



RESEARCH ARTICLE

ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF TRIPHALA EXTRACTS

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ARTICLE INFO

Article History:

Received 15th June, 2016

Received in revised form

10th July, 2016

Accepted 28th August, 2016

Published online 30th September, 2016

Key words:

Antioxidant,
Antibacterial,
Triphala,
DPPH,
Ayurvedic.

ABSTRACT

Triphala (Digestive Support) is a composite herbal preparation containing equal proportions of the fruits of three myrobalans, *Embolia officinalis*, *Terminalia chebula* and *Terminalia bellerica*. This preparation is known to be a safe hypoglycemic agent. Triphala prevents aging, imparts immunity and improves mental faculties. It helps to detoxify the liver, restore digestion and purify blood. Triphala is widely used in a large number of medicinal preparations. Phytochemical screening reveals that the major constituents of Triphala, Amla, Baheda & Harad extract are phenolic, alkaloid, and flavanoid, compounds. Phenolic compounds which may be responsible for the activation of antioxidant. DPPH radical scavenging activity: Triphala & its individual fruit extracts had significant scavenging effect on the DPPH free radical which increases with increasing concentration from 20-10 μ g/ml. The anti microbial activity was found maximum with the methanolic extract of Triphala followed by Amla then Baheda and Harad. The antimicrobial activity and may be due to the extracted phytochemicals in methanolic extracts. Triphala, Amla, and Baheda & Harad shows positive results for phenolic component in the methanolic extract thus we can assume that the phenols may be responsible for the antimicrobial activity. But further chemical characterization is needed to confirm the molecule responsible for the activity. Triphala as a mixture shows better antibacterial activity as compared with individual fruit extracts therefore Triphala can be used in therapy as it has multiple other health benefits also.

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Citation: Gajendra Singh Pushkar Choudhary Syed Asif Yaqoob Rajveer Singh Rawat and Dr. Bhanwar Lal Jat, 2016. "Antibacterial and antioxidant activity of triphala extracts" *International Journal of Current Research*, 8, (09), 38335-38348.

INTRODUCTION

Ayurvedic System of Medicine

Ayurvedic medicine (also called Ayurveda) is one of the world's oldest medical systems. It originated in India and has evolved there over thousands of years. The term "Ayurveda" combines the Sanskrit words ayur (life) and Veda (science or knowledge). Thus, Ayurveda means "the science of life." Ayurvedic medicine also treats specific physical and mental health problems. A chief aim of Ayurvedic practices is to cleanse the body of substances that can cause disease, thus helping to reestablish harmony and balance.

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Two ancient books, written in Sanskrit more than 2,000 years ago, are considered the main texts on Ayurvedic medicine-Charaka Samhita and Sushruta Samhita.

Aim of Ayurveda

- The aim of Ayurvedic medicine is to integrate and balance the body, mind, and spirit. This is believed to help prevent illness and promote wellness.
- Ayurvedic medicine uses a variety of products and techniques to cleanse the body and restore balance. Some of these products may be harmful if used improperly or without the direction of a trained practitioner. For example, some herbs can cause side effects or interact with conventional medicines. (iii) Before using Ayurvedic treatment, ask about the practitioner's training and experience.

- Tell your health care providers about any complementary and alternative practices you use. Give them a full picture of what you do to manage your health. This will help ensure coordinated and safe care.

Indian Herbs

Indian herbs are renowned all over the world for their medicinal properties. India is the second highest producer of medicinal herbs in the world after China. Himalayas, Aravalis, and Nilgiri mountains are the greatest reserves of medicinal herbs in India. Ayurveda, the traditional Indian form of medicine, has been using herbs for thousands of years. These Ayurvedic herbs are very effective in increasing the body resistance and are used in the treatment of various diseases. Indian herbs are a significant part of the history of Ayurveda, the system of traditional medicine which is now popularly called alternative medicine? Herbs are more commonly called plants that have some value with them. They are used because of their qualities such as flavours, scent or some other properties. Different herbs are obtained from different parts of a plant they may come from roots, leaves, barks, seeds or flowers of a plant. However, the herbal extracts obtained from plants are used in very small quantities.

Phytochemicals

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produces these chemicals to protect them but recent research demonstrates that they can also protect humans against diseases. There are more than thousand known phytochemicals. Some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soya and Havanoids in fruits. Other common name: antioxidants, flavonoids, flavones, isoflavones, catechins, anthocyanidins isothiocyanates, carotenoids, polyphenols. Some phytochemicals have either antioxidant or hormone-like actions. Phytochemicals are promoted for the prevention and treatment of many health condition, including cancer, heart disease, diabetes, and high blood pressure.

Alkaloid

Alkaloids are a group of nitrogen-containing bases. Alkaloids were among the earliest isolated pure compounds with biological activity.

List of some important medicinal Indian herbs:-

Common Name	Botanical Name	Plant Parts	Medicinal use
Amla	<i>Emblica officinalis</i> Family- Euphorbiaceae	Fruit	Vitamin –C, Cough, Diabetes, Cold, Laxative, hyper acidity
Ashok	<i>Saraca indica</i> Family–Caesalpinaceae	Bark Flower	Menstrual Pain, uterine, disorder, Diabetes.
Aswagandha	<i>Withania somnifera</i> Family : Solanaceae	Root, Leafs	Restorative Tonic, Stress, nerves disorder, Aphrodisiac
Bach Sweet Flag	<i>Acorus calamus</i> Family : Araceae	Rhizome	Sedative, analgesic, epilepsy, hptensivess
Bhimi Amla	<i>Phyllanthus amarus</i> Family : Euphorbiaceae	Whole Plant	Anemic, jaundice, Dropsy
Brahmi	<i>Bacopa monnieri</i> Family : Scrophulariaceae	Whole Plant	Nervous, Memory enhancer, mental disorder
Gritkumari	<i>Aloe vera</i> Family : Liliaceae	Leaves	Laxative, Wound healing, Skin burn & care, Ulcer
Gorakhmundi	<i>Sphaeranthus indicus</i> Family : Asteraceae	Root, Bar, Leafs, flowers and seeds	Antidiabetic, anti inflammatory, antioxidant, leprosy, fever
Gudmar	<i>Gymnema sylvestre</i> Family : Asclepediaceae	Leaves	Diabetes, hydrocil, Asthama
Madhunaini, Guggul (T)	<i>Commiphora wightii</i> Family : Burseraccac	Gum resin	Rheumatized, arthritis, paralysis, laxative
Guluchi / Gioe	<i>Tinospora cordifolia</i>	Stem	Gout, Pile, General debility, fever, jaundice
Kalmegh / Bhui neem	<i>Andrographis paniculata</i> Family : Scanthaccac	Whole plant	Fever, weakness, release of gas
Long peeper / Pippali	<i>Piper longum</i> Family : Piperaceae	Fruit, Root	Appetizer, enlarged spleen, Bronchitis, Cold antidote
Makoi / Kakamachi	<i>Solanum nigrum</i> Family : Solanaceae	Fruit / Whole Plant	Dropsy, General debility, Diuretic, Anti dysenteric
Neem	<i>Azadirachata – indica</i> Family : Malvaceae	Rhizome	Sedative, analgesic, epilepsy, hypertensive
Sandal Wood	<i>Santalum album</i> Family : Santalinaccac	Heart wood, Oil	Skin disorder, burning sensation, jaundice, Cough
Sarpa Gandha	<i>Rauwolfia serpentina</i> Family : Apocynaceae	Root	Hyper tension, insomnia
Satavari	<i>Asparagus racemosus</i> Family : Liliaceae	Tuber, root	Enhance lactation, general weakness, fatigue, Cough
Sada bahar	<i>Vinca rosea/ catharanthus</i> Family : Apocynaceae	Whole Plant	Leukemia, Hypotensive, Antispasmodic, Atidot.
Tulsi (Perennial)	<i>Ocimum sanctum</i> Family : Lamiaceae	Leaves / Seed	Cough, Cold, Bronchitis, and Expectorant.
Vai Vidanka	<i>Embelia ribes</i> Family : Yretaceae	Root, Fruit, Leafs	Skin disease, Snake Bite, Helminthiasis
Vringraj	<i>Eclipta alba</i> Family : Compositae	Seed / Whole	Anti-inflammatory, Digestive, hair tonic

