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RESEARCH ARTICLE

EXTRACTION AND PHYTOCHEMICAL EVALUATION OF AMLA FOR ANTIMICROBIAL ACTIVITY AND ANTI-DIABETIC ACTIVITY IN MICE

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ABSTRACT

Herbs are plants grown for culinary, medicinal, or in some cases even spiritual value. The green, leafy part of the plant is typically used. General usage differs between culinary herbs and medicinal herbs. A medicinal herb may be a shrub or other woody plant, where as a culinary herb is a non-woody plant. By contrast, spices are the seed, berries, bark, root, fruit, or other parts of the plant, even leaves in some cases; although any of these, as well as any edible fruits or vegetables, may be considered "herbs" in medicinal or spiritual use. Culinary herbs are distinguished from vegetables in that they are used in small amounts and provide flavour (or spices) rather than substance to food. Ayurveda is the oldest surviving complete medical system in the world. Derived from its ancient Sanskrit roots – 'ayus' (life) and 'ved' (knowledge) – and offering a rich, comprehensive outlook to a healthy life, its origins go back nearly 5000 years. To when it was expounded and practiced by the same spiritual rishis, who laid the foundations of the Vedic civilisation in India, by organising the fundamentals of life into proper systems. The main source of knowledge in this field therefore remain the Vedas, the divine books of knowledge they propounded, and more specifically the fourth of the series, namely Atharvaveda that dates back to around 1000 BC of the few other treatises on Ayurveda that have survived from around the same time, the most famous are CharakaSamhita and the Sushruta Samhita which concentrate on internal medicine and surgery respectively. From this study we can assess that though the plant powder was procured from the authentic source but still for the confirmation we have done the organoleptic study under Pharmacognostic characteristics of Drug. The powdered drug was subjected to extraction protocol soxhalation. The extract so obtained was tested for the presence of phytochemicals like alkaloid, carbohydrate, amino acid, Glycosides, Phenolic compounds and Tannins that shows positive results for the extract. The antimicrobial activity of the powder extract was done with Chloroform, Petroleum ether and Methanol. The result indicates that the antimicrobial activity of the methanolic extract of amla shows the maximum activity. This shows the amla has an antimicrobial activity and this may be due to the extracted phytochemicals in methanolic extract. The amla shows positive results for tannins in the methanolic extract and even alkaloids may be responsible for the maximum antimicrobial activity. But further chemical characterization is needed to confirm the molecule responsible for the activity. The antimicrobial activity of this herbal formulation was even comparable with standard antibiotics like penicillin G and Oflaxacin

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INTRODUCTION

Herbal Medicine sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savoury qualities.

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Herb plants produce and contain a variety of chemical substances that act upon the body. Herbalists use the leaves, flowers, stems, berries, and roots of plants to prevent, relieve, and treat illness. From a "scientific" perspective, many herbal treatments are considered experimental. The reality is, however, that herbal medicine has a long and respected history. Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. Today, science has

isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted and analysed. Many plant components are synthesizes in large laboratories for use in pharmaceutical preparations. For example, vincristine (an antitumor drug), digitalis (a heart regulator), and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants.




History of Herbal Medicine

Herbal medicine is the oldest form of health care known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provide food, clothing, shelter and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, well into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. Indeed, about 25% of the prescription drugs dispensed in the United States contain at least one active ingredient from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound.

Undisputedly, the history of herbology is inextricably intertwined with that of modern medicine. Many drugs listed as conventional medications were originally derived from plants. Salicylic acid, a precursor of aspirin, was originally derived from white willow bark and the meadowsweet plant. Cinchona bark is the source of malaria-fighting quinine. Vincristine, used to treat certain types of cancer, comes from periwinkle. The opium poppy yields morphine, codeine and paregoric, a treatment for diarrhoea Laudanum, a tincture of the opium poppy, was the favoured tranquilizer in Victorian times. Even today, morphine – the most important alkaloid of the opium poppy-remains the standard against which new synthetic pain relievers is measured. Prior to the discovery and subsequent synthesis of antibiotics, the herb Echinacea (which comes from the plant commonly known as purple coneflower) was one of the most widely prescribed medicines in the United States. For centuries, herbalists prescribed Echinacea to fight infection. Today, research confirms that the herb boosts the immune system by stimulating the production of diseases-fighting white blood cells. The use of plants as medicine is older that recorded history. As mute witness to this fact marshmallow root, hyacinth and yarrow have been found carefully tucked around the bones of a Stone Age man in Iraq. These three medicinal herbs continue to be used today.

Marshmallow root is a demulcent herb, soothing to inflamed or irritated mucous membranes, such as a sore throat or irritated digestive tract. Hyacinth is a diuretic that encourages tissues to give up excess water. Yarrow is a time- honoured cold and fewer remedy that may once have been used much as aspirin is today. In 2735 B.C, the Chinese emperor ShenNong wrote an authoritative treatise on herbs that is still use today. ShenNong recommended the use of Ma Huang (known as ephedra in the western world), for example, against respiratory distress. Ephedrine, extracted from ephedra is widely used as a decongestant. You will find it in its synthetic form, pseudoephedrine, in many allergy, sinus and cold-relief medications produced by large pharmaceutical companies. The records of King Hammurabi of Babylon (c. 1800 B.C) include instructions for using medicinal plants.

Hammurabi prescribed the use of mint for digestive disorders. Modern research has confirmed that peppermint does indeed relieve nausea and vomiting by mildly anesthetizing the lining of the stomach. The entire Middle East has a rich history of herbal healing. There are texts surviving from the ancient cultures of Mesopotamia, Egypt and India that describe and illustrate the use of many medicinal plant products, including castor oil, linseed oil and white poppies. In the scriptural book of Ezekiel, which dates from the sixth century B.C, we find this admonition regarding plant life: "… and the fruit there of shall be for meat, and leaf there for medicine." Egyptian hieroglyphs show physicians of the first and second centuries A.D treating constipation with Siena pods, and using caraway and peppermint to relieve digestive upsets. Throughout the middle Ages, home-grown botanicals were the only medicines readily available, and for centuries, no self-respecting house hold would be without a carefully tended and extensively used herb garden. For the most part, herbal healing lore was passed from generation by word of mouth. Mother taught daughter; the village herbalist taught a promising apprentice. By the seventeenth century, the knowledge of herbal medicine was widely disseminated throughout Europe. In 1649, Nicholas Culpeper wrote A Physical Directory, and a few years later

produced The English Physician. This respected herbal pharmacopeia was one of the first manuals that the layperson could use for health care, and is still widely referred to and quoted today. Culpeper has studied at Cambridge University and was meant to become a great doctor, in the academic sense of the word. Instead, he choose to apprentice to an apothecary and eventually setup his own shop. He served the poor people of London and became known as their neighbourhood doctor. The herbal he created was meant for the layperson. The first US pharmacopeia was published in 1820. This volume included an authoritative listening of herbal drugs, with description of their properties, uses, dosages, and tests of purity. It was periodically revised and became the legal standard for medical compounds in 1906. But as Western Medicine evolved from an art to a science in the 19th century, information that had at one time been widely available became the domain of comparatively few. Once Scientific methods were developed to extract and synthesise the active ingredients in plants, pharmaceutical laboratories took over from providers of medicinal herbs as the producers of drugs. The use of herbs, which for most of history had been mainstream medical practice, began to be considered unscientific, or at least unconventional, and to fall into relative obscurity.

