Comparison of anti-bacterial activity of the leaves extract of moringa olifera and murraya koenigii

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ABSTRACT:

The present study was to performed to compare the the antibaterial activity of crude extract of leaves of moringa oliefera and murraya koenigii against staphylococcus aureus by using agar steak plate method ,hydroalcoholic solvent is used during the study .moringa oliefera showed effective aantibacterial activity than murraya koenigii.priliminary phytochemical investigation on crude extracts of leaves of moringa and murraya are performed, it showed alkaloids,tannins, glycocides, flavonoids and aminoacids presence of these secondary metabolites indicates the pharmacological activity of plant leaves . on the basis of this finding, the extracts demonstrating antibacterial activity could results in discovery of novel anti-bacterial agents.

KEYWORDS: Moringaoliefera, murraya koenigii, hydroalcholic, flavonoids, antibacterial activity

INTRODUCTION

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection . In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections. The aim of this study was to evaluate the antimicrobial activity of some medicinal plant used in Ayurveda and traditional medicinal system for treatment of manifestations caused by microorganisms

India is frequently known by enormous biodiversity of medicinal plants. Among them Murraya konigii have a lot of bio microbial principles due to which plant has been proven as the medicinally important plant but least or no attention received by the scientist. Murraya koenigii is proven as the natural medicinal plant.[1] There are different forms of Murraya koenigii due to which they are found as the useful plant such as extract, essential oil, or directly used due to the presence of following active constituent bismahanine, murrayanine, murrayafoline-A, bi-

koeniquinone-Abismurrayaquinone,mukoenine-A,mukoenine-B,mukoenine-C,murrastifoline, Murrayazolinol, murrayacine, murrayazolidine, murrayazoline, mahanimbine, girinimbine, koenioline, xanthyletin, koenigine-Quinone A and koenigine-Quinone B for therapeutic purpose by folk people.[2]2–5 Many medicines such as digitalis, vinblastine, aspirin and quinine has plant as a source of origin for example foxglove (Digitalis purpurea), willow bark (Salix spp.), quinine bark (Cinchona officinalis). For therapeutic or prophylactic purposes medicinal plant are used[3]. For the therapeutic properties of medicinal plants presence of secondary metabolites plays a very important role such as alkaloids, flavonoids, terpenoid's, vitamins, tannins etc., these all are the secondary metabolites of the plant as active constituent.[4] These all secondary metabolites of plant physiologically affect the body at different stages of abody development and make the body disease free[5]. The plant Murraya koenigii belonging to the family Rutaceae is largely growing plant throughout the spring, summer and in rain fall season in every part of the tropical region up to the height of 1500 to 1655m from sea level.6,7 It is also known as Curry Leaf English, Mitha Neem in Hindi, and Karuveppilei in Tamil Nādu and Surabhinimba in Sanskrit.[6]

Moringa oleifera belonging to the family of Moringaceae is an effective remedy for malnutrition.[7] Moringa is rich in nutrition owing to the presence of a variety of essential phytochemicals present in its leaves, pods and seeds. In fact, moringa is said to provide 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yoghurt, 15 times more potassium than bananas and 25 times more iron than spinach[8]. The fact that moringa is easily cultivable makes it a sustainable remedy for malnutrition. Countries like Senegal and Benin treat children with moringa . Children deprived of breast milk tend to show symptoms of malnutrition[9]. Lactogogues are generally prescribed to lactating mothers to augment milk production. The Lactogogues, made of phytosterols, acts as a precursor for hormones required for reproductive growth. Moringa is rich in phytosterols like stigmasterol, sitosterol and kampesterol which are precursors for hormones. These compounds increase the oestrogen production, which in turn stimulates the proliferation of the mammary gland ducts to produce milk. It is used to treat malnutrition in children younger than 3 years. About spoonfuls of leaf powder can meet a woman's daily iron and calcium requirements, during pregnancy[9]. This study provides an overview on the cultivation, nutritional values, medicinal properties for commercial use and pharmacological properties of moringa. There are no elaborate reports on treatment of diabetes and cancer using moringa.

The Moringa plant has been consumed by humans throughout the century in diverse culinary ways. Almost all parts of the plant are used culturally for its nutritional value, medicinal properties and for taste and flavour as a vegetable and seed. The leaves of M. oleifera can be eaten fresh, cooked, or stored as a dried powder for many months without any major loss of its nutritional value[10]. Studies have indicated that M. oleifera leaves are a good source of nutrition and exhibit anti-tumour, anti-inflammatory, anti-ulcer, anti-atherosclerotic and anti-convulsant activities. The antimicrobial properties of plants have been investigated by a number of workers worldwide and many of them have been used as therapeutic alternatives. Plants have many secondary metabolites such as alkaloids, phenolic compounds, etc. In the present study attention has been focused on anti-bacterial activity of M. oleifera on S. aureus; P. aeruginosa

and E.coli, with the broader objective of providing cheap and safe remedy for human health problems.

Presently, huge numbers of people in developing countries depend on medicinal plants for healthcare, skin care, economic benefits, and cultural development. For centuries, medicinal plants have been widely used in traditional medicine in countries like India, China, Germany, Thailand, etc. The World Health Organization (WHO) projected that 80% of the population relies on traditional medicine, which is clearly elucidated by the 19.4 billion USD global revenue for herbal remedies in 2010. Moreover, the demand for traditional medicinal plants is increasing; for instance, the market for medicinal plants is expanding at an annual rate of 20% in India. Likewise, in China, 30% to 50% of the total medicinal consumption is from preparations of traditional medicine. Nearly 76.7% of the citizens of Thailand have reported mainly using traditional herbal medicine for their primary healthcare. Around 90% of the German population uses natural remedies for certain health issue. Therefore, the medicinal plants used in traditional medical treatments are significant in both developing and industrialized countries. This is clearly demonstrated by the worldwide market for traditional medicine. This market continues to gradually increase.

