplished by covalently conjugating the tooth-binding moieties to the ends of pluronic copolymer using “click chemistry”.\textsuperscript{87}

Nanotechnology has been enormously applied in forensic science, agriculture, fiber, textiles, electronics, space and medical therapeutics.\textsuperscript{88} The application of nanotechnology in medicine gave the birth of a new concept nanomedicine. So far, many nanomedicine formulations have been developed, like nanoparticles, nanocapsules, micellar systems and dendrimers.\textsuperscript{88} These nanoscaled formulations
improve drug bioavailability, prolong drug in vivo circulating half-live and decrease drug size effect as a drug delivery system. In addition, nanomedicine has specific advantages due to its nanoscaled size and targeted drug delivery that is achieved without difficulty.

Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm. They consist of macromolecular materials and could be used therapeutically as adjuvant in vaccines or drug carriers, in which the active ingredient was dissolved, entrapped, encapsulated, adsorbed or chemically attached. Polymers used to form nanoparticles could be both synthetic and natural polymers. Amongst water-soluble polymers available, chitosan is one of the most extensively studied. This is because chitosan possesses some ideal properties of polymeric carriers for nanoparticles such as biocompatible, biodegradable, nontoxic and inexpensive. Furthermore, it possessed positive charge and exhibited absorption enhancing effect. These properties render chitosan a very attractive material as a drug delivery carrier. In the last two decades, chitosan nanoparticles (chitosan NP) have been extensively developed and explored for various applications.

Chitosan is an abundant polysaccharide and a cationic polyelectrolyte present in nature.\textsuperscript{89} It is a linear polysaccharide, which has gained a lot of attention in the area of drug delivery due to the advantageous biological properties which included non-toxicity, biocompatibility, biodegradability, cationic nature, bio-adhesiveness and permeability-enhancing effect.\textsuperscript{89-92} Its intrinsic antimicrobial properties and biodegradability made it an ideal candidate for antimicrobial substances. Chitosan has progressively attracted attention due to its numerous bioactivities, such as antimicrobial.\textsuperscript{90,92-94} and antitumor.\textsuperscript{95} Particularly, antibacterial activity of chitosan has been followed with immense interest. Chitosan inhibited the growth of a fairly diverse range of bacteria\textsuperscript{94,96} and thus offered a great benefit to a wide variety of applications like medical\textsuperscript{97} and agricultural.\textsuperscript{98}

Chitosan nanoparticles have gained more attention as drug delivery carriers because of their better stability, low toxicity, simple and mild preparation method, and providing versatile routes of administration. As a new drug delivery system, they have attracted increasing attention for their wide applications in loading protein drugs, gene drugs and anticancer chemical drugs and via various routes of administration including oral, nasal, intravenous and ocular.
1.7.1 Advantages of nanoparticulate drug delivery

- Simple and inexpensive to manufacture and scale-up.
- No heat or high shear forces or organic solvents involved in their preparation process.
- Reproducible and stable.
- Applicable to a broad category of drugs; small molecules, proteins and polynucleotides.
- Ability to lyophilize and non toxic.
- Stable after administration.

1.7.2 Preparation methods of chitosan nanoparticles (NP)

The chitosan NP preparation technique has been developed based on chitosan microparticles technology. There are at least four methods available: ionotropic gelation, microemulsion, emulsification solvent diffusion and polyelectrolyte complex. The most extensively developed methods are ionotropic gelation and self assemble polyelectrolytes complex formation. These methods offer many advantages such as simple and mild preparation method without the use of organic solvent or high shear force.

1.7.2.1 Ionotropic gelation

Chitosan NP prepared by ionotropic gelation method was first reported by and has been extensively examined and developed. The mechanism of chitosan NP formation is based on electrostatic interaction between amine group of chitosan and negatively charged group of polyanion such as tripolyphosphate. When chitosan solution is in an appropriate concentration range, the opalescent suspension is obtained in which nanoparticles can be further proved. Initially, chitosan can be dissolved in acetic acid in the absence or presence of stabilizing agent, such as poloxamer, which can be added in the chitosan solution before or after the addition of polyanion. Polyanion or anionic polymer was then added and nanoparticles were spontaneously formed under mechanical stirring at room temperature. The size and surface charge of particles can be modified by varying the ratio of chitosan and stabilizer. The major disadvantage of the chitosan nanoparticles produced by this method was drug burst release effect and low particles yield.

1.7.2.2 Emulsification solvent diffusion method

The preparation of chitosan nanoparticles has been reported by emulsion solvent
diffusion method. An oil-in-water emulsion was obtained by adding an organic solvent partially miscible into chitosan solution containing a stabilizing agent under magnetic stirring, followed by high-speed homogenization. The resulting emulsion was diluted in a large amount of water to extract the organic solvent. Nanoparticles were obtained as a result of the diffusion of the organic solvent into water. The main drawbacks of this method included ruthless processing conditions (e.g., the use of organic solvents) and the high shear forces used throughout nanoparticle preparation.

1.7.2.3 Microemulsion method
Chitosan NP prepared by microemulsion method was first reported. This technique was based on formation of chitosan NP in the aqueous core of reverse micellar droplets and afterwards cross-linked through glutaraldehyde. In this method, a surfactant was dissolved in n-hexane. Subsequently, chitosan in acetic solution and glutaraldehyde were added to surfactant/hexane mixture under continuous stirring at room temperature. Nanoparticles were formed in the presence of surfactant.

1.7.2.4 Emulsion-droplet coalescence method
An emulsion-droplet coalescence technique to prepare chitosan nanoparticles was first reported by Tokumitsu et al. Herein both chitosan and sodium hydroxide solutions were emulsified into the same oil phase under the conditions to prepare two emulsions. The two emulsions were then mixed and stirred at high rotating speed to afford chitosan nanoparticles. The hydrophilic drug- gadopentetic acid encapsulated chitosan nanoparticles had a diameter of 452 nm and were proved suitable for intravenous injection.

1.7.3 Applications of chitosan nanoparticles
1.7.3.1 Oral drug delivery
An idea that nanoparticles might protect labile drugs from enzymatic degradation in the gastrointestinal tract lead to the development of nanoparticles as oral delivery systems for macromolecules, proteins and polynucleotides. Amongst polymeric nanoparticles, chitosan NP proved to be attractive carriers for oral delivery vehicle as they endorse absorption of drug Sarmento et al. reported that alginate/chitosan nanoparticles administered orally to diabetic rats were found effective for oral insulin delivery whereas, Zhang et al. reported that water-soluble chitosan nanoparticles enhanced and prolonged intestinal absorption of bovine serum albumin. Pan et al. mentioned that hypoglycemic effect was observed in induced diabetic rats after oral
administration of chitosan nanoparticles. Moreover, chitosan can be employed as a coating material for liposomes, nanocapsules to improve their residence time, thereby improving drug bioavailability.\textsuperscript{110-111}

1.7.3.2 Pulmonary and nasal drug delivery

Pulmonary and nasal routes were considered as promising routes to deliver peptides and proteins because they possessed very large surface areas and manifested less intracellular and extracellular enzymatic degradation.\textsuperscript{112} It may be administered as solution or powder with absorption enhancing agent to slow down mucociliary clearance process and thereby prolong the contact time between the formulation and nasal tissue. Recently, chitosan was demonstrated to promote absorption of insulin in rats as well as the sheep nasal mucosa. On the other hand, the insulin-chitosan powder, chitosan blended with insulin using pestle and mortar, endowed to have bioavailability greater than chitosan NP containing insulin.\textsuperscript{113} Shahnaz \textit{et al.}\textsuperscript{114} reported a thiolated nanoparticle to enhance the bioavailability for the nasal application of leuprolide. The inter- and/or intramolecular disulfide formation inside the NPs network stabilized obtained NPs and accomplished a sustained release of leuprolide over six hours.