Indian Herbs and Ayurveda

Herbs are plants grown for culinary, medicinal, or in some cases even spiritual value. The green, leafy part of the plant is typically used. General usage differs between culinary herbs and medicinal herbs. A medicinal herb may be a shrub or other woody plant, where as a culinary herb is a non-woody plant. By contrast, spices are the seed, berries, bark, root, fruit, or other parts of the plant, even leaves in some cases; although any of these, as well as any edible fruits or vegetables, may be considered "herbs" in medicinal or spiritual use. Culinary herbs are distinguished from vegetables in that they are used in small amounts and provide flavour (or spices) rather than substance to food. Ayurveda is the oldest surviving complete medical system in the world. Derived from its ancient Sanskrit roots – 'ayus' (life) and 'ved' (knowledge) – and offering a rich, comprehensive outlook to a healthy life, its origins go back nearly 5000 years. To when it was expounded and practiced by the same spiritual rishis, who laid the foundations of the Vedic civilisation in India, by organising the fundamentals of life into proper systems. The main source of knowledge in this field therefore remain the Vedas, the divine books of knowledge they propounded, and more specifically the fourth of the series, namely Atharvaveda that dates back to around 1000 BC of the few other treatises on Ayurveda that have survived from around the same time, the most famous are Charaka Samhita and the Sushruta Samhita which concentrate on internal medicine and surgery respectively. The Astanga Hridayam is a more concise compilation of earlier texts that was created about a thousand years ago. These between them forming a greater part of the knowledge base on Ayurveda as it is practiced today. The art of Ayurveda had spread around in the 6th century BC to Tibet, China, Mongolia, Korea and Srilanka, carried over by the Buddhist monks travelling to those lands. Although not much of it survives in original form, its effects can be seen in the various new age concepts that have originated from there. No philosophy has had greater influence on Ayurveda than Sankhaya's philosophy of creation and manifestation. Which professes that behind all creation there is a state of pure existence or awareness, which is beyond time and space, has no beginning or end, and no qualities. Within pure existence, there arises a desire to experience itself, which results in disequilibrium and causes the manifestation of the primordial physical energy and the two unite to make the "dance of creation" come alive. Imponderable, indescribable and extremely subtle, this primordial energy – which and all that flows from its existence only in pure existence – is the creative force of all action, a source of form that has qualities. Matter and energy are so closely related that when energy takes form, we tend to think of it in terms of matter only. And much modified, ultimately leads to the manifestation of our familiar mental and physical worlds. It also gives rise to consciousness, which is the universal order that pervades all life. Individual intelligence, as distinct from the everyday intellectual mind, is derived from and is part of this consciousness. It is the inner wisdom, the part of individuality that remains unsaved by the demands of daily life, or by Ahamkara, the sense of 'I-ness. A Sanskrit word with no exact translation, Ahamkara, is a concept not quite understood by everyone as it is often misleadingly equated to 'ego'. Embracing much more than just that, it is in essence that part of 'me' which knows which parts of the universal creation is 'me'. Since 'I' am not separate from the universal consciousness, but 'I' has an identity that differentiates and defines the boundaries of 'me'. All creations therefore have Ahamkara, not just human beings. There arises from Ahamkara a two-fold creation. The first is Satwa, the subjective world, which is able to perceive and manipulate matter. It comprises the subtle body (the mind), the capacity of the five sense organs to hear, feel, see, taste and smell, and for the five organs of action to speak, grasp, move, procreate and excrete. The mind and the subtle organs providing the bridge between the body, the Ahankara and the inner wisdom, which three together is considered the essential nature of humans. The second is Tamas, the objective world of the five elements of sound, touch, vision, taste and smell – the five subtle elements that give rise to the dense elements of ether or space, air, fire, water and the earth – from which all matter of the physical world is derived. All it is Rajas, the force or the energy of movement, which brings together parts of these two worlds.

Phytochemical

Phytochemicals are chemical compounds, such as beta-carotene to cite one well known example, that occur naturally in plants. The term is generally used to refer to those chemicals that may affect health, but are not established as essential nutrients. While there is abundant scientific and government support for recommending diets rich in nutrients from fruits and vegetables, there is only limited evidence that physiological effects result from any specific phytochemicals. Phytochemicals have been used as drugs for millennia. For example, Hippocrates may have prescribed willow tree leaves to abate fever. Salicin, having anti-inflammatory and pain - relieving properties, was originally extracted from the bark of the white willow tree and later synthetically produced became the staple over-the-counter drug called aspirin.

Commonly used herbs and minerals

Botanical Name	Common Name (English)	Sanskrit Name	Functions or Properties
Acacia concinna	Acacia	Shikakai	Jaundice, Malarial Fever
Acacia nilotica	Indian gum Arabic Tree	Babool	Gonorrhoea, Leucorrhoea, Diarrhoea
Allium sativum	Garlic	Lahsuna	Used to control excess conversion of Lipids and Cholesterol
Aloe vera	Indian Aloe	Kumari	Spleen and Liver disorders, Cysts, poisons, hydrocele, kaphha fever, vatadosha
Azadirachtaindica	Neem	Neem	Is an anti-bacterial and anti-fungal agent
Bacopa monnier	Bacopa	Brahmi	Improves alertness
Cinnamomumcamphora	Camphor	Karpoora	Used in kaphha, thirst, obesity
Citrus limon	Lemon	Nimbaka	Used as food for increased taste
Cucumissativus	Cucumber	Trapusha	Diuretic, useful in hematemesis, haemorrhages
Daucuscarota	Carrot	Garjira	Seeds: useful in flatulence, renal disorders
Ficusbengalensis	Banyan tree	Vata	Kaphha pitta shamak, nausea.
Mangiferaindica	Mango	Amra	Sour, tasteful, increases vata and pitta
Jasminumofficinale	Jasmine	Jati	Cold nature, bitter taste, increases vata
Malus domestic	Apple	Seva	Sour, tasteful

There is evidence from laboratory studies that phytochemicals in fruits and vegetables may reduce the risk of cancer, possibly due to dietary fibres, polyphenol antioxidants and anti-inflammatory effects. Specific phytochemicals, such as fermentable dietary fibres, are allowed limited health claims by the US Food and Drug Administration. Some phytochemicals with physiological properties may be elements rather than complex organic molecules. Abundant in many fruits and vegetables, selenium, for example, is involved with major metabolic pathways, including thyroid hormone metabolism and immune function. Particularly, it is an essential nutrient and cofactor for the enzymatic synthesis of glutathione, an endogenous antioxidant. The term “”phytochemicals” refer to a wide variety of compounds produced by plants. They are found in fruits, vegetables, beans, grains, and other plants. Scientists have identified thousands of phytochemicals, although only small fractions have been studied closely. Some of the more commonly known phytochemicals include beta-carotene, ascorbic acid (vitamin C), folic acid and vitamin E. some phytochemicals have either antioxidant or hormone – like actions. There is some evidence that a diet rich in fruits, vegetables and whole grains reduces the risk of certain types of cancer and other diseases and researchers are looking for specific compounds in these foods that may account for the beneficial effects in humans. Available scientific evidence does not support claims that taking phytochemical supplements is as helpful as consuming the fruits, vegetables, beans and grains from which they are taken.

Carotenoids

Carotenoids, with their polyisoprenoid structure and differing degrees of conjugated double bonds, are abundant in most colourful plant foods. Carotenoid intake has been associated with a reduced risk of cardiovascular diseases, age –related cataract and macular degeneration and some forms of cancer. Although, 600 carotenoids have been characterized in nature, those found most concentrated inhuman blood and tissues are α-and β-carotene β-cryptoxanthin, lutein, lycopene and zeaxanthin. Although low concentrations of carotenoids are present in several nuts, they are not a major source of dietary carotenoids; however, β-carotene and lutein are found in pistachios at 0.21 and 2.32 mg/100 g dry weight.

Phenols

Plant phenols, including simple phenolic acids, flavonoids, stilbenes and a variety of other polyphenolic compounds, possess hydroxyl groups conjugated to an aromatic hydrocarbon group. Phenolic compounds are ubiquitous in plant foods with total daily intakes estimated at 500-1000 mg. The reduction in the risk of several chronic diseases associated with the consumption of plant phenols has been attributed to their array of bio-mechanisms, including anti-oxidation, anti-inflammation, carcinogen detoxification and cholesterol reduction. The total phenol content among nuts varies widely, with pecans, pistachios and walnuts being the richest sources and Brazil nuts, macadamias and pine nuts containing the lowest concentrations.

Phenolic Acids

Cranberry juice is rich in phenolic acids, which reduces adherence of bacteria to teeth and the cells lining the bladder – thereby reducing urinary tract infection and dental carries. Sweetening reduces the anti-adhesion properties of phenolic acids.

Caffeic Acid Ester

Phenolic acids reduce oxidation of LDL cholesterol. Phenolic acids reduce the formation of cancer-promoting nitrosamines from dietary nitrates and nitrites. The most important phenolic compounds in grapes (red wine, grape juice, raisons) are proanthocyanidins, resveratrol and ellagic acid.

Ellagic Acid

Rich in strawberries, but 50% more in raspberries (mainly ellagitannins) reduces oesophageal and colon cancers inhibits the formation of DNA adducts inhibits phase 1 enzymes and potentiates phase 2 enzymes.

Chlorogenic Acid

Very high in blueberries, tomatoes and bell peppers found in the flesh of grapes, along with ellagic acid most frequently an ester of Caffeic acid. Caffeic acid is a hydroxycinnamic acid,

reduces mutagenicity of polycyclic aromatic hydrocarbons, major contributor to the anti-oxidant activity of coffee. Caffeic acid can regenerate oxidized Vitamin E, may be pro-oxidant in the propagation phase of LDL oxidation, roasting coffee increases anti-oxidant activity.

Resveratrol

Resveratrol is a stilbene and. Like many other polyphenols acts in the plant as a phytoalexin. In addition to sharing anti-oxidant and other bioactivities common to polyphenols, resveratrol appears capable of extending the lifespan of yeast and mice. In addition to the presence of resveratrol in red vine and the skins of red grapes, it has been found in peanuts and pistachios at 84 and 115µg/100 gm. This concentration is in contrast to the 98-1803ug/100mL in red wines. Resveratrol has not been found in other nuts.

Flavonoids

Flavonoids are comprised of six principle classes, anthocyanin, flavanone, flavone, flavanol, flavonol and isoflavone. Flavonoids have been identified in most nuts with their glycone profiles included in the USDA database. The highest total flavonoid concentrations are found in pecans at 34, almonds at 15 and pistachios and hazelnuts at 12mg/100 g respectively. No flavonoid have detected in Brazil or macadamia nuts. It is worth noting that some nuts can contribute total flavonoids to the diet in amounts comparable to some fruit and vegetables. E.g., almonds have a similar quantity of total flavonoids as are delicious apples at 15 and apricots at 1 mg/100g.