INTRODUCTION

Staphylococcus aureus is a major bacterial human pathogen that causes a wide variety of clinical manifestations. Infections are common both in community-acquired as well as hospital-acquired settings and treatment remains challenging to manage due to the emergence of multi-drug resistant strains such as MRSA (Methicillin-Resistant Staphylococcus aureus). S. aureus is found in the environment and is also found in normal human flora, located on the skin and mucous membranes (most often the nasal area) of most healthy individuals S. aureus does not normally cause infection on healthy skin; however, if it is allowed to enter the bloodstream or internal tissues, these bacteria may cause a variety of potentially serious infections. Transmission is typically from direct contact. However, some infections involve other transmission methods[11]

ETIOLOGY

Staphylococcus aureus is Gram-positive bacteria (stain purple by Gram stain) that are cocci-shaped and tend to be arranged in clusters that are described as “grape-like.” On media, these organisms can grow in up to 10% salt, and colonies are often golden or yellow (aureus means golden or yellow). These organisms can grow aerobically or anaerobically (facultative) and at temperatures between 18 C and 40 C. Typical biochemical identification tests include catalase positive (all pathogenic Staphylococcus species), coagulase positive (to distinguish Staphylococcus aureus from other Staphylococcus species), novobiocin sensitive (to distinguish from Staphylococcus saprophyticus), and mannitol fermentation positive (to distinguish from Staphylococcus epidermidis). MRSA strains carry a mec gene on the bacterial chromosome, which is a component of the larger Staphylococcal chromosomal cassette mec (SCCmec) region, conferring resistance to multiple antibiotics depending on the SCCmec

type.The mec gene encodes the protein PBP-2a (penicillin-binding protein 2a). PBP-2a is a penicillin-binding protein (PBP), or essential bacterial cell wall enzyme that catalyzes the production of the peptidoglycan in the bacterial cell wall. PBP-2A has a lower affinity to bind to beta-lactams (and other penicillin-derived antibiotics) when compared to other PBPs, so PBP-2A continues to catalyze the synthesis of the bacterial cell wall even in the presence of many antibiotics. As a result, S. aureus strains that synthesize PBP-2A can grow in the presence of many antibiotics, and these MRSA strains are resistant to many antibiotics. MRSA strains tend to be resistant to methicillin, nafcillin, oxacillin, and cephalosporins.[11]

EPIDEMOLOGY

Staphylococcus aureus (including drug-resistant strains such as MRSA) are found on the skin and mucous membranes, and humans are the major reservoir for these organisms.It is estimated that up to half of all adults are colonized, and approximately 15% of the population persistently carry S. aureus in the anterior nares. Some populations tend to have higher rates of S. aureus colonization (up to 80%), such as health care workers, persons who use needles on a regular basis (i.e., diabetics and intravenous (IV) drug users), hospitalized patients, and immunocompromised individuals. S. aureus can be transmitted person-to-person by direct contact or by fomites.

PATHOPHYSIOLOGY

S. aureus are one the most common bacterial infections in humans and are the causative agents of multiple human infections, including bacteremia, infective endocarditis, skin and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (e.g., pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections. Depending on the strains involved and the site of infection, these bacteria can cause invasive infections and/or toxin-mediated diseases .The pathophysiology varies greatly depending on the type of S. aureus infection. Mechanisms for evasion of the host immune response include the production of an antiphagocytic capsule, sequestering of host antibodies or antigen masking by Protein A, biofilm formation, intracellular survival, and blocking chemotaxis of leukocytes. Binding of the bacteria to extracellular matrix proteins and fibronectin in infectious endocarditis is mediated by bacterial cell wall-associated proteins such as fibrinogen-binding proteins, clumping factors, and teichoic acids. Also, Staphylococcal superantigens (TSST-1 or toxic shock syndrome toxin 1) are important virulence factors in infectious endocarditis, sepsis, as well as toxic shock syndrome. Pneumonia infections are associated with the bacterial production of PVL (Panton-Valentine leukocidin), Protein A, and alpha-hemolysin, and infections are more common following influenza virus infection as well as a diagnosis of Cystic Fibrosis. Prosthetic device infections are often mediated by the ability of S. aureus strains to form biofilms as well as communicate using quorum sensing in a bacterial cell den

PLANT PROFILE

plant profile

MORINGA OLIEFERA

Taxonomical classification:-

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Brassicales
Family	Moringaceae
Genus	Moringa
Species	M. olelifera[12]

fig.1 moringa oliefera

VERNACULAR NAMES

Telugu	Tella-Munaga, Mulaga
Hindi	Munaga, Mungaara, Sahijna, Sarinjna, Segra, Shajmah
Tamil	Murungai, Murunkak-kai, Morunga
Urdu	Sahajna[13]

DESCRIPTION

M. oleifera is a fast-growing, deciduous tree that can reach a height of 10–12 m (32–40 ft) and trunk diameter of 45 cm (1.5 ft).The bark has a whitish-grey color and is surrounded by thick cork. Young shoots have purplish or greenish-white, hairy bark. The tree has an open crown of drooping, fragile branches, and the leaves build up a feathery foliage of tripinnate leaves.

The flowers are fragrant and hermaphroditic, surrounded by five unequal, thinly veined, yellowish-white petals. The flowers are about 1.0–1.5 cm (1/2 in) long and 2.0 cm (3/4 in) broad. They grow on slender, hairy stalks in spreading or drooping flower clusters, which have a length of 10–25 cm.

Flowering begins within the first six months after planting. In seasonally cool regions, flowering only occurs once a year in late Spring and early Summer (northern hemisphere between April and June, southern hemisphere between October and December). In more constant seasonal temperatures and with constant rainfall, flowering can happen twice or even all year-round.

The fruit is a hanging, three-sided brown capsule of 20–45 cm size, which holds dark brown, globular seeds with a diameter around 1 cm. The seeds have three whitish papery wings and are dispersed by wind and water.

In cultivation, it is often cut back annually to 1–2 m (3–6 ft) and allowed to regrow so the pods and leaves remain within arm's reach[13].

BIOLOGICAL SOURCE

Moringa, native to parts of Africa and Asia, is the sole genus in the flowering plant family Moringaceae. The name is derived from murungai, the Tamil word for drumstick, and the plant is commonly referred to as the drumstick tree .It contains 13 species from tropical and subtropical climates that range in size from tiny herbs to massive trees. Moringa species grow quickly in many types of environments.