Alkaloids have diverse and important physiological effects on humans and other animals. Among the most famous of the alkaloids are the Solanaceae or tropane alkaloids, quinine extracted from *Cinchona succirubra*, and morphine alkaloids, derived from the opium poppy, *papaver somniferum*. Many alkaloids are poisonous in nature; some are used medicinally as analgesics (pain relievers) or anaesthetics and for other uses.

Flavones

Flavones are polyphenolic compounds present in plants as secondary metabolites. It is a class of flavonoids based on the backbone of 2-phenylchromen-4-one. It is most commonly known for their antioxidant activity. It is present in green vegetables, fruits, berries and beverages such as tea, red wine and fruit juices. Naturally occurring flavones includes Apigenin, Luteolin and Tangeritin. It provides many health benefits like protect against damage in blood vessels, thus decreasing the risk of cardiovascular diseases, prevent cancer and enhances immune system of body.

Saponin

Saponins are natural surfactants, or detergents, found in many plants, especially certain desert plants. Saponins are glycosides of steroids with a distinctive foaming characteristic. Saponins have long been known to have strong biological activity. They consist of a polycyclic aglycone that is either a choline steroid or triterpenoid attached via C3 and an ether bond to a sugar side chain. The antifungal and antibacterial properties of saponins are important in cosmetic applications, in addition to their emollient effects.

Tannin

Tannins are naturally occurring plant polyphenols. They are composed of a very diverse group of oligomers and polymers. Their main characteristic is to bind and precipitate proteins. Tannins are located mainly in the vacuoles or surface wax of the plants. It is mainly used in tanning animal hides into leather. *Acacia catechu* is an example of a plant having high tannin content.

Terpenes

Terpenes are natural organic solvents widespread in nature. Terpenes are varied class of hydrocarbons with a basic unit of isoprene. It is biosynthesized by plants mainly conifers. These are antibacterial, antitumour, anti-inflammatory, expectorant, diuretic and sedative. It is used in pharmaceuticals and perfumeries.

Volatile oil

A substance of oily consistency and feel, derived from a plant and containing the volatile aroma compounds from plants. They are also known as essential or ethereal oils. A volatile oil evaporates when exposed to the air and thus is capable of distillation; it may also be obtained by expression or extraction; many volatile oil's, identical to or closely resembling the natural oil's, can be made synthetically.

Volatile oils are used in medicine as stimulants, stomach aches, and correctives, carminatives, in perfumes and cosmetics and for purposes of flavoring.

Phytosterols

Phytosterols are plant sterols that occur in most plant Species but appear to be most abundant in the seeds of green and yellow vegetables. They are important in the human diet because they help to reduce the amount of dietary cholesterol absorbed by the body by blocking uptake in the intestine. They also facilitate cholesterol excretion from the body. This is especially important as cholesterol is a well established risk factor for heart disease.

Carotenoids

These are plant pigments found in bright yellow, orange and red fruits and vegetables. They are responsible for the pink colour of flamingoes and shellfish and the colour of egg yolk. There are more than 600 naturally occurring carotenoids divided into two distinct types: carotenes and xanthophylls. Carotenoids are generally well known as vitamin A precursors, meaning that once ingested, the body converts the compounds into vitamin A. However, less than 10% of carotenoids function in this way. In the carotene group only alpha, beta and epsilon carotene function as vitamin-A precursors. Beta carotene is the most active of the three. Xanthophylls protect vitamin A, other carotenoids and vitamin E from oxidation.

Advantages of herbal medicine

All forms of herbal medicines are made from various kinds of extracts from plants, animals, minerals and other natural substances also and are good for the body. The benefits of herbal medicine are common nowadays and there is also a common belief that herbs can treat the diseases where chemicals and other drugs have failed. There are a number of advantages associated with using herbal medicines as opposed to pharmaceutical products. Examples include the following:-

Reduced risk of side effects

Most herbal medicines are well tolerated by the patient, with fewer unintended consequences than pharmaceutical drugs. Herbs typically have fewer side effects than traditional medicine, and may be safer to use over time.

Effectives with chronic conditions

Herbal medicines tend to be more effective for long-standing health complaints that don't respond well to traditional medicine. One example is the herbs and alternative remedies used to treat arthritis. Vioxx, a well-known prescription drug used to treat arthritis, was recalled due to increased risk of cardiovascular complications.

Lower cost

Another advantage to herbal medicine is cost. Herbs cost much less than prescription medications.

Research, testing, and marketing add considerably to the cost of prescription medicines. Herbs tend to be inexpensive compared to drugs.

Widespread availability

Yet another advantage of herbal medicines is their availability. Herbs are available without a prescription. You can grow some simple herbs, such as peppermint and chamomile, at home. In some remote parts of the world, herbs may be the only treatment available to the majority of people.