Proanthocyanidins

Proanthocyanidins, flavanol oligomers, are comprised principally of (+) – catechin and (-) – epicatechin linkage with single interflaven bonds between C4 of the "upper" extension units and C6 or C8 of the "lower" unit (C4-C6, C4-C8, B Type) or a single C-C bond plus a second ether linkage between C-2 of the upper unit and an A-ring hydroxyl group of the lower unit (A type). Some Proanthocyanidins are formed from less common flavanols such as afzelechin, epiafzelechin, gallocatechin and epigallocatechin. The size of proanthocyanidins varies widely in plants foods and is determined by the degree of polymerization.

Catechins

Flavanols antioxidant found in dark chocolate lost in drying grapes to raisins inhibits catechol – O – methyltransferase norepinephrine degradation increases metabolic rate ("burns fat" while increasing free-radical production) can halt the initiation and progression of cancer may strengthen capillaries can protect against DNA damage, therefore useful for patients undergoing chemotherapy or radiation therapy.

Antimicrobial Activity

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoans. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Disinfectants are antimicrobial substances used on non-living objects. The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that the reason one bacterium failed to growth, of another microorganism. Of course, in today's common usage, the term antibiotic is used to refer to almost any drug that attempts to rid your body of a bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well. The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. Before penicillin became a viable medical treatment in the early 1940s, no true cure for gonorrhoea, strep throat or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials. However, the future effectiveness of antimicrobial therapy is somewhat in doubt. Microorganisms, especially bacteria, are becoming resistant to more and more antimicrobial agents. Bacteria found in hospitals appear to be especially resilient, and are causing increasing difficulty for the sickest patients –those in hospitals. Currently bacterial resistance is combated by the discovery of new drugs. However, microorganisms are becoming resistant more quickly than new drugs are being made available; thus, future research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials or how to treat infections with alternative means, such as species – specific phage.

Main Classes of Antimicrobial:-Antibiotics

Antibiotics are generally used to treat bacterial infections. The toxicity to humans and other animals from antibiotics is generally considered to below. However, prolonged use of certain antibiotics can decrease the number of gut flora, which can have a negative impact on health. Some recommend that, during or after prolonged antibiotic use, one should consume probiotics and eat reasonably to replace destroyed gut flora.

Antivirals

Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics, specific antivirals are used for specific viruses. They are relatively harmless to the host and therefore can be used to treat infections. They should be distinguished from vermicides, which actively deactivate virus particles outside the body. Antiviral drugs work by inhibiting the virus before it enters the cell, stopping it from reproducing, or in some cases, preventing it from existing the cell. However, like antibiotics, viruses may evolve to resist the antiviral drug.

Antifungals

An antifungal drug is medication used to treat fungal infections such as athlete's foot ringworm, candidiasis (thrush), serious systematic infections such as cryptococcal meningitis and others.

Antiparasitic

Antiparasitics are a class of medications which are indicated for the treatment of infection by parasites, such as, nematodes, cestodes, trematodes, infectious protozoa and amoebae. Like antifungals, they must kill the infecting pests without serious damage to the host.

Non-pharmaceutical antimicrobials

A wide range of chemical and natural compounds are used as antimicrobials. Organic acids are widely used as antimicrobials in food products, e.g., lactic acid, citric acid, acetic acid and their salts, either as ingredients, or as disinfectants. For example, beef carcasses often are sprayed with acids and then rinsed or steamed, to reduce the prevalence of E.coli O157:H7Traditional healers long have used plants to prevent or cure infectious diseases. Many of these plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit the growth of pathogenic microorganisms. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal. So, it is worthwhile to study plants and plant products for activity against resistant bacteria.

Essential oils

The antimicrobial properties of 21 plant essential oils and two essences were investigated against five food-borne pathogens, Campylobacyter jejuni, Salmonella enteriditis, Escherichia coli, Staphylococus aureus and Listeria monocytogenes. The oils of bay, cinnamon, clove and thyme were the most inhibitory, each having a bacteriostatic concentration of 0.075% or less against all five pathogens. (A. Smith-Palmer, J. Stewart and L. Fyfe). Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens.

Cations and elements

Many heavy metal cations such as Hg^{2+}, Cu^{2+} and Pb^{2+} have antimicrobial activities, but are also very toxic to other living organisms, thus making them unsuitable for treating infectious diseases. Colloidal Silver is commonly used as an antimicrobial in alternative medicine without clear scientific proof of effectiveness. Medicinal plants are proving their potential for the treatment of many dreadful diseases including cancer. The basic advantage of using the drug from the plant origin is, it is free from any side effect. The phytochemicals are regularly explored for the identification of its effectiveness against the diseases. As India is very rich in medicinal flora, the drug research in this area has a wide potential and many industries are involved in Research and development activities like Dabur, Himalaya etc. The present study was focussed on determining the antimicrobial and analgesic activity of plant extract of Emblica Officinalis. For this the literature reviewed was divided into following parts – Pharmacological Studies, Antimicrobial Activity, Phytochemical Analysis, Evaluation of Phytochemical, Biological activity of Amla, Indian Herb.

Use of Herbs

Many people have turned away from conventional medicines, with the belief that natural substances like herbs are safer than synthetic substances. This belief is augmented by many other unwarranted claims such as herbal products do not contain chemicals while conventional medicines do, thus contributing to the latter's side effects. The increasing use of herbal medicines has resulted in concern about the efficacy and safety of these products. Herbs can be hazardous in many ways. They may be intrinsically toxic or toxic when taken in combination with other preparations (Hussin, Abashj, 2001). Memory is the ability of an individual to record sensory stimuli, events, information, etc., retain them over short or long periods of time and recall the same at a later date when needed. Poor memory, lower retention and slow recall and are common problems in today's stressful and competitive world. Age, stress, emotions are conditions that may led to memory loss, amnesia, anxiety, high blood pressure, dementia, to more ominous threat like schizophrenia and Alzheimer's diseases. Ayurveda, the ancient science of life has more than one reference of the plant for the enhancement of memory and mental agility of mankind. The famous Charaka Samhita written in the 1st century refers Brahmi (nerve tonic), a perennial creeping plant (Bacopa monnieri) for curing mental retardation leading to psychosis (Bhowmik. Debjit *et al*., 2010).

Herbal and its Extraction

The medicinal plants are used in traditional treatments to cure variety of diseases for thousands of years. The aim of this study was to identify such plants with antimicrobial and antioxidant efficacy for controlling some food borne pathogens. The locally available plants viz., Ficusreligiosa, Leucasaspera, Holarrhenaantidysenterica and Psidiumguajavai were selected for the study. Various methods were tried to standardize the extraction of antimicrobial agents (Preethi *et al*., 2010). Polyherbal formulations available with a wide range of indications like protective to liver, appetite and growth promoters, gastrointestinal and hepatic regulator, as treatment for hepatic dysfunction, for hepatic regeneration as well as liver stimulant and tonic. Despite the widespread use, there is a lack of scientific evidence of their efficacy and safety. This study was undertaken to evaluate the heptoprotective activity of sic commercially available formulations, namely Liv 52, Livergen, Livokin, Octogen, Stimuliv and Tefroliv in acute liver toxicity in mice model induced by paracetamol(PCM) (Girish *et al*., 2009).

Pharmacological Studies

Emblica Officinalis (Amla) are widely used in the Indian system of medicine and believed to increase defence against diseases. This article discusses and summarizes important medicinal values of Emblica Officinalis (EO). In this communication, we reviewed the applications of EO in cancer, diabetes, liver treatment, heart diseases, ulcer, anaemia and various other diseases. The use of EO as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastroprotective are also reviewed (Khan *et al*., 2009).

Antimicrobial Activity

India, fruits of *Phyllanthus Emblica* L are the most common ingredients of almost all Ayurvedic preparations like Lehya, Choorna etc. *Phyllanthus Emblica* L fruits have been used for various disorders. This has led to the investigation of antimicrobial activity of *Phyllanthus Emblica* (Raghu *et al*., 2010). Present investigations focussed on antimicrobial potential of aqueous infusions and aqueous decoctions of Emblica Officinalis (amla) and Coriandrumsativum (coriander) against 186 bacterial isolates belonging to different genera of G +ve bacterial population and 2 isolates of Candida albicans isolated from urine specimens. The well diffusion technique was employed. Aqueous infusion and decoction of Emblica Officinalis exhibited potent antimicrobial activity against Staphylococcus aureus (80), S. haemolyticus (8), S. saprophyticus (65), Micrococcus varians (12), M. lylae (6), M. roseus (3), M. sedenterius (2), M. halobius (1), Bacillus subtilis (8), B. megaterium (1) and Candida albicans (SaeedSabahat *et al*-2007). Methanolic extracts of dried leaves of Ocimumtenuiflora, Azadirachtaindica, *Phyllanthus Emblica* were used for the comparative study of antimicrobial and antioxidant activity. Antioxidant activity of these sources of drugs that's why we have conducted our research to find out the biological activity of the alkaloids of a plant that is the amalaki (Bole B. Shivaji *et al*., 2010).