1.7.3.3 Gene delivery

Albeit viruses could efficiently transfer genes into cells, concerns such as host immune response, residual pathogenicity and potential induction of neoplastic growth following insertional mutagenesis have led to the exploration of non-viral gene transfer systems.\textsuperscript{115} Katas \textit{et al.}\textsuperscript{116} reported a 100\% protection of siRNAs from nuclease degradation by chitosan nanoparticles. On the other hand, usually gene transfection efficiency of chitosan nanoparticles was lower than that of viral gene carriers. Mansouri \textit{et al.}\textsuperscript{117} mentioned folic acid modified chitosan nanoparticles to improve gene transfection efficiency. Their results revealed that the folic acid-modified chitosan nanoparticles exhibited low cell toxicity and were able to condense DNA effectively with ideal size and zeta potential. Chitosan mediated efficient \textit{in vitro} gene transfer at nitrogen to phosphate ratio (N/P, 3:5). Sato \textit{et al.},\textsuperscript{118} found that \textit{in vitro} chitosan-mediated transfection depended on the cell type, serum concentration, pH and molecular weight of chitosan.

1.7.3.4 Ocular drug delivery

De la Fuente \textit{et al.},\textsuperscript{119} studied hyaluronic acid-chitosan nanoparticles to deliver genes

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1.7.3.5 Vaccine delivery
Chitosan nanoparticles have been used in vaccine delivery owing to their bioadhesive, biocompatible, biodegradable and permeation-enhancement properties. They can be effectively up taken from phagocytotic cells, inducing strong systemic and mucosal immune responses against antigens. Wen et al.,\textsuperscript{122} investigated the immune stimulation mechanisms of ovalbumin (a frequently used antigen model) loaded chitosan nanoparticles. They suggested that chitosan nanoparticles had a strong potential to increase both cellular and humoral immune responses.

1.7.4 In situ gelling system

In situ is a latin phrase which translated literally as “In process”. In situ gels were drug delivery systems that were in solution forms before administration in the body. Once administered they underwent gelation in situ to form a gel. Alternatively, in situ gel referred to polymer solution which could be administered as a liquid. It underwent a phase transition to semisolid gel upon exposure to the physiological environment.\textsuperscript{123} It was basically a polymeric drug delivery system. Administration routes for in situ gels were oral, ocular, nasal, rectal and vaginal.\textsuperscript{124-129} The gelation could be triggered by temperature, pH change, ionic change and UV as well as solvent exchange induced gelation.\textsuperscript{130-132}

1.7.4.1 Importance of in situ gelling system

- Ease of administration and reduced frequency of administration.
- Improved local bioavailability.
- Improved patient compliance and comfort.
- Accurate administration of drugs.

to the cornea and conjunctiva. Both chitosan and hyaluronic acid are the polysaccharides with excellent bioadhesive and permeability enhancement properties. In addition, hyaluronic acid was known for its implication in several processes, such as the regeneration of corneal and conjunctival epithelial cells, through an interaction with the CD44 receptor. The results of this study revealed that hyaluronic acid-chitosan nanoparticles were able to target and further transfer genes to the ocular surface. Felt et al.,\textsuperscript{120} established that chitosan solutions, prolonged the cornea resident time of antibiotic in rabbits. Similar effects were also observed employing chitosan NP as verified by De Campos et al., which revealed that chitosan NP remained attached to the rabbit cornea and the conjunctiva for at least twenty four hours.\textsuperscript{121}
Formulation is less complex, which lowers the investment and manufacturing cost.\textsuperscript{133-134}

1.7.4.2 Ideal characteristics of polymer for \textit{in situ} gelling system

A polymer used for \textit{in situ} gels should have the following characteristics

- It should be biocompatible
- It should have a pseudo plastic behaviour
- It should be non hazardous
- It should be capable of adherence to mucus membranes
- It should be inert

Two particular problems common to many periodontal drug delivery systems are short retention time and difficult as well as time consuming application.\textsuperscript{135} The limitation of oral mucosal drug delivery is the dilution and rapid elimination of topically applied drugs due to the flushing action of saliva. The local delivery can make drug reside in periodontal pockets, however, the use of some agents can lead to patients’ pain, resulting in poor compliance and treatment effect.

The delivery system in which the drug is incorporated is therefore an important consideration and should be formulated to prolong the retention of the drug in the oral cavity. Bio-adhesive polymers have been utilized in gel forms to prolong the residence time on oral mucosa and to reduce the frequency of application and the amount of drug administered. This might improve the patient’s compliance and acceptance of the drug product.\textsuperscript{133}

\textit{In situ} gel formulations a current novel idea of delivering drugs to patients as a liquid dosage form, yet achieve the sustained release of drugs for the desired period.\textsuperscript{136-140}

Different delivery systems based on polymers have been developed, which are able to increase the residence time of the formulation at absorption site of drugs.

In recent years, there has been an increasing interest in water-soluble polymers that are able to form gels after application to the delivery site. These polymers are often water soluble and when used in a dry form, they attract water from the mucosal surface and this water transfer leads to a strong interaction. These polymers also form viscous layers when hydrated with water, which increases the retention time over the mucosal surfaces and leads to adhesive interactions.\textsuperscript{141}

Poloxamer consist of more than thirty different non-ionic surface active agents. These polymers are ABA –type triblock co-polymers composed of PEO (A) and PPO units.
Pluronic are commercially available in a range of molecular weights, composition ratios, and forms; it would be useful to mention the nomenclature rules for these copolymers. The letter in the notation stands for liquid (L), paste (P), or flakes (F), whereas the first two numbers indicate the molecular weight of the PPO block and the last number the weight fraction of the PEO block. For example, the one commonly used in biomedical application F127 has a weight percentage of seventy percent PEO and a molecular weight of PPO around 4000. Carbopol is a renowned pH-dependent polymer, which stays in solution form at acidic pH, however, forms a low viscosity gel at alkaline pH. Carbopols, which are very high molecular weight polymers of acrylic acid, have been used mainly in liquid or semi-solid pharmaceutical formulations, such as gels, suspensions and emulsions, as thickening agents, in order to modify the flow characteristics. These so-called in situ gelling polymers are highly advantageous compared with other polymers because, in contrast to very strong gels, they can be easily applied in liquid form to the site of drug absorption. At the site of drug absorption, they swell to form a strong gel that is capable of prolonging the residence time of the active substance. Therefore in situ gel, a novel sustained-release delivery system, can resolve above problems. Based on polymer material happens in response to the external stimulus, in situ gel offer polymer dispersion or a reversible conformational change under physiological conditions and the solution transforms to gel completely. In situ gel can be injected into periodontal pockets by small needles. Upon contact with the physiological environment, the liquid product solidifies and adheres to the periodontal pockets, then allows for sustained release of drugs, thus ensuring a high effective drug concentration in the periodontal pockets, improving the bioavailability, increasing drug efficacy and reducing side effects. The agents can exhibit a self-elimination, avoiding the need to remove the polymer system from the site of implantation after its use, thereby increasing patient compliance.

### 1.8 Achyranthes aspera

Achyranthes aspera (Amaranthaceae) a perennial rigid, erect herb, grows up to 1m height. Stems are square, leaves elliptic, ovate or broadly rhombate, 5.22 cm long, 2.5 cm broad, and pubescent. Flowering time is in summer. Flowers are greenish white, numerous in small, dense auxiliary heads or spikes, bracts and bracteoles persist ending in a spine. Main root is long, cylindrical, thick; secondary and tertiary roots
are present which are slightly ribbed and yellowish brown in color; odour is mild, taste is slightly sweet and mucilaginous; stem is yellow brownish, erect, branched, cylindrical hairy about 60 cm high. Seeds are sub cylindrical, truncate at apex, rounded at base, black and shining. The plant is distributed throughout India up to an altitude of 3000 feet. The plant is prevalent in the world as a weed.\textsuperscript{146}

The antibacterial activity of \textit{Achyranthes aspera} seed has been mentioned.\textsuperscript{147} Methanolic extract was found to reveal significant inhibitory activity against the pathogenic \textit{B. subtilis, E. coli} and \textit{K. pneumoniae}. The best MIC was found in methanol extract against \textit{B. subtilis}.