Triphala

Triphala (Digestive Support) is a composite herbal preparation containing equal proportions of the fruits of three myrobalans, *Embllica officinalis*, *Terminalia chebula* and *Terminalia bellerica*. This preparation is known to be a safe hypoglycemic agent. Triphala prevents aging, imparts immunity and improves mental faculties. It helps to detoxify the liver, restore digestion and purify blood. Triphala is widely used in a large number of medicinal preparations. It is also a laxative that rejuvenates the membrane lining the digestive tract and contributes to effective cleansing of the colon, a key condition in Ayurveda to maintaining optimum health

Therapeutic use of Triphala

Triphala; An age-old Ayurvedic Medicine: - (i) No other herb or group of herbs, finds such repeated mention in the Ayurvedic system of medicine as Triphala. It has become a household name in our country. (ii) As the name suggests, Triphala is a combination of three important herbal fruits amla (*Embllica officinalis*), harada (*Terminalia chebula*), and baheda (*Terminalia bellerica*). (iii) The popularly known Triphala Churna is the powder of dried fruits of these herbs without their seed part. Usually, these herbs are mixed in equal quantity but references are found when their ratio has been modified as harad one part, baheda two parts and amla three parts. (iv) The first of these fruits, Amla, is the richest source of Vitamin C. It also contains tannic acid, resinous matter, glucose, protein, cellulose and calcium. Amla is useful as stomachic, antipyretic, hair tonic, alterative and nerve-brain tonic. Anemia, hyperacidity, urine anomalies, hemorrhages, epistaxis and gynecological disorders are among the indications where amla is prescribed as medicine and also as a preventive, restorative and curative. (v) The next among this triad, Baheda or vibhitaka, is a pungent, acrid and bitter fruit. It is rich in Vitamin A and has astringent, digestive, laxative, anti-allergic, anthelmintic (killer of intestinal worms) and expectorant properties. Baheda, in fruit form, is used in a number of ailments like cough, bronchitis, biliousness, inflammatory conditions of the small intestines, problems of the eye, dropsy and in the enlargement of liver and spleen. (vi) Finally, Harada or haritaki which is also known as pathya in Sanskrit is also a very prestigious herb of Ayurveda. It is a carminative, a killer of intestinal worms, a laxative, and is not only a general tonic but also a protector of the lungs. Harad contains tannin up to 30 percent, chebulinic acid and a sufficient amount of Vitamin B complex. The use of harad is beneficial in a number of diseases like asthma, constipation,

piles and sinus allergies. (vii) Triphala helps one to recover from anemia, indigestion, bowel toxicity and constipation. Its use is also beneficial in chronic lung disease, skin disorders, eye problems, hypertension and conditions where cholesterol is raised.

Triphala: The Miracle Drug: - (i) deeply embedded in the Indian ethos; it was trusted as a multi-purpose medicine by the ancient people and is relied upon by millions even today. Known as "phalatrik" I and "vara" in Sanskrit, Triphala is a combination of the pulp of three famous fruits or herbs - amla (*Embllica officinalis*), harad (*Terminalia chebula*) and baheda (*Terminalia bellerica*). (ii) Amla contains tannic acid, resinous matter, glucose, protein, cellulose and calcium. Amla has cooling, astringent, digestive, carminative, diuretic, anti-pyretic, aphrodisiac and anti-oxidant properties. It is considered useful in tackling a vast array of diseases like acid dyspeptic disease, urinary anomalies, skin problems, chest diseases, jaundice, hemorrhagic conditions, emaciation, hair and eye diseases. It is also regarded as a tonic. (iii) Harad, the other constituent of Triphala, is dry, light and hot in effect and has carminative, digestive, anti-inflammatory, anthelmintic, cardiogenic, aphrodisiac and restorative properties. It is said to be endowed with the same healing effects as those of amla, but is additionally beneficial in flatulence, constipation, piles, cough and colds. Harad is also a drug of choice in sinus allergies, lung diseases, liver and splenic enlargements and obesity. (iv) Baheda is very useful in disorders of the respiratory tract like cough, bronchitis and asthma and is also given to treat insomnia and general debility.

Triphala: A Unique Herbal Combination

- Triphala strengthens immunity, neutralizes the activity of carcinogens and supports balanced digestion, absorption and assimilation. As an anti-oxidant and tonic, it can be used in three different ways.
- The Triphala decoction mixed with a little honey also serves the same purpose. Besides having properties to lower the high cholesterol level, Triphala is an excellent medicine to treat the raised uric acid level also.
- Triphala can be effectively used to strengthen the respiratory system and to treat various sinus allergies, cough, bronchitis and chest diseases.
- It can be taken mixed with 500mg each of giloy satva and lauh bhasma to treat blemishes, pimples and anemia. In unidentified skin allergies, inflammations, infections and stubborn conditions like psoriasis, concomitant use for Triphala enhances the efficacy of the basic treatment. A wash with Triphala decoction is beneficial for hair and scalp care and conjunctivitis, gargles in mouth ulcers and if taken internally helps treat liver diseases.
- Many experienced physicians start the treatment of certain chronic ailments only after a few days of administration of Triphala. In some cases, where it is used for a longer period as a laxative, the drying effect of harad can cause dependence. It should be given with care to pregnant women. It is contraindicated in conditions like diarrhoea, dysentery and IBS.

Antimicrobial Activity:-An anti-microbial 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 (microbiostatic). Disinfectants are antimicrobial substances. 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 grow was that the other bacterium was producing an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the 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.

Antibiotics: - Antibiotics are generally used to treat bacterial infections. The toxicity to humans and other animals from antibiotics is generally considered to be low. 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. The term antibiotic originally described only those formulations derived from living organisms, but is now applied also to synthetic antimicrobials, such as the sulfonamides. The discovery, development, and clinical use of antibiotics during the 20th century have decreased substantially the mortality from bacterial infections. The antibiotic era began with the pneumatic application of nitroglycerine drugs, followed by a "golden" period of discovery from about 1945 to 1970, when a number of structurally diverse, highly effective agents were discovered and developed. However, since 1980 the introduction of new antimicrobial agents for clinical use has declined, in part because of the enormous expense of developing and testing new drugs. Paralleled to this there has been an alarming increase in bacterial resistance to existing agents. Antibiotics are among the most commonly used drugs. For example, 30% or more hospitalized patients are treated with one or more courses of antibiotic therapy. However, antibiotics are also among the drugs commonly misused by physicians, e.g. usage of antibiotic agents in viral respiratory tract infections. The inevitable consequence of widespread and injudicious use of antibiotics has been the emergence of antibiotic-resistant pathogens, resulting in a serious threat to global public health. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics.

One of the possible strategies towards this objective is the rational localization of bioactive phytochemicals.

Antimicrobial agents in the treatment of infectious disease: Most microbiologists distinguish two groups of antimicrobial agents used in the treatment of infectious disease: antibiotics, which are natural substances produced by certain groups of microorganisms, and chemotherapeutic agents, which are chemically synthesized. A hybrid substance is a semi synthetic antibiotic, wherein a molecular version produced by the microbe is subsequently modified by the chemist to achieve desired properties. Furthermore, some antimicrobial compounds, originally discovered as products of microorganisms, can be synthesized entirely by chemical means. In the medical and pharmaceutical worlds, all these antimicrobial agents used in the treatment of disease are referred to as antibiotics, interpreting the word literally. The modern era of antimicrobial chemotherapy began in 1929, with Fleming's discovery of the powerful bactericidal substance, penicillin, and Domagk's discovery in 1935 of synthetic chemicals (sulfonamides) with broad antimicrobial activity. In the early 1940's, spurred partially by the need for antibacterial agents in WWH, penicillin was isolated and purified and injected into experimental animals, where it was found not only to cure infections but also to possess incredibly low toxicity for the animals. This fact ushered into being the age of antibiotic chemotherapy, and an intense search for similar antimicrobial agents of low toxicity to animals that might prove useful in the treatment of infectious disease. The rapid isolation of streptomycin, chloramphenicol and tetracycline soon followed, and by the 1950's, these and several other antibiotics were in clinical usage.