The most widely cultivated species is Moringa oleifera, native to the foothill of the Himalayas in northwestern India, a multipurpose tree cultivated throughout the tropics and marketed as a dietary supplement, health food or source for herbalism practices .The fruit pods of Moringa oleifera ("drumsticks") are increasingly consumed as food in many parts of the world, but particularly in South Asia. The leaves are commonly used to make tea. Oils are made from the seeds, while powders can be made from the leaves and roots[14].

CHEMICAL CONSTITUENTS

Moringa species contain various phytoconstituents such as alkaloids, saponins, tannins ,steroids ,phenolic acids ,glucosinolates, flavonoids & terpenoids The leaves are the most nutritious part of the plant, being a significant source of B vitamins, vitamin C, provitamin A as beta-carotene, vitamin K, manganese, and protein. When compared with common foods particularly high in certain nutrients per 100 g fresh weight, cooked moringa leaves are considerable sources of these same nutrients. Some of the calcium in moringa leaves is bound

as crystals of calcium oxalate. Oxalate levels may vary from 430 mg/100g to 1050 mg/100g, compared to the oxalate in spinach.[15]

Culinary uses

The leaves can be used in many ways, perhaps most commonly added to clear broth-based soups, such as the Filipino dishes . Tender moringa leaves, finely chopped, are used as garnish for vegetable dishes and salads, such as the Kerala dish thoran. It is also used in place of or along with coriander.

For long-term use and storage, moringa leaves may be dried and powdered to preserve their nutrients. Sun, shade, freeze and oven drying at 50–60 °C are all acceptable methods, albeit variable in their retention efficacy of specific micro- and macronutrients. The powder is commonly added to soups, sauces and smoothies. Owing to its high nutritional density, moringa leaf powder is valued as a dietary supplement and may be used to enrich food products ranging from dairy, such as yogurt and cheese, to baked goods, such as bread and pastries, with acceptable sensory evaluation.[16]

Other uses

Moringa oleifera leaf powder was as effective as soap for hand washing when wetted in advance to enable anti-septic and detergent properties from phytochemicals in the leaves. Moringa oleifera seeds and press cake have been implemented as wastewater conditioners for dewatering and drying fecal sludge[17].

murraya koenigii

The curry tree, Murraya koenigii or Bergera koenigii, is a tropical to sub-tropical tree in the family Rutaceae (the rue family, which includes rue, citrus, and satinwood), and is native to Asia. The plant is also sometimes called sweet neem, though M. koenigii is in a different family to neem, Azaridecta indica, which is in the related family Meliaceae[18].

Fig .2 murraya koenigii

TAXANOMY

Kingdom Plantae

Order Sapindales

Family Rutaceae

Genus Murraya

Species M. koenigii

VERNACULAR NAMES

Telugu : karivepaku,kalemaaku

English: curry leaves

Hindi: karee patte

Tamil: karivepillai

DESCRIPTION

It is a small tree, growing 4–6 metres (13–20 ft)) tall, with a trunk up to 40 cm (16 in) diameter. The aromatic leaves are pinnate, with 11–21 leaflets, each leaflet 2–4 cm (3⁄4–1+1⁄2 in) long and 1–2 cm (1⁄2–3⁄4 in) broad. The plant produces small white flowers which can self-pollinate to produce small shiny-black drupes containing a single, large viable seed. The berry pulp is edible, with a sweet flavor.[19]

Common names

The generic name, *Murraya*, derives from Johan Andreas Murray (1740-1791),
The specific name, *koenigii*, derives from the last name of botanist Johann Gerhard König.
Curry tree is also called *curry leaf tree* or *curry bush*, among numerous local names, depending on country.[20]

Chemical constituents

Compounds found in curry tree leaves, stems, bark, and seeds include cinnamaldehyde, and numerous carbazole alkaloids, including mahanimbine, girinimbine, and mahanine.
Nutritionally, the leaves are a rich source of carotenoids, beta-carotene, calcium and iron.[21]

Uses

The fresh leaves are an indispensable part of Indian cuisine and Indian traditional medicines. They are most widely used in southern and west coast Indian cooking, usually fried along with vegetable oil, mustard seeds and chopped onions in the first stage of the preparation. They are also used to make thoran, vada, rasam, and kadhi. The fresh leaves are valued as seasoning in the cuisines of South and Southeast Asia[22]. In Cambodia, where the leaves are called sloek kontroap, the leaves are roasted and used as an ingredient in a soup, maju krueng. In Java, the

leaves are often stewed to flavour gulai. Though available dried, the aroma and flavour is greatly inferior. The oil can be extracted and used to make scented soaps.

The leaves of Murraya koenigii are also used as a herb in Ayurvedic and Siddha medicine in which they are believed to possess anti-disease properties, but there is no high-quality clinical evidence for such effects[23].

MATERIALS AND METHODS

Materials and methods:

Collection and identification of plant material:

The leaves of *Moringa oleifera* and *murraya koenigii* trees were collected from jangala kandriga Area of Nellore, Andhra Pradesh State, India. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to clean the leaves thoroughly; and dried. The plant was identified in the cognosy Unit, Department of pharmacognosy. Parts of the plant collected for identification were: leaves.

Drying and storage of plant material

The leaves of the plants were air dried under shed, and then grounded into powder with the aid of pestle and mortar.The powders obtained from the leaves of *the Moringa* and *Murraya* Plants were then sieved and stored in polythene bags prior to the analysis.

Preparation and extraction of the leaf extracts

Extraction

Extraction is the first step to separate the desired natural products from the raw materials. Extraction methods include solvent extraction, distillation method, pressing and sublimation according to the extraction principle. Solvent extraction is the most widely used method. The extraction of natural products progresses through the following stages:

(1) the solvent penetrates into the solid matrix;

(2) the solute dissolves in the solvents;

(3) the solute is diffused out of the solid matrix;

(4) the extracted solutes are collected.

Any factor enhancing the diffusivity and solubility in the above steps will facilitate the extraction. The properties of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ration, the extraction temperature and the extraction duration will affect the extraction efficiency [24].