Phytochemical Analysis

An attempt was made to determine the antimicrobial activity of crude powder, aqueous as well as methanolic extract of the fruit and leaf of Emblica Officinalis against three commonly encountered respiratory pathogens viz. staphylococcus aureus, Klebsiella pneumonia, Streptococcus pyogenes (Javale *et al*., 2010). A comparative study of wild variety and cultivated variety of Amla were carried out; simultaneously effect of different drying techniques on physicochemical properties of Amla powder were also studied. The fruits of wild variety were found smaller than the cultivated variety (Mishra *et al*., 2009).

Evaluation of Phytochemical

The present communication attempts to evaluate the physiochemical and preliminary phytochemical studies on the fruit of Emblica Officinalis Gaertn, Euphorbiaceae family. Amla is one of the most celebrated herbs in the Indian traditional medicine system, Ayurveda. Amla is becoming increasingly well known for its unusually high levels of Vitamin C, which is resistant to storage and heat damage due to cooking (Meena *et al*., 2010).

Biological Activity of Amla

Aqueous extract of EO was found to be cytotoxic to L929 cells in culture in a dose dependent manner. Concentration needed for 50% inhibition was found to be 16.5 mg:ml. Emblica Officinalis and chyavanaprash (a non-toxic herbal preparation containing 50% EO) extracts were found to reduce ascites and solid tumours in mice induced by DLA cells. Animals treated with 1.25 g:kgb.wt. of EO extract increased life span of tumour bearing animals (20%) while animals treated with 2.5 g:kg b.wt. of chyavanaprash produced 60.9 5 increased in the life span. Both EO and chyavanaprash significantly the solid tumours (Jose jeena *et al*., 2001). An ethanol extract of 'Amla' Emblica Officinalis Gaertn, was examined for its antisecretory and antiulcer activities employing different experimental models in rats, including pylorus ligation Shay rats, indomethacin, hypothermic restraint stress-induced gastric ulcer and necrotizingm agents (80% ethanol, 0.2 % NaOH and 25% NaCl) (Al-Rehaily *et al*., 2002). Various chemical constituents of plants are believed to possess anti-inflammatory and antioxidant properties. However, their erythropoietic effects on test animals remain unclear. The present study aimed at investigating the erythropoietic activities of some medicinal plants found in India: Aegelmarmelos, Asparagus recemosus, Boerhaviadiffusa, Carissa congesta, Eugenia jambolana, Ficuscarica, Phoenix sylvestris, *Phyllanthus Emblica*, Spinacaoleracean and Vitusvinifera on Wistar albino rats (Lohar *et al*., 2009). Amla (*Phyllanthus Emblica* L.), apart from its food value, can be used as a gastro protective agent in non-steroidal anti-inflammatory drug (NSAID)-induced gastropathy. It has been suggested that the antioxidative property of amla is the key to its therapeutic effect. Hence, on the basis of in vitro antioxidative potential, the ethanolic extract of amla (EAE) was selected for in vivo study in NSAID-induced ulcer (Chatterjee *et al*., 2011).

Diabetes

Diabetes mellitus, often simply referred to as diabetes – is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).There are three main types of diabetes:- (i) Type 1 diabetes: Results from the body's failure to produce insulin, and presently requires the person to inject insulin. (Also referred to as insulin-dependent diabetes mellitus, IDDM for short, and juvenile diabetes). (ii) Type 2 diabetes: Results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. (Formerly referred to as non-insulin-dependent diabetes mellitus, NIDDM for short, and adult-onset diabetes).

Classification:-Type 1 diabetes

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency. The type of diabetes can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is one of the immune-mediated nature, where beta cell loss is a T-cell mediated autoimmune attack. There is no known preventive measure against type 1 diabetes, which causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect

children or adults but was traditionally termed "juvenile diabetes" because it represents a majority of the diabetes cases in children. Brittle diabetes, also known as unsuitable diabetes or labile diabetes, refers to a type of insulin-dependent diabetes characterized by dramatic and recurrent swings in glucose levels, often occurring for no apparent reason. The result can be irregular and unpredictable hyperglycaemias, frequently with ketosis and sometimes serious hypoglycaemias. Brittle diabetes occurs no more frequently than in 1% to 2% of diabetics.

Type 2 diabetes

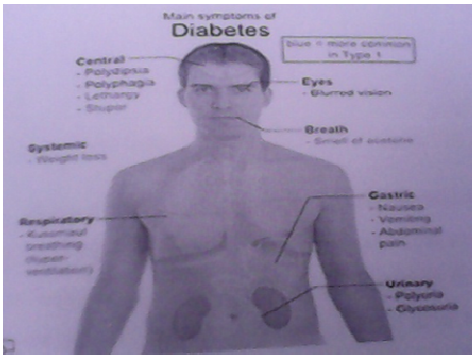
Type 2 diabetes mellitus is characterized by insulin resistance which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus due to a known defect are classified separately. Type 2 diabetes is the most common type. In the early stage of type 2 diabetes, the predominant abnormality is reduced insulin sensitivity. At this stage hyperglycaemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver.

Signs and Symptoms

The classical symptoms of diabetes are polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Symptoms may develop rapidly (weeks or months) in type 1 diabetes while in type 2 diabetes they usually develop much more slowly and may be subtle or absent. Prolonged high blood glucose causes glucose absorption, which leads to changes in the shape of the lenses of the eyes, resulting in vision changes; sustained sensible glucose control usually returns the lens to its original shape. Blurred vision is a common complaint leading to a diabetes diagnosis; type 1 should always be suspected in cases of rapid vision change, whereas with type 2 change is generally more gradual, but should be suspected. People (usually with type 1 diabetes) may also present with diabetic ketoacidosis, a state of metabolic dysregulation characterised by the smell of acetone; a rapid, deep breathing known as Kussmaul breathing; nausea; vomiting and abdominal pain; and altered states of consciousness. A rare but equally severe possibility is hyperosmolar nonketotic state, which is more common in type 2 diabetes and is mainly the result of dehydration. Often, the patient has been drinking extreme amounts of sugar-containing drinks, leading to a vicious circle in regard to the water loss. A number of skin rashes can occur in diabetes that is collectively known as diabetic dermatomes.

Causes

The cause of diabetes depend on the type. Type 1 diabetes is partly inherited and then triggered by certain infections, with some evidence pointing at Coxsackie B4 virus. There is a genetic element in individual susceptibility to some of these triggers which has been traced to particular HLA genotype (i.e., the genetic 'self' identifiers relied upon by the immune system). However, even in those who have inherited the susceptibility, type 1 diabetes mellitus seems to require an environment trigger. Type 2 diabetes is due to primarily to lifestyle factors and genetics.



Medicinal Plants Used for Study Amla (Emblica officinalis)

The Indian Gooseberry is also called Amalaka in Sanskrit, Amla in Hindi, Usirikayi in Telugu, Nellikayi in Tamil and Kannada, Nellika in Malayalam. You will be hearing much more about this unique, yet sour super fruit in the years ahead. The Emblica Officinalis, aka Amla, aka Indian gooseberry is enjoying a great deal of attention from the natural sciences community as a super fruit with extra ordinary health benefits. Learn more about the amla fruit in this super fruits article and find out what powerful benefits it may hold. Known for its edible fruit of the same name, the Indian Gooseberry (*Phyllanthus Emblica*, syn. Emblica Officinalis) is a deciduous tree of the Euphorbiaceae family.In its natural regions, the Indian Gooseberry is known under many different names including Amla and Amla, the tree grown to about 15 feet high and is found throughout Nepal. It is also found in India, Bhutan, Sri Lanka, South China and Southeast Asia. The fruit is sour, bitter, astringent and is quite fibrous, ripening in autumn when it is harvested by hand after climbing to the upper branches where most of the fruit is borne. Being so sour, in India, it is common to eat Amla steeped in salt water and turmeric to make them palatable. In folk medicine, dried and fresh fruits of the plant are used. All parts of the various Ayurvedic/Unani Medicine (Jawarish Amla) herbal preparations, including the fruit, seed, leaves, root, bark and flowers.

Classification of Emblica Officinalis (amla)

Kingdom- Plantae	Division-Angiospermae	Class-Dicotyledonae
Order-Geraniales	Family-Euphorbiaceae	Genus-Emblica
Species-Officinalis geartin		

Botanical Description

A small to medium sized deciduous tree,8-18 meters height with thin light grey bark exfoliating in small thin irregular

flakes, leaves and simple, subsessile, closely s*et al*ong the branchlets, light green having the appearance of pinnate leaves; flowers are greenish yellow, in axillary fascicles, unisexual, males numerous on short slender pedicles, females few, subsessile, ovary 3-celled; fruits globose, fleshy, pale yellow with six obscure vertical furrows enclosing six trigonous seeds in 2-seeded 3 crustaceous coccil.