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 onto the agar. (ii) Turbidimetric assessment is used less widely than agar 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 treatment. Here, a nutrient broth is inoculated capillary network through which the antibiotic components diffuse. Where the antibiotic concentration is sufficiently high, microbial growth is inhibited, but where the 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 standards and unknowns 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°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 neat from all residues of previous use. Occasional washing in nitric acid (2M) or chromic acid (200gm sodium dichromate 100ml water, 1500ml sulphuric acid) is ideal, but must be performed with safety in mind. The measurement of diameter should be taken through the center 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.

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 behavior of the active principles towards the solvent system. Following is a schematic representation of the procedure of obtaining powdered drugs:- Plant collected → authenticating by Taxonomist → Drying → subjected to powdering → Subjected to Suitable methods of Extraction. The following methods are used for extraction: Infusion; Decoction; Maceration; Percolation; Successive extraction; Supercritical fluid extraction; Steam distillation.

Infusion:-The method is only applicable for soft drugs & drugs containing water-soluble Constituents. Then the drug is mixed with water & it is allowed to stand for 15 to 20 minutes. Shaking or stirring is done if required. Drug + H₂O → Filtrate. Heat is not applied in this method & it is then filtered & the filtrate is called as infusion. The Infusion should be used within 24 hrs.

Decoction:-Decoction is applicable only for water-stable & heat-stable drugs obtained~ from hard & woody sources. Then the drug is mixed with water & heated for 15 to 20 minutes. Drug + H₂O → Filtrate. It is filtered & mare is pressed & volume is adjusted then the filtrated is called as Decoction. It should be consumed within 24 hrs.

Maceration:-During maceration the plant material is soaked in the solvent & 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 & the number of times the plant has been macerated. Usually, the solute-solvent proportion is 1:10. & unorganized drugs like gums, resins, gum-resins and oleo gum resins are also extracted by the maceration process. There are three types of maceration.

- Simple (Single) maceration
- Double maceration
- Triple maceration.

Digestion:-Digestion is a 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 & are rarely used for extraction of drugs with few exceptions.

Percolation:-In a 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 that is- (i) Simple percolation (cold percolation). (ii) Hot percolation.

(i) Simple percolation (Cold percolation):- In cold percolation we performed powdering the drug & inhibition of the drug that is moisturing the drug with a little menstruum for 4hrs 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 & allowed to saturate the drug. When the drops start to come down through the nozzle, the nozzle is closed and the moistened drug allowed standing for 24 hrs.

Percolation: The percolate is collected drop-wise 7 the process is continued till complete extraction has taken place. Complete extraction can be observed in the following ways: take the final drop & allow evaporating then if no residue remains, complete extraction has taken place. Test the specific gravity of the final percolate and menstruum. 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 may 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 analyses. Thus, in contrast to percolation, the total amount of solvent is limited. In spite of what is sometimes through, a soxhlet extraction can be far from complete, due to channeling or the presence of air in the semi-permeable thimble containing the plant material. Suitability of Soxhlet method: for heat-stable substances only. And also for active constituents which are less soluble in the menstruum in the absence of heat.

Solvent Extraction by Soxhlet extraction Method: - 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 distill 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 extracting chamber differ from that originally used in the collector & this fact may introduce errors when trying to reproduce the experiment using other extraction methods.

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

Disadvantages: - (i) Extractives are heated during the period of extraction at the boiling point of the solvent employed & thermally labile compounds such as carotenoids may hydrolyze or decompose. 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 color and consistency of the extract are noted.

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. Use of a "rotavap" therefore allows liquid solvents to be removed without excessive heating of what are often complex and sensitive solvent-solute combinations.

Antioxidant Activity:-Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease.

Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors or the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. 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 effects. 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 focused on determining the antimicrobial activity and anti-inflammatory activity of plant extract of Triphala. For this the literature reviewed was divided into following parts Introduction, advantages of Triphala, Antimicrobials, anti-oxidant, Biological activity, Phytochemical Analysis. Medicinal plants are part and parcel of human society from the dawn of civilization to combat diseases and have been considered valuable and cheap source of unique phytoconstituents which are used extensively in the development of drugs against various diseases (Gupta *et al.*, 2012). The three plants of Triphala namely *E. officinalis*, *T. bellerz'ca* and *T. chebula* are trees and belong to the family Euphorbiaceae and Combretaceae respectively. *E. officinalis* is used in the treatment of diarrhoea, gastroenteritis. (Tambekar *et al.*, 2005). *Terminalia chebula* (*T. chebula*) is a flowering evergreen tree of the family Combretaceae. It has several common names such as black myrobalan, ink tree, or chebulic myrobalan (English), haritaki (Sanskrit and Bengali), harad (Hindi), harada (Marathi and Gujrati) Karkchettu (Telgu) and Kadukkaya (Tamil). In Tibet, *T. chebula* is called as the "King of Medicine" (Aneja *et al.*, 2009).

Triphala is an ayurvedic formulation commonly prescribed by traditional health practitioners in India. It is an equal proportional mixture of fruits of three medicinal plants namely amalaki (*Embllica officinalis*), bibhitaki (*Terminalia bellerica*) and haritaki (*Terminalia chebula*) (Kulkarni *et al.*, 1995). The effect of the three fruit extracts of *E. officinalis*, *Terminalia bellerica* and *Terminalia chebula* have been studied fairly by a number of researchers using organic Solvents like ethanol, methanol and petroleum ether (Bhakshu *et al.*, 2008). Triphala contains several compounds that have been proposed to be responsible for its claimed health benefits, including gallic acid, Chebulagic acid and chebulinic acid (Reddy *et al.*, 2010). Triphala shows immunomodulatory properties and helps in improving the body's defense system. (Naik *et al.*, 2006). Triphala strengthens the different tissues of the body, prevents aging, promotes health and immunity (Jusse *et al.*, 1997). Triphala corrects constipation, cleanses and tonifies the gastrointestinal tract and also detoxifies the whole body and improves digestion and assimilation (Nadkarni *et al.*, 1976). Triphala and its constituents act as cardiogenic, control blood pressure, improve blood circulation and reduce cholesterol levels (Thakur *et al.*, 1988). *T. chebula* is used as laxative and expectorant (Rustomjee *et al.*, 1999). *Terminalia T. belleirica* is reported to provide protection from myocardial necrosis. (Tariq *et al.*, 1977). The exudates obtained from the incisions of *E. officinalis* fruits are used as an external application for ocular inflammation (Khanna *et al.*, 1963).