Anti-herpes virus activities of methanolic extract of \textit{Achyranthes aspera}.\textsuperscript{148} The results revealed that the extract inhibited HSV with EC50 of 64.4 µg/mL for HSV-1 and 72.8 µg/mL for HSV-2.

The antimicrobial potential of the methanolic extract of the dried whole plant of \textit{Achyranthes aspera} was reported.\textsuperscript{149} The methanol extract of dried whole plants of \textit{Achyranthus aspera} was evaluated against bacterial species viz., \textit{Bacillus cereus, Escherchia coli, Acinetobacter baumanii Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa} and \textit{Salmonella typhi}. All the bacterial species were found susceptible to methanolic extract of whole plants of \textit{Achyranthes aspera} however at high concentrations.

Antioxidant and antibacterial activity of \textit{Achyranthes aspera} leaf extracts has been evaluated.\textsuperscript{150} Antibacterial activity of \textit{Achyranthes aspera} leaf methanol extract was studied against four gram positive and six gram negative bacteria by disc diffusion method. It was observed that gram positive bacteria exhibited slightly greater susceptibility than gram negative bacteria to the plant extract.

The antitumor activity of \textit{Achyranthes aspera} leaf was investigated against human pancreatic tumor.\textsuperscript{151} The leaf extract significantly decreased the growth of human pancreatic BxPC- 3-luc-2 cancer cells transplanted subcutaneously in athymic mice. Validation of anticancer activity in crude preparation could justify the identification of active anti cancer compound in this plant.

Central nervous system depressant and behavioral activity of an ethanolic extract of \textit{Achyranthes aspera} in different animal models were reported.\textsuperscript{152} Results of study reflected that extract at 400 mg/kg \textit{i.p.} decreased locomotor activity, produced muscle relaxation and exhibited anxiolytic activity.
Wound healing activity of methanol extract of *Achyranthes aspera* L. Leaves has been evaluated *in vivo*.\(^\text{153}\)

The hypoglycemic effect of *Achyranthes aspera* in normal and alloxan-diabetic rabbits were mentioned.\(^\text{154}\)

The antifungal activities of the various leaf extracts of *Achyranthes aspera* were reported.\(^\text{155}\) The aqueous, ethanol and methanol leaf extracts of *Achyranthes aspera* were evaluated for antifungal activity against clinically important fungal species *viz.* *Candida albicans, C. tropicalis, C. krusei, C. kefyr, C. guilliermondi, C. glabrata, Cryptococcus neoformans, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Rhizopus oryzae*. The ethanol extract of the leaves of *Achyranthes aspera* Linn revealed an elevated antifungal activity against *C. kefyr, Cryptococcus neoformans, Aspergillus niger and Aspergillus flavus*.

The methanol extract of the leaves exhibited higher antifungal activity against *Cryptococcus neoformans* and *Aspergillus flavus*. Aqueous extract of the leaves did not show activity against tested fungal strains.

Antibacterial activities of different organic extracts of *Achyranthes Aspera* were reported.\(^\text{156}\) The antibacterial activities against *Escherichia coli, Bacillus subtilis, Vibrio cholerae, Salmonella typhi* and *Staphylococcus aureus* were evaluated. Whole plant of *Achyranthes aspera* Linn was found to enhance the induction of ovalbumin (OVA) specific humoral antibody response in mice.\(^\text{157}\)

The plant extract was found to increase the induction of OVA-specific antibody response in a dose-dependent manner. A significant elevation of IgM, IgG1, IgG3 antibodies was observed.

The larvicidal activity of saponin isolated from *Achyranthes aspera* was investigated.\(^\text{158}\) The acetone, chloroform, ethyl acetate, hexane and methanol leaf extracts of *Achyranthes aspera* were studied against the early fourth-instar larvae of *Aedes aegypti* L and *Culex quinquefasciatus*. This study investigates the potential of crude extracts from commonly used medicinal herbs in India as an environmentally protected measure to control the vector of dengue and lymphatic filariasis.

An isolation of an antifungal essential oil and a long chain alcohol from shoots of *Achyranthes aspera* has been mentioned.\(^\text{159}\) The isolated alcohol has been characterized as 17- pentatriacontanol and the oil exhibited antifungal activity against *Aspergillus carneus*. 

The eye irritation potential of aqueous leaf extract of *Achyranthes aspera* by *in vitro* and *in vivo* method were reported. The aqueous extract of *Achyranthes aspera* exhibited no eye irritation properties both in *in-vitro* and *in vivo* methods when compared with negative control whereas positive controls exhibited the eye irritation potential.

Ethanolic extracts of whole plant of *A. aspera* at doses of 50, 100 and 200 mg/kg, p.o. were screened for their effect on acute and chronic inflammation induced in mice and rats. It inhibited these inflammatory responses at doses of 100-200 mg/kg. The studies revealed that ethanolic extracts of *A. aspera* possess anti-inflammatory and anti-arthritic activity and support rationale behind the traditional use of this plant in inflammatory conditions.

The effect of alcohol extract of *Achyranthes aspera* Linn. on acute and sub acute inflammation were screened. The antiinflammatory activity of an alcohol extract of *Achyranthes aspera* was tested on carrageenan-induced paw oedema and cotton pellet granuloma models in albino male rats.

The estrogenic and pregnancy interceptory effects of *Achyranthes aspera* Linn were studied. Histopathological studies of the uterus were carried out to confirm this estrogenic activity.

The abortifacient principle of *Achyranthes aspera* Linn. extracts of the whole plant was studied and demonstrated an abortifacient effect in mice. The gastroprotective effect of *Achyranthes aspera* leaf extract on rats was investigated. The anti-ulcer assays were performed on pylorus ligation and chronic ethanol induced ulcer model.

A significant decrease in gastric fluid volume and acid output with an elevation in gastric pH observed after oral administration of ethanolic extract of *Achyranthes aspera*, indicated its antisecretory potency.

The ethanolic extract of the roots of *Achyranthes aspera* Linn for antifertility activity in proven fertile female albino rats at 200 mg/kg body weight and given orally on days 1-7 of pregnancy was mentioned. The ethanolic extract exhibited 83.3% anti-implantation activity when given orally at 200 mg/kg of body weight.

The isolation of a protein from ethanolic root extracts of *Achyranthes aspera* and its *in vitro* spermicidal action has been mentioned. The sperm immobilization studies demonstrated that about 150 µg of *Achyranthes* protein was able to immobilize sperms completely within seconds at a lower concentration than N-9 (250 µg) and can...
act as a spermicide similar to that of nonoxynol 9.
The impact of feeding an ethanolic extract of *Achyranthes aspera* on reproductive functions in male rats was reported.\(^{168}\) The results suggested that ethanolic extract of *A. aspera* caused reproductive toxicity in male rats and acted perhaps by suppressing the synthesis of androgen.
The potential antifungal as well as antibacterial activity of *Achyranthes aspera* L. petroleum ether, chloroform and methanol extract of dried leaves were screened.\(^{169}\)

**1.9 Acacia nilotica (Acacia, Babul)**

*Acacia nilotica* belonging to the family Leguminosae and the subfamily Mimosaceae is a moderate sized tree that grows up to 20m. Throughout the hot season the tree is in full leaf and its feathery foliage provides good shade. It has a flattish or umbrella shaped crown and is easily identified by its bright yellow, sweet-scented flower heads and its paired whitish spines at the base of each leaf. It is found all over the drier parts of India.