Traditional Use

The fruits are sour, astringent, bitter, acrid, sweet, cooling, anodyne, ophthalmic, carminative, digestive and tonic. They are useful in vitiated conditions of tridosha, diabetes, cough, asthma, bronchitis, cephalalgia, ophthalmopathy, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, erysipelas, skin diseases, leprosy, haematogenesis, inflammations, anaemia, emaciation, hepatopathy, jaundice, strangury, diarrhoea, dysentery, haemorrhages, leucorrhoea, menorrhagia, cardiac disorders, intermittent fevers and greyness of hair 1-6.

Pharmacology and clinical studies

Phyllembin, isolated from the ethanolic extract of the fruit pulp has been found to potentiate the action of adrenaline in vitro and in vivo. It showed a mild Emblica Officinalis depressant action on Central Nervous System and also had a spasmolytic activity. The drug also revealed mild stimulant action on isolated frog heart, short and insignificant rise in cat's blood pressure, contraction of the nictitating membrane of cat and the prolongation of the hypnosis were observed. Further studies on the action of phyllemblin revealed that the drug antagonized the spasmogenic effect of acetylcholine, bradykinin and serotonin on the guineapig ileum. It also antagonized serotonin and acetochlonine-induced contractions of oestrogenised rat uterus. It increased the amplitude of cardiac contraction and heart rate transiently. An increase in coronary flow was followed by persistent decrease. On perfused rat hind limb and rabbit ear preparation, phyllemblin in small doses, increased the amount of perfusatewhereas in larger doses it decreased the flow significantly. A triphasic response that is initial transient rise, followed by a transient fall and then sustained rise in blood pressure was seen in anaesthetized albino rats. The sustained rise was blocked by phentolamine (1 mg/Kg). The drug produced 80 percent protection against leptazol seizures in mice. It protected effectively against tremors and clonic and tonic convulsions induced by nicotine. It also antagonised tremorine-induced tremors and other cholinergic symptoms. The ether extract and 80 percent alcoholic extract of fruits acidified with hydrochloric acid, were found to have antibacterial activity. The other extract of acidified alcoholic extract showed the highest activity, inhibiting the growth of M. pyogenes var. S. typhosa and S. paratyphi at a concentration of 0.21 mg / ml and that of M. pyogenes var. albus; S. schottmmellari and S. dysenteriae at a concentration of 0.42 mg / ml. The effect of crude amla (traditionally known as amalakirasayana) on total serum protein and its fractions was studied in rabbits. The drug had no significant effect on the levels of serum protein fractions, but it raised the total protein level and increased the body weight. The studies indicated that the increase in the body weight was due to positive nitrogen balance. The drug was found to have only anabolic effect without affording resistance against diseases. Clinical studies were conducted to investigate the effect of crude amla in gastritis syndrome. The crude amla was given in 20 cases in a dose of 3 gms, 3 times a day for 7 days. The drug was found effective in 85% of the cases. It was observed that the drug did not have any significant beneficial effect in cases of hypochlorhydria. The lipid lowering and anti-atherosclerotic effects of amla fresh juice were evaluated in cholesterol fed rabbits (rendered hyperlipidemic by atherogenic diet and cholesterol feeding). Amla fresh juice was administered at a dose of 5 ml.kg body weight per rabbit per day for sixty days. Serum cholesterol, Triglycerides, phospholipid and low – density lipoprotein levels were lowered by 82%, Emblica officinalis 66%, 77% and 90% respectively. Similarly, the tissue lipid level showed a significant reduction following amla juice administration. Aortic plaques were regressed. Amla juice treated rabbits exerted more cholesterol and phospholipids, suggesting that the mode of absorption be affected. Amla juice is an effective hypolipidemic agent and can be used as a pharmaceutical tool in hyperlipidemic subjects. It is reported to have anti-cancer properties. The crude extract of Emblica Officinalis was reported to counteract hepatotoxic and renotoxic effects of metals due to anti-oxidant activity. Antioxidant of the fruit is demonstrated in several models.

Application of amla

Amla is a very rich source of Vitamin C. Its ascorbic acid content ranges from 1100 to 1700 mg per 100 grams which is said to be the second highest among all the fruits, next only to the Barbados cherry (Malpighiaglabra). The fruit, because of its high acidity and astringent taste, is not palatable for direct consumption, but its excellent and therapeutic values offer enormous potentiality for processing.

Applications of Emblica officinalis in Cancer

Triphala has been reported to exhibit chemopreventive potential. The presence of Triphala in diet had significantly lowered the benzo (a) pyrene (B(a)P) induced fore stomach papillomagenesis in mice. It was more effective in reducing tumour incidences compared to its individual constituents. Triphala also significantly increased the antioxidant status of animals which might have contributed to the chemoprevention. The breast cancer is one of the most common cancers in women. Lipid-metabolizing enzymes, lipids and lipoproteins have been associated with the risk of breast cancer. Kalpaamruthaa (KA) is a modified Siddha preparation containing EO, Semecarpusanacardium (SA) and honey. The elevated levels of free cholesterol, total cholesterol, triglycerides, phospholipids and free fatty acids and decreased levels of ester cholesterol in plasma, kidney and liver found in cancer suffering animals were reverted back to near normal levels on treatment.

Cardio-protective Activity of Emblica officinalis

The effects of chronic oral administration of fresh fruit homogenate of Amla on myocardial antioxidant system and oxidative stress induced by ischemic-reperfusion injury (IRI) were investigated on heart in rats. Chronic EO administration produces myocardial adaptation by augmenting endogenous

antioxidants and protects rat hearts from oxidative stress associated with IR.

Roles of Emblica officinalis in Reducing Cholesterol and Dyslipidaemia

Cu(2+) – induced oxidation and cholesterol-fed rats were used to investigate the effects of Amla on low-density lipoprotein (LDL) oxidation and cholesterol levels in vitro and invivo. It was concluded that Amla may be effective for hyper-cholesterolemia and prevention of atherosclerosis (54). EO and Mangiferaindica contains flavonoid which reduces the levels of lipid in serum and tissues of rats induced hyperlipidaemia. Both cause the degradation and elimination of cholesterol.

Active Roles of Emblica Officinalis in Immunomodulation

Immune activation is an effective as well as protective approach against emerging infectious diseases. Albino rats were used to access the immunomodulatory activities of Triphala of various neutrophil functions like adherence, phagocytic index, avidity index and nitro blue Tetrazolium. Oral administration of Triphala appears to stimulate the neutrophil functions in the immunized rats and stress induced suppression in the neutrophil functions were significantly prevented by Triphala.

Amla fruit is rich in Vitamin C and Pectin

It is the principal constituent of the famous Ayurvedic restorative tonic called Chayavan Prash.

Root bark	Useful in ulcerative stomatitis and gastrohelcosis. The bark is useful in gonorrhoea, jaundice, diarrhoea and myalgia.
Leaves	Useful in treatment of conjunctivitis, inflammation, dyspepsia, diarrhoea and dysentery. Useful in treatment of diabetes, cough, asthma, bronchitis, cephalalgia.
Fruits	Ophthalmopathy, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, erysipelas, skin diseases, leprosy, hematemesis, inflammations, anaemia, emaciation, hepatopathy, jaundice, strangury, diarrhoea, dysentery, haemorrhages, leucorrhoea, menorrhagia.

Cosmetics:-Hair tonic

The standard extract of Amla contain about 3% Natural Vitamin C and up to 20% Tannin. The natural mix of above is effective in skin care therapy. Natural Vitamin C lends cementing support to the intercellular spaces of the cells, thereby enhancing skin integrity, texture and sheen of the epidermal layer. Amla oil has a long history of use as an aid for improving the health of hair and scalp. In fact, it is one of the world's oldest, natural hair conditioners. The dried fruit is a detergent and is used as a shampoo. Customarily, a small amount of Amla oil is applied to the hair after washing. These not only bring forth a rich, natural shine and soft, but also help rejuvenate hair that is dull and damaged.

Analgesic Activities of Emblica officinalis

Extracts of EO fruits possess potent anti-pyretic and analgesic activities. A single oral dose of ethanolic extract and aqueous extract (500 mf/kg, i.p.) showed significant reduction in hyperthermia in rats induced by brewer's yeast. Both of these extracts elicited pronounced inhibitory effect on acetic acid-induced writhing response in mice in the analgesic test. This may be due to the presence of tannins, alkaloids, phenolic compounds, amino acids and carbohydrates.

Extraction Techniques:-Extraction

The process of separating active principle(s) from powdered crude drugs by using suitable solvents is called extraction. The basic principle behind extraction is the exceptional behaviour of the active principles toward the solvent system. Following is the schematic representation of the procedure for obtaining powdered drugs: Plant→Collected→Authentication by Taxonomist→Drying→Subjected to Powdering→Subjected to Suitable Method of Extraction. The following methods are used for extraction: Infusion; Decoction; Maceration; Percolation; Successive extraction; Supercritical Fluid extraction; Steam distillation; Headspace Technique.

Infusion

The method is only applicable for soft drugs and drugs containing water-soluble constituents. The drug is mixed with water and it is allowed to stand for 15 to 20 minutes. Shaking or stirring is done if required. Drug + water→ Filtrate. Heat is not applied in this method. It is then filtered and the filtrate is called as infusion. The infusion should be used within 24 hours.