Antimicrobials:-Triphala contains several compounds that have been proposed to be responsible for its claimed health benefits, including gallic acid, chebulagic acid, and chebulinic acid (Pawar *et al.*, 2009). Triphala exhibits antiviral, antibacterial, antifungal and anti allergic properties (Singh

et al., 2003). Triphala possesses significant antimicrobial activity against both gram-positive and gram-negative organisms (Kumar et al., 2002). The antibacterial activities of aqueous and ethanol extracts of Triphala and its individual components were tested against certain bacterial isolates (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella sonnei*, *S. flexneri*, *Staphylococcus aureus*, *Vibrio cholerae*, *Salmonella paratyphi-B*, *Escherichia coli*, *Enterococcus faecalis*, *Salmonella typhi*) obtained from HIV infected patients using Kirby-Bauer's disk diffusion and minimum inhibitory concentration (MIC) methods. Most of the bacterial isolates were inhibited by the ethanol and aqueous extracts of *T. chebula* followed by *T. belerica* and *E. officinalis* by both disk diffusion and MIC methods (Srikumar et al., 2007). The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used antimicrobials and may have a significant clinical value in treatment of resistant microbial strains (Eloff et al., 1988). The ethanolic and aqueous extract from the plants are potential source of antimicrobial agents (Srikumar et al., 2007). The chloroform, water and acetone extracts of triphala have shown distinct anti-mutagenic activity against *Salmonella typhimurium* and is found to act as purgative (Kaur et al., 2002).

Alcoholic extract of Triphala has shown *in vitro* antimicrobial activity against wound pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes*. An ointment prepared from the Triphala extract (10% w/w) was assessed for *in vivo* wound healing on infected rat model by rate of healing, bacterial count, biochemical analysis, and expression of matrix metalloproteinases. The treated group showed significantly improved wound closure. Assessment of granulation tissue on every fourth day showed significant reduction in bacterial count with significant level of collagen, hexosamine, uronic acid, and super oxide dismutase in the treated group ($P < 0.01$) (Muthusamy et al., 2008). Triphala is prescribed for anticaries agent, myocardial injury, cancer etc. (Carunanidy et al., 2007).

Antioxidant: Antioxidants are widely used as ingredients in dietary supplements and are exploited to maintain health and prevent oxidative stress mediated diseases. Antioxidant compounds like phenolic acids, polyphenols and flavonoids inhibit the mechanism that leads to degenerative diseases (Hamid et al., 2010). Anti-oxidant activity of Triphala may be due to presence of gallic-acid, thus present research work involved evaluation of antioxidant activity of gallic-acid as chief component of Triphala (consist 10% gallic-acid) with comparison to the natural reference antioxidant Curcumin (Rajani et al., 2005). Triphala exhibits analgesic and antipyretic activities without any gastric damage, increased body temperature and pain are known as the main reactions of the body against an inflammatory stimulation. Therefore, it is generally essential to possess analgesic and antipyretic activities for an anti-inflammatory compound. The analgesic, antipyretic and ulcerogenic activities of Triphala (500/1000 mg/kg body wt) were compared with then on-steroidal anti-inflammatory drug Indomethacin (10 mg/kg body wt) on the experimental models in mice and it was found that Triphala at both the dose levels produced excellent analgesic and

antipyretic effect, with the absence of gastric damage. Induction of the acetic acid writhing in mice is an effect of the acute inflammatory reaction related to the increase in the level of prostaglandin E2 and F2a in the peritoneal fluid (Deraedt et al., 1976). Triphala an aqueous extract of *E. officinalis* was found to be a potent inhibitor of lipid peroxide formation and as a scavenger of hydroxyl and superoxide radicals *in vitro*. (Vapaatalo et al., 1993). Triphala has been tested as an antioxidant and also as a radio protector in mice (Jagetia et al., 2002). There is preliminary evidence that Triphala contains compounds with antioxidant properties in isolated cells and rats, however this has not yet been demonstrated in people (Reddy et al., 2010). Antioxidants can give free radicals which become companions to their unpaired electrons, thus eliminating the threat of gene alteration which can lead to cancer. (Narayanan et al., 2003). Free radicals have been implicated in the causation of several disorders, which includes diabetes, and the agents that scavenge free radicals may have great potential in ameliorating these diseases. The most frequently encountered free radicals are the hydroxyl radical ($\cdot OH$), the superoxide radical ($O_2^{\cdot -}$), the nitric oxide radical ($\cdot NO$) and the lipid peroxy radical ($LOO\cdot$) while non-free radical species principally being H_2O_2 and singlet oxygen (1O_2). (Yildirim et al., 2000). In triphala carbonyl groups are responsible for free radical scavenging activity. Free radicals are atoms or groups of atoms with an odd number of electrons and can be formed when oxygen interacts with certain molecules. Once highly reactive free radicals are formed, they can start chain reactions. Their major threat comes from the damage they can do when they react with important cellular components such as DNA or cell membranes. Cells may function poorly or die if this occurs. To prevent free radical damage, the body has a defense system of antioxidants. (Thomas et al., 2000).

The evaluation of the *in vitro* antioxidant activity of aqueous extract of the fruits of *Embllica officinalis*, *Terminalia chebula* and *Terminalia belerica* and their equi-proportional mixture, Triphala, has indicated their strong ability to scavenge free radicals such as DPPH and super oxide. Results of DPPH reduction have shown that Triphala had a synergistic effect, compared to each individual constituent, and it may be useful for free radical induced disorders such as paracetamol toxicity, heavy metal and radiation. (Naik et al., 2005). Methanolic extract of Triphala (70%) has shown high antioxidant activity in the *in-vitro* studies. Some reports have shown the radio protective activity of Triphala in mice exposed to gamma radiation (Baliga et al., 2002). In Triphala the methanolic extracts (75%) of *Terminalia chebula*, *Terminalia belerica*, *Embllica officinalis* and their combination named Triphala were found to inhibit lipid peroxide formation and to scavenge hydroxyl and super oxide radicals *in vitro*. The concentration of plant extracts that inhibited 50% of lipid peroxidation induced with Fe^{2+} -ascorbate were found to be 85.5, 27, 74 and 69 $\mu g/ml$, respectively. The concentration needed for the inhibition of hydroxyl radical scavenging were 165, 71, 155.5 and 151 $\mu g/ml$, and that for super oxide scavenging activity were found to be 20.5, 40.5, 6.5 and 12.5 $\mu g/ml$, respectively. (Sabu et al., 2002). In Triphala the phenolic extract present in these extracts are mostly responsible for their radical scavenging activity, the total phenolic content present in these

extracts has been determined and found to vary from 33% to 44% in terms of Gallic acid equivalents. These studies revealed that all three constituents of Triphala are active and they exhibit slightly different activities under different conditions. *Embllica officinalis* shows greater efficiency in lipid peroxidation and plasmid DNA assay, while *Terminalia chebula* has greater radical scavenging activity. Thus their mixture, Triphala, is expected to be more efficient due to the combined activity of the individual components (Vani et al., 1997).