Generally, the finer the particle size is, the better result the extraction achieves. The extraction efficiency will be enhanced by the small particle size due to the enhanced penetration of

solvents and diffusion of solutes. Too fine particle size, however, will cost the excessive absorption of solute in solid and difficulty in subsequent filtration. Preparation of medicinal plants for experimental purposes is an initial step and key in achieving quality research outcome. It involves extraction and determination of quality and quantity of bioactive constituents before proceeding with the intended biological testing. The primary objective of this study was to evaluate various methods used in the preparation and screening of medicinal plants in our daily research. Preparation of medicinal plants for experimental purposes is an initial step and key in achieving quality research outcome. It involves extraction and determination of quality and quantity of bioactive constituents before proceeding with the intended biological testing. The primary objective of this study was to evaluate various methods used in the preparation and screening of medicinal plants in our daily research. Although the extracts, bioactive fractions, or compounds obtained from medicinal plants are used for different purposes, the techniques involved in producing them are generally the same irrespective of the intended biological testing. The major stages included in acquiring quality bioactive molecule are the selection of an appropriate solvent, extraction methods, phytochemical screening procedures, fractionation methods, and identification techniques. The nitty-gritty of these methods and the exact road map followed solely depends on the research design. Solvents commonly used in extraction of medicinal plants are polar solvent (e.g., water, alcohols), intermediate polar (e.g., acetone, dichloromethane), and nonpolar (e.g., n-hexane, ether, chloroform). In general, extraction procedures include maceration, digestion, decoction, infusion, percolation, Soxhlet extraction, superficial extraction, ultrasound-assisted, and microwave-assisted extractions. Fractionation and purification of phytochemical substances choice of an appropriate extraction method depends on the nature of the plant material, solvent used, pH of the solvent, temperature, and solvent to sample ration. It also depends on the intended use of the final products[25]This study aimed to assess various solvents of extractions, methods of extraction, fractionation, purification, phytochemical screening, and identification of bioactive compounds in medicinal plants

Solvent extraction

According to the solubility of the bioactive compounds there are a large number of inorganic, organic, polar and non-polar solvents to perform a good extraction, also in combination among them. If the substance of our interest is lipophilic, the organic solvents of choice will be non-polar, ranging from those with a very low polarity such as hexane, to those that are less non-polar like chloroform and dichloromethane. For example, the apolar solvents cyclohexane, hexane, toluene, benzene, ether, chloroform and ethyl acetate are currently used to extract alkaloids, coumarins, fatty acids (FAs), flavonoids and terpenoids. On the contrary, for hydrophilic compounds the choice will fall on a polar solvent which may be non-protic such as acetone, or protic such as ethanol, methanol or even water. In fact acetone, acetonitrile, butanol, propanol and ethanol are the solvents for the extraction of flavonols, lectins, alkaloids, quassinoids, flavones, polyphenols, tannins and saponins. One of the major pros of this procedure is the use of simple equipment and its limited cost[26].

Definitions of terms

Medicinal plant It refers to a plant comprising active ingredients or secondary metabolites that possess biological activity. A whole plant may be medicinally active or plant parts[27]

Menstruum It is a liquid or a suitable solvent chosen for an effective extraction process[27].

Marc. It is an insoluble or inert drug material that is left behind at the end of the extraction process[28].

Micelle It is the mixture of both the extracted drug material and the solvent of extraction.

Primary plant constituents These are mainly nutritional components of plants such as common sugars, amino acid, proteins, and chlorophyll. These have little or no medicinal properties[28].

Secondary plant constituents These are also known as secondary metabolites such as alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, and tannins. These are responsible for many biological or pharmacological activities.[28]

PROPERTIES OF SOLVENT OF EXTRACTIONS

Water. It is the most polar solvent and is used in the extraction of a wide range of polar compounds.

Advantages. It dissolves a wide range of substances; it is cheap, nontoxic, nonflammable, and highly polar.

Disadvantages. It promotes bacterial and mold growth; it may cause hydrolysis, and a large amount of heat is required to concentrate the extract.[29]

Alcohol. It is also polar in nature, miscible with water, and could extract polar secondary metabolites.

Advantages. It is self-preservative at a concentration above 20%. It is nontoxic at low concentration, and as small amount of heat is required for concentrating the extract. Disadvantages. It does not dissolve fats, gums, and wax; it is flammable and volatile.

Factors to be considered in selecting solvents of extraction

Various factors enumerated below should be taken into consideration when choosing a solvent of extraction.[30]

Selectivity. The ability of a chosen solvent to extract the active constituent and leave the inert material.

Safety. Ideal solvent of extraction should be nontoxic and nonflammable.

Cost. It should be as cheap as possible.

Reactivity. Suitable solvent of extraction should not react with the extract.

Recovery. The solvent of extraction should be quickly recovered and separated from the extract.

Viscosity. Should be of low viscosity to allow ease of penetration.

Boiling temperature. Solvent boiling temperature should be as low as possible to prevent degradation by heat[31]

METHODS USED IN EXTRACTION OF MEDICINALPLANTS

Factors to be considered in choosing extraction method

Stability to heat. Heat-stable plant material is extracted using Soxhlet extraction or microwave-assisted extraction, whereas plant materials that are not heat stable are extracted using maceration or percolation.[31]

Nature of solvent. If the solvent of extraction is water, maceration is a suitable method but for volatile solvent percolation and Soxhlet extraction are more appropriate.

Cost of the drug. Cheap drugs are extracted using maceration, whereas costly drugs are preferably extracted using percolation.

Duration of extraction. Maceration is suitable for plant material requiring long exposure to the menstruum, whereas techniques such as microwave- or ultrasound-assisted extraction are used for a shorter duration.[32]

Final volume required. Large volume products such as tinctures are prepared by maceration, whereas concentrated products are produced by percolation or Soxhlet extraction.