**Leaves:** The leaves are bipinnate, pinnate 3-10 pairs, 1.3-3.8 cm long, leaflets 10-20 pairs and 2-5mm long.\(^{170}\)

**Stem:** Stems are typically dark to black coloured, profound longitudinally fissured, with grey-pinkish slash, exuding a reddish low quality gum.\(^{171}\)

**Bark:** The bark has a tinge of orange and/or green (young tree), but older trees have dark, rough bark and tend to lose their thorns.\(^{172}\)

**Gum:** The gum varies in color from very pale yellowish brown to dark reddish brown depending on the tannin content in the sample. The lighter, more highly valued gums are soluble in water and very viscous; the tannins in darker gum reduce the solubility. The gum has a moisture content of about 13% and is slightly dextrorotatory.\(^{173}\)

The cytotoxicity and anti-viral activities against Peste des petits ruminants virus (PPRV) by adopting MTT colorimetric assay and anti-viral assay using a Vero cell line of aqueous extracts from the bark, leaves and pods of *Acacia nilotica* were investigated.\(^{174}\)

The aqueous extract from leaves presented significantly better anti-PPRV activities in comparison to pods extract. In contrast, bark extract did not show any anti-viral activity. The data presented in the study could pave a way towards the discovery of a novel anti-viral compound in the plants against PPRV and other viral diseases.

Antimicrobial activities of methanolic extract of leaves of *Acacia nilotica* L against
one gram-positive *Bacillus subtilis* and three gram-negative *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia* were screened.\textsuperscript{175} These results exhibited that plant extract has potential against bacteria, while against fungi their activity is not much effective. Therefore findings of this study revealed that methanol extract has promising antibacterial potential.

The inhibitory effect of *Acacia nilotica* leaf extract and \(\gamma\)-sitosterol on cell proliferation, the apoptotic effect and cell cycle arrest in breast and lung cancer cells were mentioned.\textsuperscript{176} The results indicated that \(\gamma\)-sitosterol, bioactive ingredient of extract exerted potential anticancer activity.

The various extracts of *Acacia nilotica* was investigated against *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Escherichia coli* test organism for their antimicrobial properties by bioassay method using the disk diffusion test.\textsuperscript{177} The findings indicated that *Acacia nilotica* possessed antimicrobial potential.

An isolation of acanilol A and acanilol B from the stem bark of *Acacia nilotica* was reported.\textsuperscript{178} The new compounds were tested as kinase inhibitors against CDK1, GSK3, CK1, and DYRK1A. The results of this study exhibited good kinase inhibitory activity.

The antioxidant and anti-quorum (Anti-QS) sensing activities of different extracts of green pods of *Acacia nilotica* was reported.\textsuperscript{179} The extracts of *A. nilotica* pod exhibited strong and effective \textit{in vitro} and \textit{in vivo} antioxidant potential by chelation to metal ions in addition to scavenging free radicals and anti-QS activity. Presence of polyphenols was held responsible for their overall antioxidant potential. It could also prevent strand break formation in supercoiled plasmid DNA and protein oxidation. Green pod extracts could also inhibit QS-regulated violacein pigment production in bacteria without interfering its growth.

The antiplatelet aggregatory activity of the extract of *Acacia nilotica* was reported.\textsuperscript{180} The findings of study exhibited that the antiplatelet aggregatory activity was mainly due to blockade of calcium channels, albeit evidence also suggested the involvement of protein kinase C.

The potential of *Acacia nilotica* as an adsorbent material for the removal of cadmium and lead from aqueous solution was reported.\textsuperscript{181} Almost 100 percent removal of lead was possible at a pH value of 4 and 80.5% removal of cadmium was possible at a pH value of 5 under the batch test conditions.
The effect of aqueous methanol extract (150 and 300 mg/kg body weight) of *Acacia nilotica* pods in streptozotocin-induced diabetic rat was investigated.\(^{182}\) The results of this study exhibited that diabetic rats receiving *Acacia nilotica* pods extract had a reduction of their blood glucose in comparison to diabetic control rats.

The chemopreventive activity of aqueous extracts of flower and leaf of *Acacia nilotica* on 7,12-dimethylbenz(a)anthracene (DMBA) induced skin papillomagenesis in male swiss albino mice was mentioned.\(^{183}\) The activity of the leaf extract was most significant followed by the flower extract. The contractile effect of the seeds of *Acacia nilotica* on the isolated guinea-pig ileum was reported.\(^{184}\) The extract displayed sustained dose-related activity with the involvement of calcium.

The findings of a survey of plants used for the treatment of sexually transmitted diseases (STDs) were mentioned.\(^{185}\) The finding suggested that *Acacia nilotica*, *Solanum incanum*, *Cassia abbreviate*, *Zanha africana*, *Vernonia amygdalina* and *Dichrostachys cinerea* were the most commonly used plants for the treatment of STDs.

An anti-free radical activity of kaempferol isolated from *Acacia nilotica* was investigated.\(^{186}\) The antioxidant potential of the kaempferol was demonstrated in several *in vitro* assays: measuring the proton radical scavenging activity (DPPH scavenging assay), hydroxyl radical scavenging activity (deoxyribose degradation assay), metal chelating activity, reducing power and inhibition of lipid peroxidation. It was found that the effect of the compound kaempferol was strongly dose dependent up to the concentrations 1–50 µg/mL in DPPH assay and 1–100 µg/mL in the deoxyribose degradation assay.

The *in vivo* antitypanosomal activity of *Acacia nilotica* against *Trypanosoma brucei* was evaluated.\(^{187}\) This study had justified the claim that methanol extracts of stem bark could be useful in the management of trypanosomiasis.

An association of the antimutagenic and chemopreventive activity of the barks of two usually observed plants viz. *Acacia nilotica* and *Acacia auriculiformis* by means of the AMES antimutagenicity assay and the mouse mammary gland organ culture (MMOC) model was presented.\(^{188}\) Results of this study exhibited a good correlation between the antimutagenesis assay and MMOC model, suggesting that these plants may contain active chemopreventive agents.
An anthelmintic potential of Ziziphus nummularia (bark) and Acacia nilotica (fruit) against Trichostrongylid nematodes of sheep were reported. The plants exhibited dose- and time-dependent antihelminthic effects by causing mortality of worms, and inhibiting egg hatching and larval development. Acacia nilotica (LC\textsubscript{50} = 512.86 and 194.98\,\mu\text{g/mL}) was found to be more potent than Ziziphus nummularia (LC\textsubscript{50} = 676.08 and 398.11\,\mu\text{g/mL}) in egg hatch test and larval development assay, respectively. The data justified their use in traditional veterinary medicine.

Immunomodulating activity using luminol/lucigenin-based chemiluminescence assay was reported. Results obtained exhibited that the fruits and barks of Acacia nilotica, possess average inhibitory effects on both types of phagocytes. The inhibitory effects on hepatitis C virus protease using \textit{in vitro} assay methods of methanol and aqueous extracts of different parts of 71 plants commonly used in Sudanese traditional medicine were mentioned. Thirty-four extracts exhibited significant inhibitory activity. The eight extracts, methanol extracts of \textit{Acacia nilotica}, \textit{Quercus infectoria}, \textit{Embelia schimperi}, \textit{Boswellia carterii}, \textit{Trachyspermum ammi} and aqueous extracts of \textit{Piper cubeba}, \textit{Syzygium aromaticum} and \textit{Q. infectoria} were most active.

Isolation of niloticane, a new bioactive cassane diterpene from the bark of \textit{Acacia nilotica subspecies Kraussiana} was reported. The isolated niloticane was tested for antibacterial activity using the micro-dilution assay; antiinflammatory activity using the cyclooxygenase-1(COX-1) and COX-2 assays and investigated for inhibitory effect against acetylcholinesterase using the microplate assay.