Phytochemical Analysis:- Triphala is traditional Ayurvedic herbal formulation, consisting equal parts of three medicinal plant fruits namely 71 chebula, *T. bellirica* and *officinalis*. According to Indian traditional medical system (Ayurveda). Triphala strengthens the different tissues of the body, prevents aging, promotes health and immunity. It corrects constipation, cleanses and notifies the gastrointestinal tract and also detoxifies the whole body and improves digestion and assimilation. It exhibits antiviral, antibacterial, antifungal and antiallergic properties. (Singh et al., 2003). Phytochemical test, quantitative analysis, *in-vitro* antioxidant test, antimicrobial analyses are some of the basic tests done. Quantitative estimation showed more amounts of phenols present in all the plants. Neem showed high anti microbial activity when compared to other plants. Extracts from Turmeric, Neem, African spider plant, Triphala, Wheatgrass showed varying antioxidant (free standard ascorbic acid. The H2O2 scavenging activity was found to be more in neem extract. Triphala and turmeric showed high reducing power activity. Wheatgrass and turmeric was found to have high total antioxidant capacity. *Azadirachta indica* and *Vitis vinifera* (Brown raisins) showed more antioxidant activity than the other plant extracts. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable molecule. The plant extracts are made to react with the stable radical DPPH-I, in ethanol solution. The decrease in the absorbance at 517 nm shows the reduction capability of DPPH radical (Mittal et al., 2012). The common chemical compounds on review present in this drug are Tannin, Gallic acid, Chebulagic acid, Ellagic acid, Phenols and Glycosides. Phenolic acids, flavonoids and tannins are the most commonly found polyphenolic compounds in plant extracts.

The total Phenolic content in Triphala using spectrophotometric methods has been evaluated and the phytochemical analysis showed that Triphala is rich in phenols polyphenols (38.3%) and tannins (35.13%), while flavonoids were found to be absent. HPLC analysis (column, C18 PCX 500; mobile phase, aqueous acetonitrile (10%)-HCl (0.05 M)-KCl (0.1M); detection, absorbance at 260nm) showed that Triphala contains 73.5mg Gallic acid per gram of Triphala, which was found to increase to 150.5mg/gm upon acid hydrolysis. Tannins are naturally occurring, high molecular weight plant polyphenols. They are usually subdivided into two groups. hydrolysable tannins and condensed tannins. The total tannin content present in Triphala was measured using a colorimetric Fella-Denis method. The measurements were compared with standard tannic acid sample and results

expressed in terms of % tannic acid equivalents. The tannin content in Triphala was found to be 35.3%. Analysis of total flavonoid content in Triphala has been done using colorimetric method and quercetin as standard flavonoids. The results showed that Triphala did not contain any significant amount of flavonoids (Priyadarsini et al., 2006). Triphala contains 73.5 mg Gallic acid per gram of Triphala, which was found to increase to 150.5 mg/g upon acid hydrolysis. Tannins are naturally occurring, high molecular weight plant polyphenols. They are usually subdivided into two groups, hydrolysable tannins and condensed tannins.

The total tannin content present in Triphala was measured using a colorimetric Folin-Denis method. The measurements were compared with standard tannic acid sample and results expressed in terms of % tannic acid equivalents. The tannin content in Triphala was found to be 35.3%. Analysis of total flavonoid content in Triphala has been done using colorimetric method and quercetin as standard flavonoid. The results showed that Triphala did not contain any significant amount of Flavonoids. (Naik et al., 2006). The ethanol extracts of the plants and water extract of raisins obtained were subjected to preliminary test, flavonoids (Alkaline reagent test), carbohydrates (Molisch's test), glycosides (Legal's test), saponins, tannins, phytosterol (Salkowski test), triterpenoid (Liebermann-Burchard test), proteins and amino acids (Ninhydrin test), biuret test, anthrax quinones, steroids, catechol, reducing sugars (Fehling's Test), acidic compounds, lipids/ fats, phlobatannins and resins were (Poudel et al. 2008). Triphala inhibits lipid peroxidation and also acts as scavenger of free radicals. It is rich in active ingredients like tannins, carbohydrates, saponins, ellagic acid, sorbitol and ascorbic acid (Bhattacharya et al., 1999).

Plan of work: - Collection of the powdered of the Triphala Amla Baheda & Harad from sources. → Solvent extraction protocol for the extraction of phytoconstituents of the drug. The extraction will be done with the help of Methanol. The Extraction to be followed 1 concentration & recovery of Solvent using Rotatory Vacuum Evaporator. → The Phytochemical examination of the extract for the identification of phytochemicals like glycosides, saponins, gums, carbohydrates, proteins etc. → The antimicrobial activity of each methanolic extract (Triphala, Amla, Baheda & H.) by using disc diffusion method against the bacteria obtained from MTCC & Determination of Zone of Inhibition → Anti-oxidant activity of each methanolic extract (Triphala, Amla, Baheda & Harad) by DPPH Assay.

Materials: - Material required: Microorganisms: MTCC Cultures of *E coli* MTCC 739, *S aureus* MTCC 737

Glasswares:- Petri plates, Pipettes (1 ml & 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 Zone measurement), Dropper, Aluminum foil, Rubber band, Pipette bulbs, test tube stand, 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, Wagner's Reagent, Ninhydrin solution etc.

Instruments:- (i) Soxhlet Assembly (J-Sil, 50/42, Borosil glass):- For extracting the phytochemicals of powdered drug with the help of solvents. (ii) Vacuum Rotary Evaporator (Sciencetech):- For evaporation of the solvents. (iii) Heating Mantle (Labtech):- For heating the solvents. (iv) Digital Balance (Denver, Germany):- For weighing chemicals in micro quantities. (v) Digital pH Meter (Eutech, Singapore):-For adjusting the pH of the medium and buffer. (vi) Autoclave Vertical (Sciencetech):- For sterilization of the media before inoculation and disposal of the fermented Materials. (vii) Hot Air Oven (Sciencetech, 325 L):-For drying the glassware's after washing. (viii) Laminar Air Flow Chamber Horizontal (Sciencetech):-For maintenance of aseptic condition by using UV light for 15 minutes before Inoculation. (ix) Incubator (Sciencetech):-For the growth of the microorganism. (x) Cyclomixer (Remi):- For mixing the suspensions. (xi) Antibiotic Zone Scale (Hi Media Laboratories Limited, Mumbai):-For Measurement of Zone. (xii) Composition of selected Media for Culture & Antimicrobial Activity of Microorganism.

Nutrient Agar Media (NAM):- (i) Beef Extract: 3gm (ii) Peptone: 5gm (iii) Agar: 15gm (iv) NaCl: 5gm (v) Distilled water 1 liter (vi) pH = 7

Nutrient Broth:- (i) Beef extract: 3gm (ii) Peptone: 5gm (iii) NaCl: 5gm (iv) Distilled water 1 Liter (v) pH=7

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.	Triphala powder	Triphala	100gm.
2.	<i>Embelica officinalis</i>	Amla	100gm
3.	<i>T. erminalia chebula</i>	Baheda	100gm
4.	<i>Terminalia bellerica</i>	Harad	100gm

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

Extraction Methods Used:- Soxhalation:- 250ml of solvent Methanol was taken in a round bottom flask. Then 50gm of the individual drug powder was taken. It was wrapped in filter paper to make a thimble .h was then placed in the central compartment & it was heated for boiling into 50-60°C in heating mantle. After heating the vapor passes through the side arm up into the reflux condenser. Here the vapor condenses liquefies & drips into the thimble containing the material to be extracted. The warm solvent percolate through the material & the wall of the thimble & 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 & back into the lower round bottomed flask. Then process is then repeated.