Decoction

Decoction is applicable only for water-stable and heat-stable drugs obtained from hard and woody sources. Than the drug is mixed with water and heated for 15 to 20 minutes. Drug + water→ Filtrate. It is filtered and marc is pressed and volume is adjusted. The filtrate is then called as decoction. It should be consumed within 24 hours.

Maceration

During maceration, the plant material is soaked in the solvent and in this process the extraction time can vary from several minutes up to weeks. Other variables include the amount of agitation, plant-solvent ratio, moisture content, temperature and the number of times the plant has been macerated. Usually the solute-solvent proportion is 1:10. Unrecognized drugs like gums, resins, gum-resins and oleo gum-resins are also extracted by the maceration process. There are three types of maceration:- (i) Simple (Single) maceration. (ii) Double maceration. (iii) Triple maceration.

Digestion

Digestion id the modified form of maceration. The solvent action in case of digestion is enhanced by the application of heat. This process is suitable only for heat-stable substances. Processes like infusion, decoction and digestion are now obsolete and are rarely used for extraction of drugs with few exceptions.

Percolation

In the percolation type of extraction, the plant material is continuously flushed with fresh solvent. The extraction is continued until sufficient compound is extracted. If necessary, the same material can be re-extracted with a second solvent. There are three types of percolation:- (i) Simple percolation (cold percolation). (ii) Hot Percolation.

Simple Percolation (Cold Percolation)

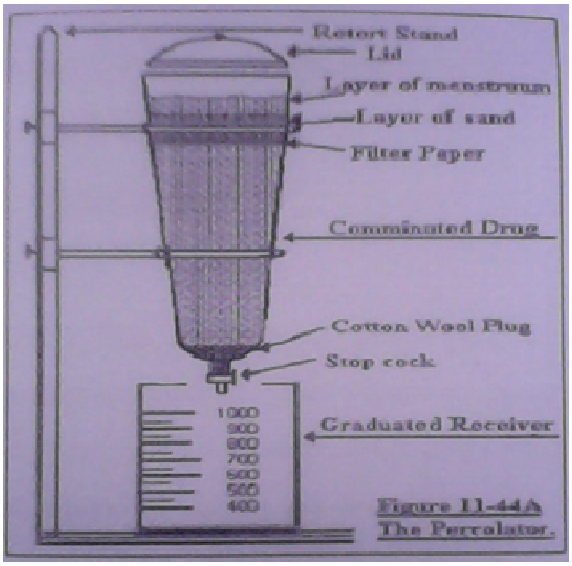
In cold percolation, we performed powdering the drug and inhibition of the drug that is moisturing the drug with a little menstruum for 4 hours. In this extraction packing, maceration and then percolation was performed. Packing:-The drug is packed up to 2/3rd capacity of the percolator. Maceration: Sufficient menstruum is added and allowed to saturate the drug. When the drops start to come down through the nozzle, the nozzle is closed and the moistened drug is allowed to stand for 24 hours.

Percolation

The percolate is collected drop-wise and the process is continued till complete extraction has taken place. Complete extraction can be observed in the following ways:- (i)Take the final drop and allow to evaporate then if no residue remains, complete extraction has taken place. (ii)Test the specific gravity of the final percolate and menstruum. (iii) Test the final percolate for absence of the phytoconstituents concerned. This method is suitable for heat-sensitive, hard and woody drugs. It is not suitable for drugs which bay block the percolator.

Hot Percolation

Hot Percolation is a type of soxhlet extraction. In this extraction, the plant material is continuously flushed with fresh solvent. But the fresh solvent is formed by boiling the solvent containing the extracted analytes. Thus, in contrast to percolation, the total amount of solvent is limited. Inspite of what is sometimes through, a soxhlet extraction can be far from complete, due to channelling or the presence of air in the semi-permeable thimble containing the plant material. Suitability of Soxhlet method: for heat substances only. And also for active constituents which are less soluble in the menstruum in the absence of heat.



Successive Solvent Extraction:-By Soxhlet Extraction

In 1879 Franz Von Soxhlet was the scientist who discovered the term known as soxhlet assembly. The use of commercially soxhlet extractor is a convenient way to prepare crude plant extracts, this procedure is used mainly with pure solvents although some author ternary solvent mixtures. Mixed solvents suffer the inconvenience that individual components may distil at different temperature, so that the resulting mixture in the chamber containing the drug is enriched in the solvent of lower boiling point. Thus actual solvent properties in the extraction chamber differ from that originally used in the collector and this fact may introduce errors when trying to reproduce the experiment using other extraction methods.

Advantages

- It is an automatic, continuous method that does not require further manipulation other than concentration of the extractive and saves solvent by recycling it over the sample.
- This method is not time consuming since for a standard-sized sample (500g), the extraction time is less than 24 hours.

Disadvantages

Extractives are heated during the period of extraction at the boiling point of the solvent employed and thermally labile compounds such as carotenoids may hydrolyse or decompose. The air-dried powdered drug is extracted by the Soxhlet extractor with successively different solvents, in increasing order of polarity. Chloroform→acetone→Ethanol→ Methanol. Finally the drug is macerated with chloroform-water. Before extracting with a new solvent, the powdered material is dried in hot air oven at temperature below 50 ^{0}C.Each extract is concentrated by distilling off the solvent and then evaporating the solvent is weighed. Its percentage yield is calculated in terms of the air-dried weight of the plant material. The colour and consistency of the extract are noted. In this method, the material to be extracted is placed in a 'Thimble' made of cellulose or cloth in a central compartment with a siphoning

device and side-arm both connected to a lower compartment. The solvent is placed in a lower compartment and a reflux condenser is attached above the central sample compartment. The solvent in the lower container (a round-bottomed flask) is heated to boiling and the vapour passes through the side arm up into the reflux condenser. Here the vapour liquefies and drips into the thimble containing the material to be extracted. The warm solvent percolate through the material and the wall of the thimble and the extract gradually collects in the central compartment. Once the height of the extract reaches the top of the siphon, the entire liquid in the central compartment flows through this and back into the lower solvent container. The process is then repeated.

In this method, the extract collects in the lower vessel gradually becoming more and more concentrated. Assuming that no volatile substances are present, the vapour rising from the heated extract is pure solvent vapour and so the lipid dripping into the material from the condenser is essentially pure solvent, though derived from the extract, thus although a relatively small volume of solvent is needed. The effective volume of solvent is used for the extraction is proportional to the time for which the process is allowed to continue. This process is useful for the exhaustive extraction of plant material with a particular solvent. An apparatus for extracting components from a solid (e.g., extracting natural products from plant material). The material used is placed in a thimble made of thick filter paper and this is held in a specially designed reflux condenser with a suitable solvent. The chamber holding the thimble fills with warm solvent and this is led back to the source via a side arm. The apparatus can be operated for long periods, with components concentrating in the source vessel. It is named after Franz Soxhlet, who devised it in 1879.

Evaporation of Extract by Rotary Vacuum Evaporator

A simple rotary evaporator system was invented by Lyman C. Craig. It was first commercialized by the Swiss company Buchi in 1957. Vacuum evaporators as a class function because lowering the pressure above a bulk liquid lowers the boiling points of the component liquids in it. Generally, the component liquids of interest in applications of rotary evaporation are research solvents that one desires to remove from a sample after an extraction, for instance, following natural product isolation or a step in an organic synthesis. Use of a 'rotavap' therefore allows liquid solvents to be removed without excessive heating of what are often complex and sensitive solvent-solute combinations. Rotary evaporation is most often and conveniently applied to separate "low boiling" solvents such as a n-hexane or ethyl acetate from compounds which are solid at room temperature and pressure. However, careful application also allows removal of a solvent from a sample containing a liquid compound if there is minimal co-evaporation (azeotropic behaviour), and a sufficient difference in boiling points at the chosen temperature and reduced pressure.

Antibiotics Assay

The antibiotic bioassay still required by major pharmacopoeias such as the British (BP 1999), European (EP 1997) and United States (USP 1995), is the major survivor of a bygone era in which a biological assay of a natural substances was ultimate measure of its suitability for use.

Principles

Both agar diffusion (Plate assays) and Turbidimetric assays permit an estimate of antibiotic potency through direct comparison of a test antibiotic with an approved, well calibrated, reference substance. Agar diffusion is usually the method of choice whenever the nature of an antibiotic permits (in particular an antibiotic must be water-soluble for assay by diffusion). (i) In the plate method, active components of antibiotics diffuse, during a defined period at optimum temperature through an appropriate medium which is seeded with a sensitive organism. The medium is gelled by agar, a carbohydrate extract of certain seaweeds, whose chain-like molecules provide a framework in which water forms a continuous on the surface, or absorbed into discs placed on to the agar. (ii) Turbidimetric assessment is used less widely than afar diffusion, although it might be considered to resemble more closely the clinical situation where growth of an organism in a liquid culture is directly challenged by antibiotic components diffuse. Where the antibiotic concentration is insufficient, microbial growth is displayed. The border of these effects defines a clear boundary and a zone of inhibition is created. With most antibiotics, a linear relationship exists between the diameter or area of this zone of inhibition and the logarithm of the antibiotic concentration producing the zone. Generally, the potent the antibiotic, the larger is the zone of inhibition.