Intended use. Extracts intended for consumption by human are usually prepared by maceration, whereas products intended for experimental testing are prepared using other methods in addition to maceration.[32]

Commonly used methods in the extraction of medicinal plants

Maceration. This is an extraction procedure in which coarsely powdered drug material, either leaves or stem bark or root bark, is placed inside a container; the menstruum is poured on top until completely covered the drug material. The container is then closed and kept for at least three days.[33] The content is stirred periodically, and if placed inside bottle it should be shaken time to time to ensure complete extraction. At the end of extraction, the micelle is separated from marc by filtration or decantation. Subsequently At the end of extraction, the micelle is separated from marc by filtration or decantation. Subsequently, the micelle is then separated from the menstruum by evaporation in an oven or on top of water bath. This method is convenient and very suitable for thermolabile plant material.

Infusion. This is an extraction process such as maceration. The drug material is grinded into fine powder, and then placed inside a clean container. The extraction solvent hot or cold is then poured on top of the drug material, soaked, and kept for a short period of time. This method is suitable for extraction bioactive constituents that are readily soluble. In addition, it is an appropriate method for preparation of fresh extract before use. The solvent to sample ratio is usually 4:1 or 16:1 depending on the intended use[33]

Decoction. This is a process that involves continuous hot extraction using specified volume of water as a solvent. A dried, grinded, and powdered plant material is placed into a clean container. Water is then poured and stirred. Heat is then applied throughout the process to hasten the extraction. The process is lasted for a short duration usually about 15min. The ratio of solvent to crude drug is usually 4:1 or 16:1. It is used for extraction of water soluble and heat stable plant material.

Percolation. The apparatus used in this process is called percolator. It is a narrow-cone-shaped glass vessel with opening at both ends. A dried, grinded, and finely powdered plant material is moistened with the solvent of extraction in a clean container. More quantity of solvent is added, and the mixture is kept for a period of 4h. Subsequently, the content is then transferred into percolator with the lower end closed and allow to stand for a period of 24h.[34] The solvent of extraction is then poured from the top until the drug material is completely saturated. The lower part of the percolator is then opened, and the liquid allowed to drip slowly. Some quantity of solvent was added continuously, and the extraction taken place by gravitational force, pushing the solvent through the drug material downward.The addition of solvent stopped when the volume of solvent added reached 75% of the intended quantity of the entire preparations. The extract is separated by filtration followed by decantation. The marc is then expressed and final amount of solvent added to get required volume.

Soxhlet extraction :

This process is otherwise known as continuous hot extraction. The apparatus is called Soxhlet extractor made up of glass. It consists of a round bottom flask, extraction chamber, siphon tube, and condenser at the top. A dried, grinded, and finely powdered plant material is placed inside porous bag (thimble) made up of a clean cloth or strong filter paper and tightly closed[34]. The extraction solvent is poured into the bottom flask, followed by the thimble into the extraction chamber. The solvent is then heated from the bottom flask, evaporates, and passes through the condenser where it condenses and flow down to the extraction chamber and extracts the drug by coming in contact. Consequently, when the level of solvent in the extraction chamber reaches the top of the siphon, the solvent and the extracted plant material flow back to the flask.[35] The entire process continues repeatedly until the drug is completely extracted, a point when a solvent flowing from extraction chamber does not leave any residue behind. This method is suitable for plant material that is partially soluble in the chosen solvent and for plant materials with insoluble impurities. However, it is not a suitable method for thermolabile plant materials[36].

Advantages. Large amount of drug can be extracted with smaller amount of solvent. It is also applicable to plant materials that are heat stable. No filtration is required, and high amount of heat could be applied.Disadvantages. Regular shaking is not possible, and the method is not suitable for thermolabile materials.[37]

Extraction of hydroalcoholic leaf extract :

Fifty grams (50g) of the powdered leaves were weighed and poured into 500 ml conical flask in which 200 ml of distilled water was added. The mixture was kept for 3days with constant stirring at 2 hours intervals. The extract was filtered using Whatman No.1 filter paper. Extracts (filtrate) were concentrated at 40oC under reduced pressure using evaporator, and then kept in a glass flask. The semi solid extract (residue) obtained was stored in a refrigerator for further use.[38]

we extracted the plant by using maceration technique as it is most effectively used and the process is easy to perform also thermolabile contituents of drug can be extracted with ease

5.4.preparation of culture media:

Nutrient broth preparation

Nutrient Agar is a general purpose, nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for the bacterial growth.

Composition of Nutrient Agar

- *0.*5% Peptone

It is an enzymatic digest of animal protein. Peptone is the principal source of organic nitrogen for the growing bacteria.

- 0.3% beef extract/yeast extract

It is the water-soluble substances which aid in bacterial growth, such as vitamins, carbohydrates, organic nitrogen compounds and salts.

- 1.5% agar

It is the solidifying agent.

0.5% NaCl

The presence of sodium chloride in nutrient agar maintains a salt concentration in the medium that is similar to the cytoplasm of the microorganisms.

- Distilled water

Water is essential for the growth of and reproduction of micro-organisms and also provides the medium through which various nutrients can be transported.

- pH is adjusted to neutral (7.4) at 25 °C.[39]

Preparation of Nutrient Agar

1. Suspend 28 g of nutrient agar powder in 1 litre of distilled water.

2. Heat this mixture while stirring to fully dissolve all components.

3. Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes.

4. Once the nutrient agar has been autoclaved, allow it to cool but not solidify.

5. Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified.