Niloticane exhibited antibacterial activity against gram-positive bacteria \textit{Bacillus subtilis} and \textit{Staphylococcus aureus} with MIC values of 4 and 8 \,\mu\text{g/mL}, respectively. With gram-negative bacteria, niloticane exhibited weak activity. MIC values obtained were 16 and 33\,\mu\text{g/mL} against \textit{Klebsiella pneumonia} and \textit{Escherichia coli}, respectively. In the cyclooxygenase test, niloticane possessed activity with IC\textsubscript{50} values of 28 and 210 \,\mu\text{M} against COX-1 and COX-2, respectively. IC\textsubscript{50} values observed with indomethacin (positive control) were 3.6 \,\mu\text{M} for COX-1 and 189 \,\mu\text{M} for COX-2. In the acetylcholinesterase test, niloticane exhibited anti-cholinesterase activity with an IC\textsubscript{50} value of 4 \,\mu\text{M}.

The molluscicidal activity of the pods and stem bark of \textit{Acacia} subspecies \textit{nilotica}, \textit{tomentosa} and \textit{astringens} against the snail species \textit{Bulinus truncatus} and...
Biomphalaria pfeifferi was studied. The spray-dried powders of the pods as well as stem bark of Acacia nilotica subspecies nilotica, tomentosa and astringens proved to be promising vegetable molluscicides. Free radical scavenging activity from the leaves of Acacia nilotica (L.) Wild. ex Delile was investigated. All the extracts in this study exhibited different extent of antioxidant activity, but the ethanol extract exhibited a higher potency than ascorbic acid, catechin, quercetin and tocopherol in scavenging DPPH free radical. The high scavenging property of Acacia nilotica may be due to hydroxyl groups existing in the phenolic compounds that can scavenge the free radicals. An aqueous extracts of Acacia nilotica for anti-inflammatory, analgesic and antipyretic activities was evaluated. The phytoconstituents like flavonoids, polysaccharides and organic acids may be responsible for pharmacological activities. The effect of an aqueous extract of Acacia nilotica on milk production in rats was reported. The extract found to stimulate the synthesis and release of prolactin could consequently have the properties claimed for lactating women. The in vitro anti-uveal melanoma activity of phenolic compounds from the Egyptian medicinal plant Acacia nilotica was investigated. The compounds were gallocatechin 5-O-gallate in addition to methyl gallate, gallic acid, catechin, catechin 5-O-gallate, 1-O-galloyl-β-D-glucose, 1,6-di-O-galloyl-β-D-glucose and digallic acid. The compound gallocatechin 5-O-gallate exhibited in vitro activity and selectivity towards uveal melanoma cell lines compared to that of the known compound EGCG from green tea. Antispasmodic action of Acacia nilotica was mediated through the calcium channel blockade and was responsible for the blood pressure lowering effect in the in vivo studies was reported. An isolation of strong antioxidant phenolics from Acacia nilotica was reported. The fragmentation pattern obtained evidence in Acacia nilotica pods of galloylated catechin- and gallocatechin derivatives along with galloylated glucose derivatives.

1.10 Ruta graveolens

Ruta graveolens commonly known as sadab belongs to the family Rutaceae. Rutaceae is one of the prevalent plant families with around 150 genera and 1,500 species distributed largely in tropical and subtropical parts of the world. This family is known throughout the world for its citrus fruits such as oranges, lemons and grape
fruit and also called citrus family. A variety of plants of the family rutaceae are used in traditional system of medicine worldwide. It is the source of rue or rue oil. Although it is native to Europe and Mediterranean region, it is distributed throughout the world. This plant is commonly cultivated in India and is commonly called as sudab or sadab. Two species of Ruta (genus) were reported to grow in India, of which Ruta graveolens (garden rue) was well known for its medicinal uses.\(^{201-202}\)

The dried leaves of Ruta graveolens were known as Barg-e-sudab, which was a crude indigenous drug of high therapeutic value. Ruta graveolens is a strong scented, erect, glabrous herb approximately 30-90 cm in height. The plant consists of 2-3 pinnate leaves and segments oblong to speculate. The plant was covered with a bloom and strongly aromatic small flowers, which have petals with dentate or wavy margins and small capsules with lobes somewhat rounded.\(^{202}\)

The pericycle in stem usually contains small isolated strands or bundles of sclerenchyma, vessels are arranged in less pronounced radial rows, pith cells are homogenous and clustered crystals of calcium are abundant in the cortex and/or pith. Petiole has an arc of separate bundles, a characteristic feature of R. graveolens not observed in any other species of the family. Stomata are present on both surfaces and hypoderm is present. In leaf; epidermal cells of the adaxial surface are larger in size and it lacks stomata. Trichomes are absent. Hypodermis is represented as one or a few layers of collenchyma in the midrib. Vascular bundle is solitary and arc shaped. No report on the presence of pith in roots.\(^{203-204}\)

The inhibitory effect of ethanolic, methanolic, chloroform and water extracts of Ruta graveolens stem against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphyloccous aureus*, *Bacillus subtilis*, *Salmonella typhimurium* and *Aeromonaes culicicola* as well as fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Penicilium chrysogenum*, *Rhizopus stolonifer* and *Fusarium oxosporium* by agar-disc diffusion method were reported.\(^{205}\) Ethanolic extract exhibited most susceptible activity against *S. aureus* and *B. subtilis* and all the extracts exhibited moderate antifungal activity except against *Fusarium oxosporium*.

An isolation of an alkaloid from Ruta graveolens stem was reported and evaluated antibacterial and antifungal activity.\(^{206}\) Antimicrobial activity of alkaloid-picrate salt of Ruta graveolens stems and leaves were evaluated against bacteria like *Escherichia coli*, *Serratia marcescens*, *Sarcina ventriculi* and *Klebsiella pneumoniae* as well as
fungi such as *Mucor and Monilia* by agar gel diffusion method. The salt exhibited most susceptible activity against *S. ventriculi*, whereas *E. coli* was the most resistant bacterial strain at a concentration of 100 µg/mL. *Mucor* was completely resistant to the extracts while *Monilia* exhibited susceptibility.

The antifungal potential of aquatic, methanolic and ethanolic extracts of *Ruta graveolens* on some mycotoxigenic fungi were reported. Antimicrobial effect of ethanolic extracts of sage and rue as a root canal irrigant (an in vivo study) was reported. Rue demonstrated antimicrobial effects on root canal microorganisms (both aerobic and anaerobic) when it was used as an endodontic irrigant in vivo.

The cytotoxic activity and antimicrobial activity of *Ruta graveolens* was investigated. In this study methanol, petroleum ether, ethyl acetate and water–methanol extracts of *Ruta graveolens* were found to possess antimicrobial and cytotoxic activities. All the extracts exhibited no activity against the gram negative strain *Escherichia coli* and the fungus *Candida albicans*. However, they exhibited inhibitory effects and a clear selectivity towards the studied gram positive microorganisms.

The antihelminthic activity against Indian earthworms (*Pheretima posthuma*) of *Ruta graveolens* L. leaf extracts were mentioned. The results of the study revealed that the methanolic extract was found to be more effective (paralysis and death time was 3.31±0.07 and 7.04±0.15 minutes respectively) at a concentration of 30mg/mL.

The anti-inflammatory effect of aqueous, ethanolic and methanolic extracts of *Ruta graveolens* in carrageenan induced paw edema in wistar male rats were investigated. Methanolic extracts of *R. graveolens* with a concentration of 20 mg/kg body weight and ethanolic extract with a concentration of 50 mg/kg body weight exhibited maximum (90.9%) inhibition on carrageenan induced rat paw edema. The methanol extract with a concentration of 50 mg/kg body weight produced 81.81% inhibition, which was also high as compared to the standard drug i.e. diclofenac.