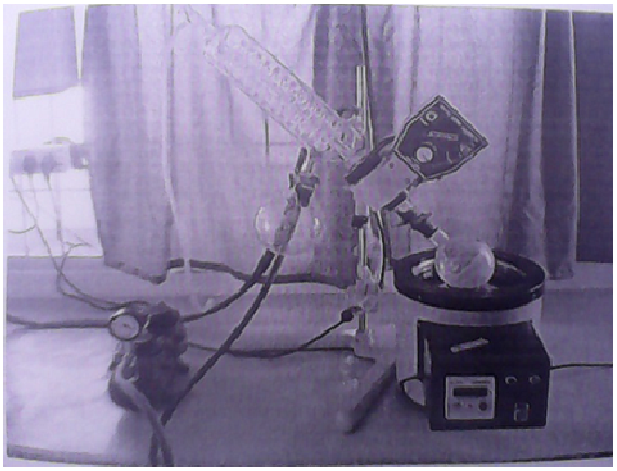
Conduct of Diffusion Assays

Solutions of standard and unknown are applied to assay plates by one of the following methods:- (i) In cavities (wells) of about 8mm diameter punched from the agar. A specially fabricated plate cutter may facilitate this process. The best defined and cleanest cavities will be obtained if the agar is cooled for a short period at 2-8 ^{0}C prior to cutting. (ii) On absorbent discs or in porcelain or stainless steel cylinders of about 6 mm diameter, balanced on the agar surface. The cylinders must be clean and free from all residues of previous use. Occasional washing in nitric acid (2M) or chromic acid (200g sodium dichromate 100 ml water, 1500 ml sulphuric acid) is ideal, but must be performed with safety in mind. (iii) The measurement of diameter should be taken through the centre of the zone which, provided that a good technique was employed earlier in the assay, should present as a perfect circle. Distorted zones may result from poorly cut wells or poor addition of solutions and should be excluded from the assay.

Plan of work

Collection of the powdered individual components of the Emblica officinalis from the authentic source→Microscopic and Pharmacognostic identification of the powdered drug→Successive solvent extraction protocol for the extraction of phytoconstituents of drug. The extraction will be done with the help of Petroleum ether, Chloroform and methanol. The

extraction to be followed by evaporation using Rotatory Vacuum Evaporator→The biochemical examination of the extract for the identification of phyto-chemicals like glycosides, saponins, gums, carbohydrates, proteins etc.→The antimicrobial activity of extract by using disc diffusion method against the bacteria obtained from MTCC→Determination of the zone of inhibition→Interpretation of the antimicrobial activity of the herbal drug "Emblica officinalis" and the antimicrobial activity of the individual plant→Anti-diabetic activity of the herbal drug "Emblica officinalis". →Interpretation of the Anti-diabetic activity of the herbal drug "Emblica officinalis".



MATERIALS METHODS

Material Required: Microorganisms: (E.Coli, S aureus. MTCC).

Glasswares

Petri Plates, Pipettes (1 ml and 2 ml), Measuring cylinder, Flask, Beaker, Jam Bottles, Glass Rod, Volumetric Flask, test tubes, Conical Flask, Funnel etc..

Miscellaneous

Cotton, Inoculation loop, Watmann filter paper, Centrifuge tubes, Micropipettes, Disk, Tips, Forceps, Hi antibiotic Zone Scale (for zome measurement), Dropper, Aluminium foil, Rubber band, Glossy papers, Pipette bulbs, Test tube stand, Wash water etc.

Chemicals required

95% Ethanol, distilled water, Nutrient Broth (NB), Agar, Nutrient Agar Media (NAM), Culture, Herbal Drug Powder, Chloroform, acetone, Ethanol, Fehling Solution, Biuret Test, Ferric chloride, Mayer's reagent, Warner's Reagent, Ninhydrin Solution etc.

Instruments

- **Soxhlet Assembly (J-Sil, 50/42, Borosil glass)-**For extracting the phytochemicals of powdered drug with the help of solvents.
- **Vacuum Rotary Evaporator (Scientech)-**For evaporation of the solvents.
- **Heating Mantle (Labtech)-**For heating the solvents.
- **Digital Balance (Denver, Germany)-**For weighing chemicals in micro quantities.
- **Digital pH meter (Eutech, Singapore)-**For adjusting the pH of the medium and buffer.
- **Autoclave – vertical (Scientech)-**For sterilization of the media before inoculation and disposal of the fermented materials.
- **Hot air Oven (Scientech, 325 L)-**For drying the glassware after washing.
- **Laminar air flow chamber – Horizontal (Scientech)-**For maintenance of aseptic condition by using UV light for 15 minutes before Inoculation.
- **Incubator (Scientech)-**For the growth of the microorganism.
- **Cyclo mixer (REMI)-**For mixing the suspensions.
- **Antibiotic Zone Scale (Hi Media laboratories limited, Mumbai)-**For Measurement of Zone.

Composition of selected media for culture and antimicrobial activity of Microorganisms

Nutrient Agar Media (NAM)	Nutrient Broth
Beef Extract-3gm	Beef Extract-3gm
Peptone-5gm	Peptone-5gm
Agar-15gm	NaCl-5gm
NaCl-5gm	
Distilled water-1 litre, pH – 7 both media	

Methodology:-Sample Collection

The samples which were collected from Vindhya Herbals at Sanjeevani Ayurveda, Bhopal are as follows:-

S.No	Botanical Name	General Name	Net Content
1	Emblica officinalis	Amla	100 grams

Then the samples were weighed accurately in digital balance for further extraction process.

Figure 1. Collection of Samples

Extraction Method Used:-Soxhalation

250 ml of solvent (Chloroform, Acetone, Ethanol) was taken in a round bottom flask. Then 25gm of the individual drug powder was taken. It was wrapped in filter paper to make a thimble. It was then placed in the central compartment and it was heated for boiling into 50 – 60 ^{0}C in heating mantle. After heating the vapour passes through the side arm up into the reflux condenser. Here the vapour condenses liquefies and drips into the thimble containing the material to be extracted. The warm solvent percolate through the material and the wall of the thimble and the extract gradually collects in the central compartment. Once the height of the extract reached the top of the siphon, the entire liquid in the central compartment flows through this and back into the lower round bottomed flask. The process is then repeated.

Figure 2. Extraction using Soxhalation method

In this method the extract collects in the lower vessel gradually becoming more and more concentrated. When the drug powder was completely extracted the solvent collecting in the present, the vapour rising from the heated extract is pure solvent vapour and so the liquid dripped into the material from the condenser is essentially pure solvent, though derived from the extract, thus although a relatively small volume of solvent is needed. The effective volume of solvent used for the extraction is proportional to the time for which the process is allowed to continue. The extraction process was repeated with all successive extraction of individual samples in chloroform, petroleum ether and menthol.

Recovery of Solvent by Rotary Vacuum Evaporator

Rotary vacuum evaporator has a water bath that was heated and then the solvent to vaporize. The extract was taken in round flask under vacuum and the vapours were trapped by a condenser and were collected for reuse. The process taken place in vacuum which helps to prevent oxidation.

Figure 3. Extraction using Rotary Vacuum Evaporator

The extracted residue further mixed with chloroform water (0.25 ml of chloroform in 100 ml of water) and resulted extract were stored in a refrigerator for further studies.

Phytochemical Extraction of Drug

Phytochemical examination were carried out for all the extracts as per the standard methods. (Brain and Turner 1975, Evans 1996)-

- Plant Constituents→Alkaloids, Test / Reagent Used→ Mayers Reagent, Preparation of Reagent→(a) 1.36g of mercuric chloride in 60ml of D.W,(b) 5g of potassium iodide in 20ml of D.W, Adjust the volume of 100 ml with D.W. Reaction→1ml filtrate was taken and few drops of Mayer's reagent was added and the formation of cream colour precipitate was observed. This confirms the presence of Alkaloids in the plant extract.
- Plant Constituents→ Carbohydrates and glycosides, Test / Reagent Used→ Fehling Solution, Preparation of Reagent→ It is used for detection of reducing sugars. Dissolve 34.66g of copper sulphate in D.W and make the volume up to 500ml (Solution- A). Dissolve 173g of potassium sodium tartarate and 50g of sodium hydroxide in D.W and make volume up to 500ml (Solution- B). Mix equal volume prior to use. Reaction→1ml filtrate was taken and few drops of Fehling solution were added and the formation of a red precipitate was observed. This confirms the presence of carbohydrates and glycosides in the plant extract.
- Plant Constituents→Phenolic compounds and Tannins, Test / Reagent Used→ Ferric Chloride Solution. Preparation of Reagent→A 5% W/V solution of ferric chloride in 90% alcohol and used for detection of

phenols.Reaction→1ml filtrate was taken and few drops of ferric chloride solution were added and the formation of a bluish black coloration was observed. This confirms the presence of Phenolic compounds and tannins.