6. Replace the lid of each Petri dish and store the plates in a refrigerator.

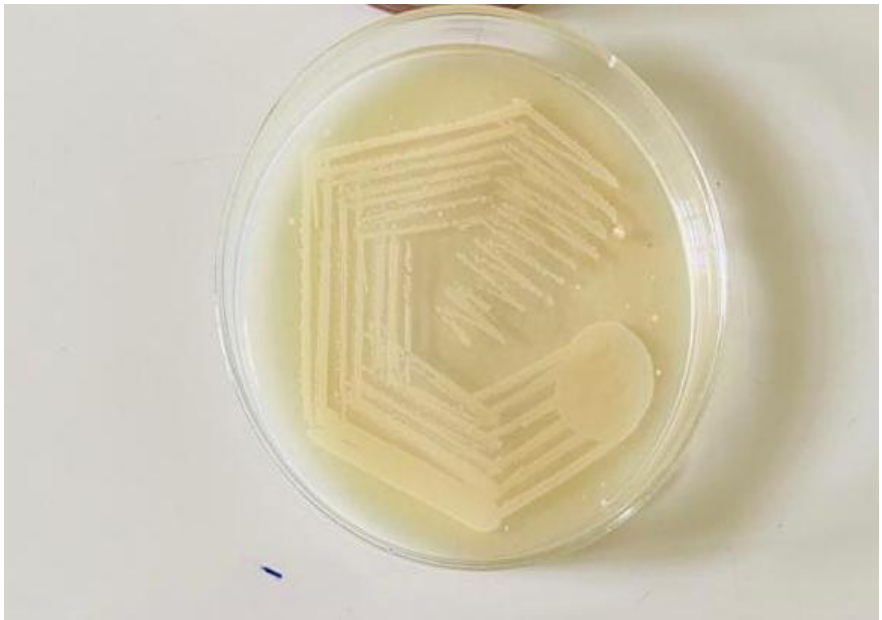

Fig.3 culture media

Uses of Nutrients Agar

1. It is frequently used for isolation and purification of cultures.

2. It can also be used as a means for producing the bacterial lawns needed for antibiotic sensitivity tests. In actuality, antibiotic sensitivity testing is typically performed on media specially formulated for that purpose.[39]

Serial dilution and inoculum preparation for isolating soil :

Serial dilution technique:

The following is the procedure for a ten-fold dilution of a sample to a dilution factor of 10-6[40]:

1.The sample/culture is taken in a test tube and six test tubes, each with 9 ml of sterile diluents, which can either be distilled water or 0.9% saline, are taken.

2.A sterile pipette is taken.

3.1 ml of properly mixed sample/culture is drawn into the pipette.

4.The sample is then added to the first tube to make the total volume of 10 ml. This provides an initial dilution of 10-1.

5.The dilution is thoroughly mixed by emptying and filling the pipette several times.

6. pipette tip is discarded, and a new pipette tip is attached to the pipette.

7.Now, 1 ml of mixture is taken from the 10-1 dilution and is emptied into the second tube. The second tube now has a total dilution factor of 10-2[41].

8.The same process is then repeated for the remaining tube, taking 1 ml from the previous tube and adding it to the next 9 ml diluents.

9.As six tubes are used, the final dilution for the bacteria/cells will be 10-6 (1 in 1,000,000).[41,4

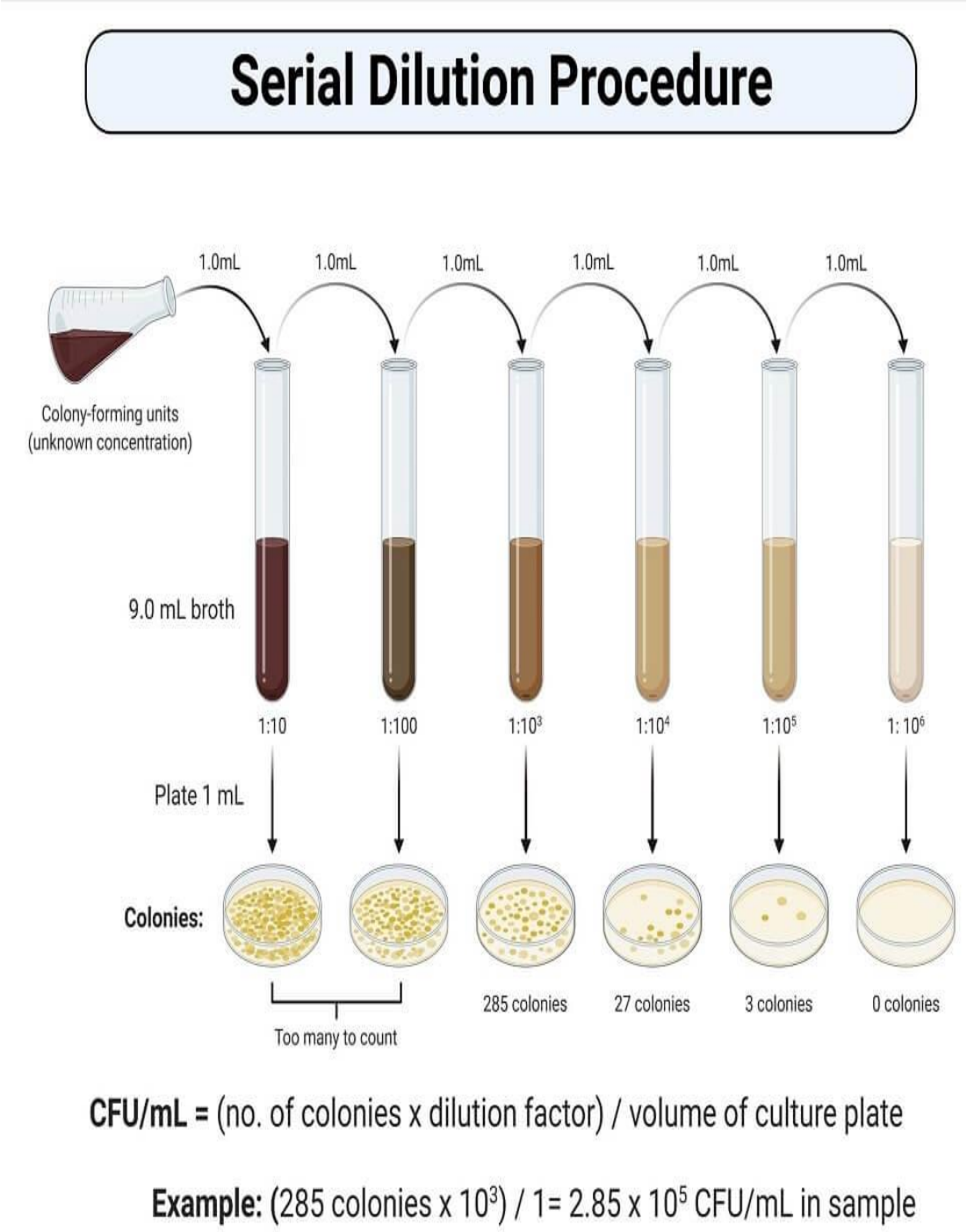

FIG.4 serial dilution

Inoculation

Inoculation meaning in microbiology is that transfer from culture for their growth. It is the direct transfer from the culture of microorganisms to inoculation needle.