Antifungal activity of natural fungicides isolated from an ethyl acetate extract of *Ruta*

graveolens leaves was reported. The isolated furanocoumarins i.e. psoralen, bergapten and xanthotoxin as novel topoisomerase I inhibitors were mentioned. The irreversible topoisomerase I mediated relaxation of plasmid in enzyme–substrate preincubation study, indicated that the observed inhibitory activity of extract constituents was not mediated through conformational changes in the DNA. Increase in inhibition of topoisomerase activity and promotion of DNA–enzyme complex was observed after enzyme–extract preincubation. The topoisomerase inhibition was mediated through both ways viz. stabilization of covalent complex and inhibition of catalytic activity of enzyme.

Cytotoxic and antiplatelet aggregation principles of Ruta graveolens were mentioned. The compounds dictamine, skimmianine, psoralen, chalepensin, clausindin and graveolinine exhibited significant inhibition of platelet aggregation, induced by arachidonic acid and collagen. On the other hand arborinine, dictamine, isopimpinellin, clausindin and graveoline exhibited cytotoxic activity against KB, Hela, DLD, NCI and Hepa tumor cell lines.

The extracts of Ruta graveolens for antimicrobial screening against selected gram-positive and gram-negative bacteria, yeasts, mold, as well as plant pathogenic fungi was tested. Ruta graveolens exhibited the highest toxicity against Rhizoctonia solani.

Anti-tumor activity of Ruta Graveolens extract was investigated. An extract of Ruta graveolens was found to be cytotoxic to Dalton’s lymphoma ascites, Ehrlich ascites carcinoma and L929 cells in culture (IC\textsubscript{100} = 16mg/mL) and also to increase the lifespan of tumor bearing animals. The Ruta graveolens extract was found to scavenge hydroxyl radicals and inhibit lipid peroxidation at low concentrations. However, at higher concentrations the extract acted as a prooxidant as inhibition of lipid peroxidation and scavenging of hydroxyl radical was minimal. These data indicate that the prooxidant activity of Ruta graveolens may be responsible for the cytocidal action of the extract and its ability to produce tumor reduction.

The algicidal and antifungal compounds from the roots of Ruta graveolens were studied. The immobilization effect of Ruta graveolens L. on human sperm was reported. The results of this study exhibited that the sperm immobilization effects of the extract appeared immediately in a dose-dependent manner and 100% of the sperms became immotile at a concentration of 100 mg/mL but other parameters were
intact. It also indicated that as the cells were alive and immotile, probably some ionic currents were blocked by a thermostable component of the plant which could be promising as a new male channel blocker contraceptive.

Antioxidant and *in vitro* antiinflammatory activity of methanol extract of *Ruta graveolens* leaves were mentioned. The methanol extract of *Ruta graveolens* exhibited 52% of free radical scavenging in the DPPH assay when compared to quercetin with 62% activity. The results of *in vitro* anti-inflammatory studies exhibited that the methanolic extract exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane was analogous to the lysosomal membrane and its stabilization implied that the extract may as well stabilize lysosomal membrane.

Antifertility effect of aqueous extract of aerial part of *Ruta graveolens* on immature female Balb/C mice was reported. The results of this study revealed that the aqueous extract of *Ruta graveolens* can interfere with reproductive system function in immature female mice by alterations in sex hormonal level and ovarian morphology and might be useful as an antifertility substance.

The antihyperglycemic, antihyperlipidemic and antioxidant activity of *Ruta graveolens* infusion and rutin in nicotinamide-streptozotocin induced diabetic rat were investigated. This study revealed that both study agents had antihyperglycemic and antihyperlipidemic efficacy which may be mediated via pancreatic and extrapancreatic effects. The involvement of gamma amino butyric acid in anticonvulsant effect of methanol extract of *Ruta graveolens* in mice was reported. The antinociceptive effect of *Ruta graveolens* extract was presented. *R. graveolens* L extract administered orally (200 mg/kg) exhibited an antinociceptive effect as measured by the tail flick and hot-plate tests and attenuated the writhing numbers in the acetic acid-induced writhing test. The results suggest that *Ruta graveolens* L extract exerts antinociceptive property in various pain models. Furthermore, this antinociceptive effect of *Ruta graveolens* L extract may be mediated by opioidergic and $\alpha_2$-adrenergic receptors, but not by serotonergic receptors.

Antidiarrhoeal activity of the ethanolic extracts of *Ruta graveolens* leaves and stem, using different experimental animal models such as castor oil induced diarrhoea, enteropooling and gastrointestinal motility test were reported. Results revealed that leaf extracts exhibited most potent antidiarrhoeal activity as compared to stem extract.
The extract produced profound decrease in intestinal transit and significantly inhibited castor oil induced enteropooling comparable to that of standard drug diphenoxylate. Anti-inflammatory effect of *Ruta graveolens* L. in murine macrophage cells (J-774) challenged with lipopolysaccharide (LPS) was investigated.\textsuperscript{225} LPS induces an inflammatory response by stimulating the production of nitric oxide and other mediators. The whole plant extract of rue was found to inhibit the nitrite level in LPS-challenged murine macrophage cells (J-774). This inhibition was much more significant than that with pure rutin, which was found in the plant extract at a concentration of 4\%. The observed decrease in *inos* gene expression associated with reduction in nitric oxide rules out the possibility of a decrease in nitric oxide level due to any cytotoxicity. Amusingly, the plant extract also had shown a decrease in COX-2 gene expression.

1.11 *Spilanthes acmella*

*Spilanthes acmella* is an important medicinal plant, found in tropical and subtropical countries, mainly India and South America. Popularly, it is known as toothache plant which reduces the pain associated with toothaches and can induce saliva secretion. *Spilanthes* (Compositae or Asteraceae) genus comprises of more than 60 species that are widely distributed in tropical and subtropical regions of the world, such as Africa, America, Borneo, India, Sri Lanka and Asia.\textsuperscript{226-227}.

*Spilanthes acmella* is native to Brazil and is cultivated throughout the year as ornamental or medicinal plant. It is an annual or short-lived herb that is 40-60 cm tall. It is grown in damp area\textsuperscript{228} and has a low rate of germination or poor vegetative propagation\textsuperscript{229}. Its flowers and leaves have pungent taste and when touched it is accompanied by tingling sensation and numbness. The plant species has been used commonly as a folk remedy, e.g. for toothache, rheumatic fever.\textsuperscript{228} The antifungal activity of different concentrations of *Spilanthes acmella* flower head extract against four different fungi: *Aspergillus niger*, *Aspergillus parasiticus*, *Fusarium oxysporum* and *Fusarium moniliform* were screened.\textsuperscript{229} All the concentrations of the test solution inhibited the fungal species with varying degree of sensitivity. Among the test organisms, high inhibitions of zones were observed in *F. oxysporium* and *F. moniliformis* followed by *A. niger* and *A. parasiticus*.

The effect of *Spilanthes acmella* hydroethanolic extract activity on the tumor cell actin cytoskeleton was investigated.\textsuperscript{230} The *S. acmella* extract at 250 mg/mL had no
significant effect on HEp-2 and L929 cell growth after 48 hours, but 500mg/mL significantly reduced cell number. At 1,000 mg/mL a highly significant reduction in cell number was observed.

The antibacterial potential of medicinal plant *Spilanthes acmella* Murr. and its *in vitro* raised callus against resistant organisms especially those harbouring *bla* genes were mentioned. The alcoholic extract of parent plant as well as its callus exhibited good antibacterial activity against gram positive and gram negative bacteria and also efficiently controlled the growth of most of the resistant bacteria harbouring *bla* genes. The MIC against gram positive bacteria ranged from 12.0 to 49.0 µg/mL, while the MIC against gram negative bacteria ranged from 1.53 to 12.0 µg/mL and MIC against resistant bacteria harbouring *bla* genes ranged from 6.1 to 98.0 µg/mL.