- Plant Constituents→Proteins and Amino Acids, Test / Reagent Used→ Ninhydrin test, Preparation of Reagent→Prepare 0.1% solution in n-butanol. Reaction→1ml filtrate was taken and few drops of Ninhydrin was added and the formation of a purple pink coloration was observed. This confirms the presence of Proteins and Amino acids.

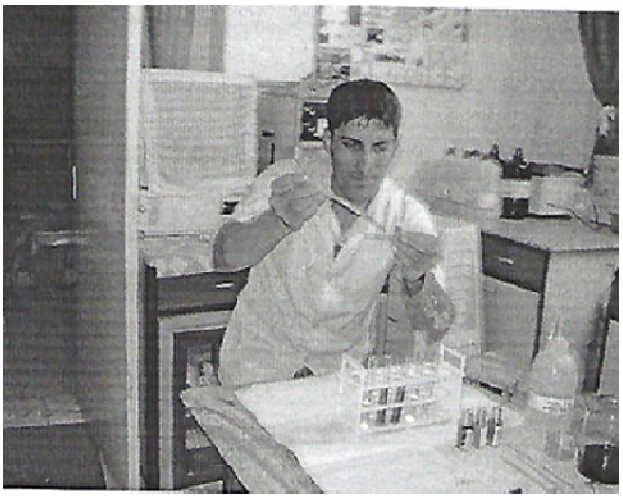


Figure 4. Phyto-Chemical Extraction of Drug

Antimicrobial Activity by Disc Diffusion Method:- Preparation of Inoculum

E.coli and S.aureus strains were used. 50ml of Nutrient broth was prepared in 100ml conical flask. It was sterilized and then inoculated with inoculum with the help of sterile loop in laminar air flow from preserved slants. They were then kept in incubator at 37 ^{0}C for sufficient period of time for organism to grow.

Preparation of Media

200 ml of NAM was prepared and the pH was maintained at 7 to 7.2.

Pour Plate Technique

1ml of prepared inoculum was poured in sterile Petri dish and then poured 15 ml of NAM in it and allowed to solidify.

Disc Diffusion Method

After solidification, the disc of Whatman filter paper imbibed with 20ul plant extract was carefully placed with the help of forceps at the centre of the Petri dish and then kept in incubator for 24 hours.

Measurement of Zones

With the help of antibiotic zone scale measures the zone of inhibition.

Antidiabetic Activity of Amla:-Procedure

- Three animals (rats) were weighed and marked for anti-diabetics.
- The three rats were divided in the following manner:- (a)Control, (b) Test, (c) Standard.
- In Control rat only diabetes was induced by Alloxan (100mg/kg) through I.P.
- In Test rat firstly diabetes was induced by Alloxan (100mg/kg) then treated with suspension of extract of amla prepared in 2 % acacia (100mg/kg) through oral dosing.
- In Standard rat firstly diabetes was induced by Alloxan (100mg/kg) then treated with the marketed drug Metformin (30mg/kg) through I.P.
- After 48 hours, the glucose level was checked by glucometer.
- The blood was collected from the eye vein of the rat with the help of capillary tube.
- One drop of the blood was put onto the glucometer strip and the level of glucose was noted from the glucometer.

RESULTS AND DISCUSSION

The samples were analysed for the pharmacognostic activity Pharmacognostic Characteristics of the Samples

Scientific Name	Common Name	Part of the Plant Used	Colour	Odour	Taste	Texture
Emblica officinalis	Amla	Fruit	Light Green	Pung	Sc	Smoo

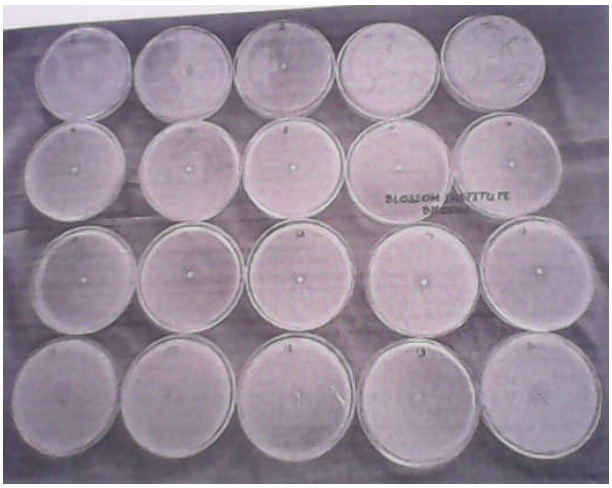
Colour of Successive Extract

S.No	Name of Reagent	Colour of Extract
1	Chloroform	Light Brown
2	Petroleum Ether	Light Brown
3	Methanol	Dark Brown

The extract were then analysed for the phytochemical constituents:-Photochemical Analysis of Drug Extract

Phytochemical Tests	Emblica Officinalis		
	Chloroform	Methanol	Petroleum Ether
Alkaloids (Mayer's Reagent)	+	+	-
Carbohydrates and glycosides (Fehling Solution)	-	+	-
Phenolic compounds and tannins (Ferric Chloride solution)	-	+	-
Proteins / Amino acids (Ninhydrin Test)	-	-	-

The Antimicrobial activities of the individual extracts were then measured by disc diffusion method



Antimicrobial Activity of Drug Extract from Soxhlet Extraction Method

(A) Chloroform Extract

S.No	Name of the drug		Zone of Inhibition (in mm)
1	Amla	E.coli	Nil
2		S.aureus	Nil

(B) Petroleum Ether Extract

S.No	Name of the drug		Zone of Inhibition (in mm)
1	Amla	E.coli	Nil
2		S.aureus	Nil

(C) Methanol Extract

S.No	Name of the drug		Zone of Inhibition (in mm)
1	Amla	E.coli	26 mm
2		S.aureus	24 mm

Antimicrobial Activity of Some Standard Antibiotics

S.No	Microorganisms	Zone of Inhibition (in mm)	
		P10	OFX5
1	E.coli	18	19
2	S.aureus	17	20

P10→Penicillin G, OFX5→ Oflaxacin

From the above study we can assess that though the plant powder was procured from the authentic source but still for the confirmation we have done the organoleptic study under Pharmacognostic characteristics of Drug. The powdered drug was subjected to extraction protocol soxhalation. The extract so obtained was tested for the presence of phytochemicals like alkaloid, carbohydrate, amino acid, Glycosides, Phenolic compounds and Tannins that shows positive results for the extract. The antimicrobial activity of the powder extract was done with Chloroform, Petroleum ether and Methanol. The result indicates that the antimicrobial activity of the methanolic extract of amla shows the maximum activity. This shows the amla has an antimicrobial activity and this may be due to the extracted phytochemicals in methanolic extract. The amla shows positive results for tannins in the methanolic extract and even alkaloids may be responsible for the maximum antimicrobial activity. But further chemical characterization is needed to confirm the molecule responsible for the activity. The antimicrobial activity of this herbal formulation was even comparable with standard antibiotics like penicillin G and Oflaxacin.

Results of anti-diabetic Activity

S.No	Treatment	Glucose level before inducing diabetes (mg/dl)	Glucose level after 48 hours (mg/dl)
1	Control	145	649
2	Test	145	337
3	Standard	145	230

Percentage difference increase in glucose level after 48 hours including diabetes in rat Control = $\frac{645-145}{145}$ $100 = 347.5\%$ Test = $\frac{337-145}{145}$ $100 = 132.4\%$. The results suggest that the Amla possesses significant anti-diabetic activity but it was less as compared to the standard drug. The literature study suggested that the mechanism of action of Amla extracts to reduce blood glucose levels is by enhancing glucose metabolism. It was further proposed that the glucose lowering effect could be explained by an antioxidant mechanism. Thus this herbal extract can be used in the formulation of anti-diabetic preparations and can be a good candidate in Polyherbal formulations.

Figure 5. Anti-Diabetic activity of Amla in Mice

Future Prospectus

The Herbal formulation has its own importance and advantages as compare to any other forms of medicines. As discussed in the present research the herbal formulations are free from any undesirable side effects and more or less they are non-habit forming. The Indian climate favours the growth of many rare varieties of medicinal plants. But the need of the hour is, these plants should be identified and much extensive research should be done on it so that new Drug discovery can be made to cure many threatful diseases. Many research organisation and Industries pursuing research on exploring the flora like CIMAP, Himalayan Drugs etc. and many success stories are daily published. But the research should be carried out in a large scale and should be region specific so that new formulations can be prepared. Much work is also going on Polyherbal Formulation, in which many herbal drugs are scientifically mixed to get the synergestic effect. The present research indicates that Emblica Officinalis shows some antimicrobial activity and anti-diabetic activity. The present study will be helpful in preparing new Polyherbal formulation for antimicrobial activity by incorporating the components of Amla. But much research on other activity of Amla can be done to generate a complete profile of Amla The present study will be helpful in preparing new Polyherbal formulation for antimicrobial activity and anti-diabetic activity by incorporating the Amla.

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