General principles:

Solid media

In general, media should be inoculated in a logical order (see below) from least

selective to most selective to avoid the inhibition of organisms by carry over of the

selective agent:

1. Media without inhibitors (for example, blood agar)

2. Indicator media (for example, CLED agar)

3. Selective media (for example, XLD agar)

4. Smears for staining

There may be occasions where it may not be advisable to inoculate media in this way.

For example, swabs for gonococcal (GC) culture may contain only small numbers of organisms. This will make the inoculation of the GC selective agar the priority. Where specimens are insufficient for a full range of culture plates, priorities should be based on origin of specimen and the range of likely organisms to be encountered[45].

For the isolation of individual colonies, the inoculum should be spread, usually by using a sterile loop (or if available, disposable loop), taking care to avoid the edges of the plate where contaminants are more likely to be located.

Liquid media

Liquid media should be inoculated first when processing fluid specimens ensuring that media are inoculated in a sequence that minimises the risk of cross contamination.However, liquid media should be inoculated after the solid media when swabs and faeces are examined, to avoid diluting the organisms contained on or in the sample and to avoid any organisms (whether viable or non-viable) present in a liquid medium being transferred to other liquid media, solid media or to slides[46].

Smears

Smears for staining are usually made after all culture media have been inoculated to avoid carry-over of contaminants that may be on the surface of the slide. However, there may be occasions where the smear is required prior to culture, for example in the case where specimens for investigation for acid fast bacilli are received. Great care should be taken to avoid contamination for example by not placing the loop back into the specimen after touching the slide[47].

Labelling of culture media

As a minimum requirement, all culture plates and containers must be labelled to identify the patient name or laboratory number or barcode. Additional labelling, including date of culture or sub-culture will be necessary for selected specimens, such as those requiring prolonged incubation or sub-culture from enrichment broth to minimise transcription errors.

To work safely and minimise risks of cross contamination, suitable racks should also be used when inoculating, incubating or storing liquid cultures or culture plates[48].

Inoculation of culture media

All culture media must be checked visually before use for contamination, significant physical imperfections (for example, uneven distribution of media, variable amounts of medium in petri dishes/tubes/bottles, colour, gross deformation of the surface on the media) and expiry date. Culture media should have an identifiable batch or quality control number and have passed QC tests before use. Plates that are beyond their expiry date, contaminated plates, and broth media appearing unusually turbid should be discarded.

For the effective detection of the bacterial content of specimens, it is important to achieve growth of individual colonies by using a good technique to inoculate the specimen on culture media. There are many variations and personal preferences for “plating out”, some of which are described in this document[49].

The initial area inoculated should cover between a quarter and a third of the total area of agar used . Whole plates, half plates, or quarter plates can be used depending on the circumstances. Specimens may be plated out for individual colonies, or seeded directly over an entire segment of a plate and incubated without further spreading.

Antimicrobial discs for identification (for example, optochin, bacitracin) may be added as appropriate. Discs should be placed near the edge of the plate, between the areas covered by the first and second spread, to avoid total inhibition of very susceptible organisms[50].

Inoculation loops are designed for quantitative procedures such as sampling, serial dilutions, as well as for bacterial inoculation. Inoculation loops can be ‘wire or disposable loops’. Disposable loops were initially used in safety cabinets to avoid sterilisation with Bunsen burners but now their use is common practice to comply with the health and safety regulations. Disposable loops are also desirable for quantitative purposes. Wire loops are rarely used in clinical microbiology laboratories in the UK to reduce the risk of infection from aerosols of pathogenic organisms and, cross contamination from improper sterilisation of the wire loops. Therefore, disposable loops are recommended in this document. For polymicrobial clinical specimens, the disposable loop should either be changed between each series of streaks, or the loop may be rotated to make the next series of streaks with the unused side of the loop. For semi-quantitative analysis of urine, the loop should be changed between streaks[51].

All media should be incubated as soon as possible after inoculation. In particular, plates for anaerobic incubation should be incubated as soon as possible to prevent loss of viability (<15

minutes)5,6. After inoculation, the specimen, or a portion of it, should be retained for at least 48 hours after the laboratory has issued the final report.

Most positive culture plates can be discarded within 24-48 hours of issuing a final authorised report. Cultures of particular epidemiological value may be retained for longer as organisms may need further work or referral to a reference laboratory.

Highly automated machines have been introduced in many clinical microbiological laboratories worldwide to contribute to more accurate, rapid, and cost-effective management of patient samples8-10[52].

Aseptic technique

When handling specimens or cultures, the use of an aseptic technique is crucial to avoid contamination and to protect the worker from infection.In-house training to develop these skills should be given to staff processing thespecimens or cultures.

The following points should be observed when culturing specimens or performing subcultures:

• caps and lids from containers should not be placed on the workbench, but retained in the hand while the sample is being processed, taking care not to contaminate the hand or cap. Caps and lids should be replaced as soon as possible

• lids from agar media should be placed on the bench to face upwards and after the plates are inoculated, the lids should be replaced immediately to avoid any contamination

• if the work is being carried out on the open bench, a disposable jar should be in close proximity to the operator in order to discard the loops

• keeping samples away from the face when opening culture containers. This can be achieved by wearing the appropriate PPE when handling cultures

• aerosol production should be minimised by:

a. opening caps of clinical specimens slowly in a microbiological safety cabinet as the contents of containers are sometimes under pressure

b. avoiding vigorous swirling or shaking of the sample prior to openingby mixing the sample gently

c.avoiding expelling the last drop from a pipette

d. removing excessfluid from a swab put in a suspension (to be inoculated on an agar plate) by turning the swab against the inside of the container when forceps or scissors are used for handling specimens, they should be autoclaved and sterilised before use. Use disposable forceps or scissors if available, and dispose after use[53].