Diuretic potential of *Spilanthes acmella* whole plant as well as fresh flowers was reported. The diuresis induced by the *Spilanthes acmella* flowers was found to be strong with an intensity similar to that of furosemide and accompanied by marked increase in both urinary Na+ and K+ levels. The results of this study revealed that the urine was slightly acidified; this suggested that it was acting as a loop diuretic.

The potential of novel vesicular carrier, ethosomes, containing the methanolic extract of *Spilanthes acmella*, for anti inflammatory action via transdermal route and to test the potential of the herbal extract for antimicrobial property when formulated as an oral mucoadhesive gel was reported. Mucoadhesive oral gels containing extract were formulated using different concentrations of polymers and extracts and tested for parameters like viscosity and mucoadhesive behavior. The optimized formulation was evaluated for antimicrobial activity against different microorganisms responsible for causing tooth decay using well diffusion method. The results fully validate the claims of the traditional medicine about the use of Akkalkara for local anti-inflammatory and anti-microbial properties and confirm the suitability of ethosomes for transdermal delivery of active constituents of this herbal formulation.

The antipyretic activity of *Spilanthes acmella* which was carried out by yeast induced method as yeast is commonly used for the induction of pyrexia was studied. The test drug in concentrations of 10% and 20% produced 70.36% and 87.02% anesthesia respectively by the intracutaneous wheal compared to 97.22% anesthetic effect produced by 2% xylocaine.

**Insecticidal toxicity of spilanthol:** Extract of spilanthol from the flower heads of
Spilanthes acmella was found to be active against *P. xylostella*. Spilanhol was shown to be toxic against adults of *P. americana*. Ethanol extract of flower heads of *Spilanthes acmella* has shown a potent ovicidal, insecticidal and pupacidal activity at dose of 7.5 ppm concentration with 100% of *Anopheles, Culex*, and *Aedes* mosquito. The hexane extract of dried flower buds of *Spilanthes acmella* (3 N-isobutylamides: spilanthol, undeca-2E,7Z,9E-trienoi c acid isobutylamide and undeca-2E-en-8,10-diynoic acid isobutylamide) was found active against *Aedes aegypti* larvae. Ethanolic extracts of *Spilanthes acmella* (whole plants) were screened against early fourth instar larvae of *Culex quinquefasciatus*. Antimicrobial cytotoxicity and phytochemical activities of *Spilanthes acmella* was investigated. The compounds β-sitosterone and stigmasterol was isolated from the dichloromethane and petroleum ether extract of the leaves of *Spilanthes acmella* respectively. The antimicrobial activities of compounds stigmasterol and β-sitosterone were moderate against *Bacillus subtilis*, *Stapylococcus aureus* and *Salmonella typhi*, while only stigmasterol exhibited significant antifugal activity against the fungi.

Antinociceptive activity of the crude ethanol extract of *S. acemella* using acetic acid induced writhing model in mice was reported. Results revealed that crude ethanol extract of *S. acemella* leaves was found to possess significant antinociceptive activity. Antimicrobial activity of *Spilanthes acmella* flower head extract was studied. The results of this study revealed that, the MIC of the flower head extract was 0.25 mg/mL for *Bacillus sphericus*, *Bacillus subtilis*, *Staphylococcus aureus* and 0.50 mg/ml *Pseudomonas aeruginosa*, *Klebesiella aerogenes*, *Chromobacterium violaceum*. The activity of the plant against both gram-positive and gram-negative bacteria may be indicative of the presence of broad-spectrum antibiotic compounds.

The phytochemical and antimicrobial studies on the leaves of *Spilanthes acmella* was mentioned.

Vasorelaxant and antioxidant activities of *Spilanthes acmella* Murr. was investigated. This study reported the effect of *Spilanthes acmella* Murr. extracts on phenylephrine-induced contraction of rat thoracic aorta as well as their antioxidant activity. Results revealed that the extracts exert maximal vasorelaxations in a dose-dependent manner, but their effects are less than acetylcholine-induced nitric oxide vasorelaxation. Significant reduction of vasorelaxations was observed in both nitro-L-
arginine methyl ester and indomethacin.
The immunomodulatory potential of ethanol extract of *Spilanthes acmella* leaves was investigated. The immunomodulatory potential was evaluated using various models like neutrophil adhesion test, haemagglutinating antibody titre and delayed type hypersensitivity response in rats. In rats immunized with sheep red blood cells (RBC), extract of *Spilanthes acmella*, enhanced the humoral antibody response to the antigen and significantly potentiated the cellular immunity by facilitating that the footpad thickness response to sheep RBC in sensitized rats.

### 1.12 Azadirachta indica (Neem)

Antibacterial and antiviral evaluation of sulfonoquinovosyldiacylglyceride (SQDG), a glycolipid isolated from *Azadirachta indica* leaves was reported. Antimicrobial activity was evaluated against *Gram-positive*, *Gram-negative* bacteria and herpes simplex virus (HSV). The SQDG exhibited significant inhibitory activity against *Salmonella typhi* and two isolates of *Shigella dysenteriae* with MIC values 32 µg/mL, while three isolates of *Salmonella typhi*, *Escherichia coli* and *Vibrio cholerae* were inhibited at 64 µg/mL. Interestingly, SQDG inhibits HSV type 1 and 2 with the EC50 of 9·1 and 8·5 µg/mL, compared with acyclovir (2·2 and 2·8 µg/mL against HSV-1 and 2). The selectivity index (SI) was found to be 12·4 against HSV-1 and 13·41 with HSV-2.

The investigation of the antiproliferative activity of ethanolic neem leaf extract alone or in combination with cisplatin by cell viability assay on human breast (MCF-7) and cervical (HeLa) cancer cells was reported. Treatment of MCF-7 on HeLa and normal cells with ethanolic extract differentially suppressed the growth of cancer cells in a dose- and time-dependent manner through apoptosis. The results of this study revealed that the chemopreventive ability of neem alone or in combination with chemotherapeutic treatment could reduce the cytotoxic effects on normal cells, while potentiating their efficacy at lower doses.

The *in vitro* antiviral property of *Azadirachta indica* polysaccharides for poliovirus was reported. The antiviral effect was determined by plaque reduction assay in different protocols. The polysaccharides did not show any cytotoxic effects on HEp-2 cells at the highest tested concentration (200 mg/mL). The compounds
demonstrated better inhibitory effect when added concomitantly with the virus infection with a dose-dependent curve inhibition. Lesser effect was observed when the compounds were added after viral infection and the least effect at pre-treatment.

The alcohol and aqueous extracts of *A. indica* flowers were tested *in vitro* for their potential antifilarial activity against whole worm, nerve muscle preparation and microfilariae of *Setaria cervi*. On whole worm, response was characterized by an initial increase in tone, rate and amplitude of contractions followed by reversible paralysis. The initial stimulant effect was likely to be due to an irritant effect on cuticle. Nerve muscle preparation responded to both extracts by inhibition of spontaneous movements followed by reversible paralysis; initial stimulation phase was absent. The inhibition was concentration related. Alcohol and aqueous extracts had an almost similar lethal effect on microfilariae of *S. cervi*, LC$_{50}$ being 15 and 18 µg/mL, respectively.

The antimicrobial potential of *A. indica* leaf extracts on some drug resistant bacteria were mentioned. The results of the study indicated that *gram-positive* bacteria were more sensitive to the extract comparing to *gram-negative* bacteria. All of the tested bacteria exhibited sensitivity at higher concentration (7mg/mL) but multi drug resistant bacteria *Klebsiella pneumoniae* was very sensitive even at very low concentration (2mg/mL).

The antibacterial potential of *Azadirachta indica* seed was reported. The *A. indica* extract was bactericidal against *S. enterica* serovar typhi at concentrations of 400 µg/mL. The ethanolic *A. indica* seed extract displayed strong anti-*S. enterica* serovar Typhi activity, suggesting the extracts could be potential sources of chemotherapeutic agents for inclusion in anti-*S. enterica* serovar typhi regimens.