In this method the extract collects in the lower vessel gradually becoming more & more concentrated. When the drug powder was completely extracted the solvent collecting in the middle compartment showed transparent color. Assuming that the no. volatile substances are present, the vapor rising from the heated extract is pure solvent vapor & 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.

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 & the vapors were trapped by a condenser and were collected for reuse. The process taken place in vacuum which helps to prevent oxidation. The extracted residue further mixed with chloroform water (0.25ml of Chloroform in 100ml of water) & resulted extract were stored in a refrigerator for further studies

Phytochemical Analysis of Extract

Phytochemical examinations were carried out for all the extracts as per the standard Methods. (Brain & Turner 1975, Evans 1996). (1) Plant Constituents (Alkaloids) → Test/Reagent Used (Mayers Reagent) → Preparation of Reagent→(a) 1.36gm of mercuric chloride in 60ml of D.W, (b) 5gm of potassium iodide in 20ml of D.W. (c) Adjust the vol. of 100ml with D.W. →Reaction:- 1ml. filtrate was taken and few drops of Mayer's reagent was added. Formation of cream colour precipitate confirms the presence of Alkaloids. (2) Plant Constituents (Carbohydrates & Glycosides) → Test/Reagent Used (Fehling Solution) →

Preparation of Reagent

It is used for detection of reducing sugars. Dissolve 34.66gm of copper sulphate in D.W. & make the volume up to 500ml (sol. A). Dissolve 173gm of potassium sodium tartarate and 50g of sodium hydroxide in D.W. & make volume up to 500ml (sol. B) . Mix equal volume prior to use. → Reaction-- 1ml filtrate was taken and few drops of Fehling solution were added. Formation of a red precipitate confirms the presence of Carbohydrates & glycosides in the plant extract. (3) Plant Constituents (Phenolic compounds & tannins) → Test/Reagent Used (Ferric Chloride solution) → Preparation of Reagent: - A5% 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 &the formation of a bluish black coloration was confirms the presence of Phenolic compounds & tannins. (4) Plant Constituents (Proteins & Amino acids) → Test/Reagent Used (Ninhydrin test) → Preparation of Reagent (Prepare 0.1% solution in n-butanol) →. Reaction:-1ml filtrated was taken and few drops of Ninhydrin was added & the Formation of a purplish pink coloration was observed. This confirms the presence of Proteins & Amino acids. (5) Plant Constituents (Flavonoids) → Test/Reagent Used (Alkaline Reagent test). →

Preparation of Reagent:-Add 1gm of Sodium Hydroxide (NaOH) in 10ml of distilled water. → Reaction:-Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids. (6) Plant Constituents (Terpenoids) → Reagent/Test Used (Acidic Reagent test) → Reaction:-Extracts were mixed with few drops of chloroform and then few drops of Conc. Sulphuric acid. A reddish brown colour indicates the presence of terpenoids. (7) Plant Constituents (Saponins) → Reagent/Test Used (Foam/Froth test) → Reaction:-Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins. (8) Plant constituents (Phlobatannis) → Reaction:-0.5gm of extract/sample was taken, 5ml of distill water was added & boiled with 5ml of 1% HCl; Deposition of a red ppt indicates presence of Phlobatannins. (9) Plant constituents (Steroids) → Reaction:-0.5ml of extract was taken, to it was added Sulphuric acid. Formation of reddish brown layer at the interface shows the presence of steroidal 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 & 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°C for sufficient period of time for organism to grow.

Preparation of Media

200ml 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 & then poured 15ml approx. of NAM in it & allowed to solidify.

Disc Diffusion Method

After solidification the disc of what man filter paper imbibed with 10ml plant extract was carefully placed with the help of forceps at the center of the Petri-dish and then kept in incubator for 24 hrs.

Measurement of Zones

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

Antioxidant Activity using DPPH (Diphenyl picryl Hydrazine) Method

The method is based on the reduction of colored solution of DPPH (1, 1diphenyl-2picryl hydrazyl) in presence of test drug measured at 517nm.

The free radical scavenging capacity of the Individual extracts was determined using DPPH method. DPPH solution (0.004% w/v) was prepared in 95% methanol. Ascorbic acid solution preparation: 1000µg/ml stock solution was prepared by dissolving 10mg of ascorbic acid in 10 ml of methanol. From this 20, 40, 60, and 80, 100µg/ml ascorbic acid solutions were prepared. The extracts were mixed with 95% methanol to prepare the stock solution (10mg/1000ml). The concentration of this Ext solution was 10mg [100ml].

Stock solution 2ml, 4ml, 6ml, 8ml & 10ml of this solution were taken in five test tubes & by serial dilution with same solvent were made the final volume of each test tube up to 10ml whose concentration was then 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml & 100µg/ml respectively. 2ml of the Dilutions of Standard 8: Samples were taken in respective test tubes. Then 0.5ml of freshly prepared DPPH solution was added to each of the test tubes. Allow it to stand for reaction for 10 min in dark conditions. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing Extraction (20µg/ml., 40µg/ml., 60µg/ml., 80µg/ml., and 1000µg/ml.) and after 10 min, the absorbance was taken at 517nm using a spectrophotometer. Control sample was prepared containing the same volume without any extract was used as blank. % scavenging of the DPPH free radical was measured using the following equation. Results are shown in table and graphically % inhibition = $[(A_0 - A_t) / A_0 \times 100]$ Where A₀ was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract.

RESULTS

The samples were analyzed for the pharmacognostic activity –

Pharmacognostic Characteristics of the Samples

Scientific name	Common Name	Part of the Plant Used	Color
Triphala powder	Triphala	Fruit	Brownish
<i>Embelica officinalis</i>	Amla	Fruit	Brownish
<i>Terminalia chebula</i>	Harad	Fruit	Yellowish Brown
<i>Terminalia belerica</i>	Baheda	Fruit	Yellowish Brown

Color of Individual Extract

S. No	Name of Reagent	Name of Drug	Color of Extract
1	Methanol	Triphala	Brownish Black
2	Methanol	Amla	Brownish
3	Methanol	Harad	Yellowish
4	Methanol	Baheda	Yellowish Brown

The extract were then analysed for the phytochemical constituents –

The Antibacterial activity of the extracts were then measured by disc diffusion method

Antimicrobial Activity of Drug Extract: Phytochemical Analysis of Drug Extract

Phytochemical tests	Methanolic Extract of Triphala	Methanolic Extract of Amla	Methanolic Extract of Baheda	Methanolic Extract of Harad
Alkaloids (Mayers Reagent)	+	+	+	+
Carbohydrates & glycosides (Fehling Solution)	-	-	-	-
Phenolic compounds & tannins (Ferric Chloride Solution)	+	+	+	+
Proteins / Aminoacids (Ninhydrin test)	-	-	-	-
Saponins	+	+	+	+
Terpenoid	+	+	-	-
Phalobatannins	+	+	-	-
Flavanoides	+	+	-	-