Various Inoculation Methods Used in Bacteriology

In Bacteriology, there are several techniques used for inoculating. Some of the most commonly used techniques are as discussed:

AGAR PLATES

Agar plates are some of the most common media which are in use for growing bacteria and other microorganisms. A mixture of agar and nutrients necessary for bacterial growth. This is then poured into circular Petri dishes where the agar solution solidifies. After this, inoculation of a solution-containing microorganism onto these plates with the help of streaking.

A small streaking loop is a dip into a solution, which contains bacterial cells that are used to streak onto the plates with the bacteria. These plates are stored at the proper temperature for bacterial growth for further study. We can also inoculate liquid media suspensions of bacteria to grow and reproduce.

A single culture of microorganism added to a small solution to form a mixture and pipette into liquid media. For Bacterial growth, we need media in which the mixture of microorganism and solution contain nutrients, compounds, and other necessary molecules.

Inoculation in microbiology is the process of introducing microbes into a culture media so that it reproduces there. Commonly, it is used in the introduction of vaccines, serum or any antigenic substance in the body so as to boost immunity against a particular disease.

This process of Inoculation is carried out in different media such as – plate culture, slant culture, agar plates, stab culture, sweep plate method and so on[53].

Streak plating method

This method is used to obtain completely isolated colonies from a culture or specimen inoculum through the creation of sections of increasing dilution on a single plate.

Inoculate clinical specimens through the use of sterile inoculation loops into the agar media. Spread the specimen gently on a section of the culture media surface Extract loop from the inoculated area and distribute into a second part

Extract the loop from the other section and disperse it to the 3rd section. Continue for the 3rd and 4th section. Make sure that sections 1 and 4 are not overlapping. Unload inoculation loop used into suitable containers Substitute the lid followed by incubating the streaked agar plate at the optimum temperature (inverted stance), so as to curb condensation

Agar stab technique

It is used in the preparation of stab cultures, from a plate select single colonies.

Select a well-isolated colony through aseptic technique using an inoculating stab needle (sterile) and stab it a few times via the agar to the base of the tube

SL.NO	DRUG USED	ZONE OF INHIBITION
1	Ofloxacin	**2.7cm**
2	Moringa leave extract	**1.7cm**
3	Murraya leave extract	**2.5cm**

Substitute the cap and secure loosely during incubation enabling exchange of gases

Incubation of this stabbed plate at the suitable temperature is carried out

Spread Plate Method

It is used for evenly spreading cells to ensure growth of the isolated separate colonies. Further, it can be used for serial dilutions. The spread plate method is used for enrichment, enumeration and screening and selection of microorganisms. Onto the agar media, with the help of a sterile spreader, inoculate the clinical specimen where we spread the bacteria gently on the whole culture media surface. This is done by rotating the plate while spreading it backwards and forward. Refrain from allowing the spreader to touch the edges of the plateSubstitute the lid and ensure the plate is standing in an upright position for drying (10-12 minutes)Now incubate the spread agar plate at the optimum temperature with the lid at the base (inverted)The biggest advantage of a spread plate method is that the morphology of the isolated bacteria can be seen vividly. The only disadvantage is that sometimes fungal colonies may grow. This was a brief on culture media used; some othermedia such as anaerobic culture techniques, liquid culture techniques and pour plates also exist[54]

Results &discussion

Murraya leaves showed greater zone of inhibition than moringa leaves, it shows that murraya leaves are effective in showing antibacterial activity when compared to moringa leaves .

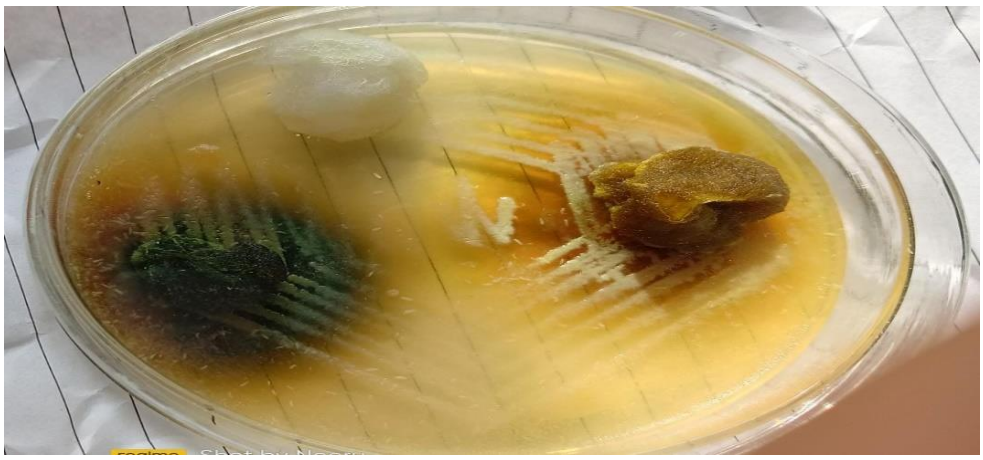

Fig.5 Drugs showing zone of inhibition

DISCUSSION:

The present study was conducted to obtain preliminary information and compare the antibacterial activity of the extracts (hydroalcoholic) of Moringa oleifera Lam. Leaves and murraya koenigii leaves . Agar Streak plate method was applied to be used in this study. The powder from fresh leaf murraya has greater antibacterial activity than moringa. The traditional method of treating a bacterial infection, maceration of the plant parts or boiling the plant in water is employed whereas, according to present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity, in accordance with the results In this investigation, highest zones of inhibition were found in powder from leaf powder of murraya than moringa against all the bacteria tested which was more effective than known antibiotic ofloxacin.

CONCLUSION:

Leaf extracts of Moringa oleifera and murraya koenigii showed varying antibacterial activity on the tested bacteria. This murraya extract is showing similar effectiveness as traditional antibiotics to combat the human pathogenic bacteria studied responsible for severe illness. The plant could be a source of new antibiotic compounds. Further work is needed to isolate the secondary metabolites and study of metabolic interchanges in bacterial metabolic pathways when applying this extract. This in vitro study demonstrated that traditional medicine can be as effective as modern medicine to combat human pathogenic bacteria. The use of these plants in traditional medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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