Effectiveness of *A. indica* leaf extract against plaque formation in males between age group of 20-30 years over a period of 6 weeks was mentioned. The study includes formulation of mucoadhesive dental gel containing ethanolic leaf extracts (25 mg/g). A 6-week clinical study was conducted to evaluate efficacy of dental gel containing extract with the commercially available chlorhexidine gluconate (0.2% w/v) mouthwash as positive control. Microbial evaluation of *Streptococcus mutans* and *Lactobacilli* species was carried out to determine the total decrease in salivary bacterial count over a period of treatment using a semi-

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quantitative four quadrant streaking method. The results of the study suggested that dental gel containing extract had significantly reduced plaque index and bacterial count than that of the control group.

Antimicrobial properties of *Azadirachta indica* leaves against certain bacterial strains causing dental caries using cup plate method and disc diffusion method were investigated.\(^{249}\) The strains of four human pathogenic bacteria causing dental caries *Micrococcus albus*, *Staphylococcus aureus*, *Proteus vulgaris* and *Pseudomonas aerogenosa* were selected for the study. The results of this study revealed that among the three extracts, the chloroform extract exhibited a higher activity than other extracts against tested dental pathogens.

The effect of *A. indica* ethanolic leaf extract on inflammatory oedema induced by chemical mediators (5-HT, histamine, bradykinin and PGE\(_1\)) to find out its possible mechanism of anti-inflammatory effect against carrageenan induced rat hind paw oedema was reported.\(^{250}\) Test material exhibited significant anti-inflammatory effect against 5-HT and PGE\(_1\) induced inflammation, but not on inflammation induced by histamine and bradykinin. Median effective doses of *A. indica* for 5-HT and PGE\(_1\) induced oedema were 1.17 g and 930 mg/ kg respectively. The result suggested that *A. indica* extract’s anti-inflammatory effect due to antagonism of the deleterious effect of 5-HT and PGE\(_1\) on blood vessels.

The larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*, the mosquito vector of dengue fever were investigated.\(^{251}\) Both seed extracts proved lethal for third to fourth instar larvae. Non-fed *A. aegypti* larvae were more susceptible to *Azadirachta* extracts at both temperatures. Under a more realistic environmental situation, namely with fed larvae at 25 °C, the death rates caused by the *Melia* extract were found to be higher; although at 30 °C the extract of *Azadirachta* had an even higher lethality.

The efficacy of *A. indica* extract against malarial infections had been reported.\(^{252}\) The effects of *Azadirachta indica* leaf extract on blood and liver glutathione, Na\(^+\)K\(^+\)-ATPase activity and thiobarbutiric acid reactive substances against paracetamol induced hepatic damage in rats with a view to elucidate possible mechanism behind its hepatoprotective action were mentioned.\(^{253}\) The results of this study indicated that the *Azadirachta indica* leaf extract is a promising hepatoprotective agent and this hepatoprotective activity of *Azadirachta*
indica leaf extract may be due to its antioxidant and normalization of impaired membrane function activity.

Antidermatophytic activity of aqueous and ethanolic extracts of A. indica leaves investigated against 88 clinical isolates of dermatophytes by agar dilution were reported. The isolates included Microsporum canis, M. audouinii, Trichophyton rubrum, T. mentagrophytes, T. violaceum, T. simii, T. verrucosum, T. soudanense, T. erinacei and Epidermophyton floccosum. Ethanolic extract was found to be more active inhibiting 90% of isolates at a concentration of 100 µg/mL.

The effect of fresh juice of tender leaves of Azadirachta indica on paracetamol-induced hepatic damage in albino rats was investigated. There was an increase in serum marker enzymes of hepatic damage (aspartate transaminase, alanine transaminase and alkaline phosphatase) after paracetamol administration. Pretreatment with the fresh juice of tender leaves of Azadirachta indica stabilized the serum levels of these enzymes. The results of this study revealed that Azadirachta indica most likely protects the liver from paracetamol induced hepatic damage by acting as an antioxidant.

The in vitro antioxidant activity of leaves, fruits, flowers and stem bark extracts from A. indica using DPPH scavenging assay, total antioxidant activity and inhibition of lipid peroxidation in Chago K1 cancer cell culture by thiobarbituric acid reactive substances method were mentioned. The results exhibited a leaf aqueous extract; flower and stem bark ethanol extracts exhibited higher free radical scavenging effect on the DPPH assay with 50% scavenging activity at 26.5, 27.9 and 30.6 µg/mL, respectively.

The chemopreventive potential of Azadirachta indica leaf extract in murine carcinogenesis model systems were reported. The effect of two different doses (250 and 500 mg/kg of body weight) of 80% ethanolic extract of the leaves of Azadirachta indica was examined on drug metabolizing Phase-I and Phase-II enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase, and lipid peroxidation in the liver of 7-week-old Swiss albino mice.

Anticarcinogenic potential of Azadirachta indica leaf extract was studied adopting protocol of benzo(a)pyrene-induced fore-stomach and DMBA-induced skin papillomagenesis. Azadirachta indica can significantly inhibit the chemical carcinogenesis at peri-initiational stages of carcinogenesis though modulation of phase II detoxification enzymes, elevation of antioxidant enzymes level and by
inhibiting lipid peroxidation and lactate dehydrogenase-induced damages. Chemopreventive potential of *A. indica* has also been evaluated on DMBA induced hamster buccal pouch carcinogenesis.\(^{258}\)

The effects of *Azadirachta indica* stem bark extract on gastric ulceration and acid secretion in rats were reported.\(^{259}\) *Azadirachta indica* extract (100–800 mg/kg p.o., 100–250 mg/kg i.p.) significantly inhibited gastric ulceration induced by indomethacin (40 mg/kg).

*Azadirachta indica* (250 mg/kg) significantly inhibited the basal and histamine-induced gastric acid secretion and cimetidine seemed to augment *Azadirachta indica* inhibition of gastric acid secretion. The results in this study suggested that the stem bark extract of *Azadirachta indica* possesses antiulcer activity, which probably acted via histamine H\(_2\) receptor.

The leaf extract of *Azadirachta indica* exhibited superior antiviral and antihyperglycemic activity *in vitro* and *in vivo* on animals.\(^{260}\) The *Azadirachta indica* leaf methanol extracts on stem cell reproduction was presented.\(^{261}\)

The methanol extracts of *Azadirachta indica* leaves at a concentration from 0.1 to 40 \(\mu\)g/mL exhibited an *in vitro* stimulatory activity in stem cell reproduction. These results suggest that the effect of methanol leaf extracts on stem cell reproduction could be of benefit to improve health.

Numerous scientific reports validate traditional uses of *Azadirachta indica* in both the maintenance of general health and skin care. Practically every part of *A. indica* (leaves, bark, fruit, flowers, oil, and gum) has been reported to be associated with various remedial properties such as, antimicrobial effects.\(^{262}\) *A. indica* is an enhancer of hepatic glutathione and glutathione-dependent enzymes.\(^{263}\) exhibiting *in vitro* antiviral activity.\(^{264}\)

An isolation of SQDG from the methanol extract of *Azadirachta indica* leaves was reported and evaluated its cytotoxic activity and DNA binding properties.\(^{265}\) The compound SQDG induces apoptosis in a dose dependent manner with IC\(_{50}\) 8.3 \(\mu\)M against acute lymphoblastic leukaemia MOLT-4 cell lines. The compound exhibited significant DNA binding properties as evidenced by the enhancement of melting temperature and perturbation of the characteristic B-form in CD evidence of calf thymus DNA and the binding process is exothermic and enthalpy driven. The findings point to its possible usefulness as an anti-cancer agent.