Methanolic Extract of Triphala

S. No.	Name of the drug	Microorganism	Zone of Inhibition (in mm)
1	Triphala	E. coli	20
		S. aureus	18

Methanolic Extract of Harad

S. No.	Name of the drug	Microorganism	Zone of Inhibition (in mm)
1	Amla	E. coli	18
		S. aureus	17

Methanolic Extract of Amla

S. No.	Name of the drug	Microorganism	Zone of Inhibition (in mm)
1	Harad	E. coli	12
		S. aureus	13

Methanolic Extract of Baheda

S. No.	Name of the drug	Microorganism	Zone of Inhibition (in mm)
1	Triphala	E. coli	14
		S. aureus	15

Antimicrobial Activity of some standard Antibiotics

S. No	Microorganism	Zone Inhibition (mm) Streptomycin
1	E. coli	24
2	S. aureus	22

Results of Antioxidant Activity

S. No	Concentration Mg/ml	% Inhibition				
		Methanolic Extract of Triphala	Methanolic Extract of Amla	Methanolic Extract of Baheda	Methanolic Extract of Harad	Ascorbic acid
1	20	16.8	14.8	12.4	9.8	25.2
2	40	23.2	22.1	20.0	18.6	33.2
3	60	31.	28.7	22.4	19.6	40.6
4	80	4.28	37.6	33.2	29.5	51.7
5	100	50.3	46.8	38.7	34.4	65.6

Antibiotic Assay of Standard Antibiotic Streptomycin

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 Methanolic extracts of Triphala, Amla, Baheda & Harad were performed. The anti microbial activity was found maximum with the methanolic extract of Triphala followed by Amla then Baheda and Hara. The antimicrobial activity and may be due to the extracted phytochemicals in methanolic extracts. Triphala, Amla, Baheda & Harad shows positive results for phenolic component in the methanolic extract thus we can assume that the phenols may be responsible for the antimicrobial activity. But further chemical characterization is needed to confirm the molecule responsible for the activity. Triphala as a mixture shows better antibacterial activity as compared with individual fruit extracts therefore Triphala can be used in therapy as it has multiple other health benefits also. The antimicrobial activity of this Triphala herbal formulation was even comparable with standard antibiotics like Streptomycin.

DISCUSSION

Phytochemical screening reveals that the major constituents of Triphala, Amla, Baheda & Harad extract are phenolic, alkaloid, and flavanoid, compounds. Phenolic compounds which may be responsible for the activation of antioxidant. DPPH radical scavenging activity: Triphala & its individual fruit extracts had significant scavenging effect on the DPPH free radical which increases with increasing concentration from 20-10 μ g/ml. The scavenging effect of samples was lower than that of Ascorbic acid. Triphala extract as good antioxidant property as compared to Amla, Baheda & Harad Extracts. This could be attributed to the presence of flavonoids, alkaloids, tannins, saponin and phenolic compounds have free radical

scavenging property. The result of this study clearly indicate the Triphala have high antioxidant activity and radical scavenging activity against various antioxidant systems in vitro. These assays have important applications for the food and pharmaceutical industry. Moreover, Triphala can be used

as an easily accessible source of natural antioxidants and as a possible food supplement.

In our present study we conclude that Triphala has good antioxidant property. Ayurveda is the science of positive health and fulfillment in life. The aim of Ayurveda is to achieve the positive health of individual. Ayurvedic herbs are very effective in increasing body resistance and are used for the treatment of various diseases. Phytochemicals are non nutritive plant chemicals that have protective or disease preventive properties. There are varieties of Phytochemicals viz. flavonoids, alkaloids, tannins, flavones etc. Advantages of herbal medicines is its wide spread availability, lower cost, effective with chronic infections with reduced side effects. *Triphala* is a composite herbal preparation containing equal proportions of the fruits of three myrobalans, *Embolica officinalis*, *Terminalia chebula* and *Terminalia bellerica*. This preparation is known to be a safe hypoglycemic agent.

Triphala prevents aging, imparts immunity and improves mental faculties. It helps to detoxify the liver, restore digestion and purify blood. Triphala is widely used in a large number of medicinal preparations. It is also a laxative that rejuvenates the membrane lining the digestive tract and contributes to effective cleansing of the colon, a key condition in Ayurveda to maintaining optimum health. The three fruits have immense medicinal properties and therefore Triphala has lots of medicinal applications. (i) The methanolic extraction of Triphala, Amla, and Baheda & Harad was performed using Soxhlet Extraction method followed by concentration by using Rotary Vacuum Evaporator Machine. (ii) The concentrated Extracts of Triphala, Amla, Baheda & Harad were then analysed for the presence of various phytochemicals. (iii) The disc diffusion antibacterial assay of the Individual extracts of Triphala, Amla, Baheda & Harad was performed using standard MTCC strains of *E. coli* & *S. aureus*.

The antibacterial efficiency of the standard antibiotic Streptomycin was also compared with. (iv) The anti microbial activity was found maximum with the methanolic extract of Triphala followed by Amla then Baheda and Harad. The antimicrobial activity and may be due to the extracted phytochemicals in methanolic extracts. Triphala, Amla, and Baheda & Harad shows positive results for phenolic component in the methanolic extract thus we can assume that the phenols may be responsible for the antimicrobial activity. But further chemical characterization is needed to confirm the molecule responsible for the activity. Triphala as a mixture shows better antibacterial activity as compared with individual fruit extracts therefore Triphala can be used in therapy as it has multiple other health benefits also. (v) The DPPH antioxidant assay was performed of individual extracts of Triphala, Amla, Baheda & Harad and comparison of % Inhibition was done with Ascorbic acid Standard. (vi) DPPH radical scavenging activity:

Triphala & its individual fruit extracts had significant scavenging effect on the DPPH free radical which increased with increasing concentration from 20-100µg/ml. The scavenging effect of samples was lower than that of Ascorbic

acid. Triphala extract has good antioxidant property as compared to Amla, Baheda & Harad Extracts. This could be attributed to the presence of flavonoids, alkaloids, tannins, saponin and phenolic compounds. It was already been reported that naturally occurring phenolic compounds have free radical scavenging property. The results of this study clearly indicate that Triphala have high antioxidant activity and radical scavenging activity against various antioxidant systems *in vitro*. These assays have important applications for the food and pharmaceutical industry.

Acknowledgements

The authors express deep sense of gratitude & heartfelt thanks to Prof. C.B. Gena Former Vice-Chancellor of M.G. S. University Bikaner Rajasthan for providing all facilities. Since thanks are extended to Shri. C.R. Choudhary, Hon'ble Minister of State for Consumer Affairs, Food and Public Distribution, Govt of India whose continuous encouragement and constant help to carry out this research work.

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