ANTIBACTERIAL ACTIVITIES OF MEDICINAL PLANT EXTRACTS AGAINST VARIOUS MICROORGANISMS AND MULTI DRUG RESISTANT PHENOTYPES.

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ABSTRACT - Interest in medicinal plants showing synergism with antibiotics or as an alternative to antibiotics as possible industrial product is receiving major importance. Medicinal plants are finding their way into pharmaceuticals, cosmetics, and neutraceuticals. Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies. Today’s microbial infections, resistance to antibiotic drugs have been the biggest challenges, which threaten the health of societies. It has become widely recognized that antimicrobial resistance(AMR) is one of the biggest health threats that mankind faces encompassing huge health and economic burdens on governments and societies in every region of the globe. Plant based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials is the need of the hour. Plant-derived antimicrobials have a long history of providing the much needed novel therapeutics. So the purpose of this review is to evaluate the antimicrobial property of various indigenous medicinal plants and traditional herbal medicines for their potential application in treatment and/or prevention of infectious diseases in humans, as well as providing as an alternative to antibiotics against disease.

KEYWORDS – Medicinal plants, Antimicrobial resistance, Plant – derived antimicrobials, Novel therapeutics.
INTRODUCTION - Infectious diseases of bacterial origin are a recurrent problem in public health and have a substantial impact on morbidity and mortality in populations in general (Chandak., 2018). Although, for the last 60 years, antibiotics played a major role in the treatment of infectious diseases caused by bacteria and fungi, the occurrence of dangerous and antibiotic-resistant bacteria have been observed to increase in frequency over the past several decades. Drug resistance can be executed by multiple mechanisms; hence overcoming such problem is not an easy task. Reasons for the emerging antibiotic resistance include the irresponsible, unfit or too common use of antibiotics in fields, such as medicine, veterinary, and especially in agriculture (Saleem M, 2010). Antimicrobial resistance is a global health challenge with the increasing drug-resistant organisms even in community (Vidushi Chaturvedi, 2019). Therefore, in recent years, the pharmaceutical industry has been prompted to develop new antibiotic drugs, in particular because of the emergence of microorganisms resistant to conventional drugs (Chandak., 2018). The overall cost burden on the health system with increased stay in the hospitals warrants urgent attention to the problem. With no new drugs in the pipeline, it is high time to search for some alternatives to the antimicrobials which can replace or boost the activity of existing antimicrobials (Chandak., 2018). Therefore there is dire need to search for new classes of antibacterial substances to which there is lesser resistance (Chandak., 2018).

Plants have formed the basis of sophisticated traditional medicine and their natural products led for new drug development. Approximately, 80% of the world inhabitants rely on traditional medicine for their primary health care and medicinal plants play important roles on the remaining 20% population (M.E.A Dawoud, 2013). Traditional medicine is in practice for many centuries by a substantial proportion of the population of many centuries. It is recognized that in some developing countries, plants are the main medicinal source to treat various infectious diseases. Plant extracts represent a continuous effort to find new compound against pathogens. Approximately 20% of the plants are found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semisynthetic resources (Kedarnath, 2013).

MEDICINAL PLANTS AS ANTIMICROBIALS: Today’s, microbial infections, resistance to antibiotic drugs, have been the biggest challenges, which threaten the health of societies. Microbial infections are responsible for millions of deaths every year worldwide. In 2013, 9.2 million deaths have been reported because of infections i.e. about 17% of total deaths. The occurrence of the evolution of resistance has caused the existing antibacterial drugs to become less effective or even ineffective. In recent years, various strategies have been suggested to overcome the resistance of antibiotics. One of the recommended strategies to achieve this goal has involved the combination of other molecules with the failing antibiotics, which apparently restores the desirable antibacterial activity. These molecules can be non-antibiotic drugs with potential antibacterial properties that can create opportunities for innovative therapeutic approaches. In regards to this case, phytochemicals have exhibited potent activities while many researchers have used natural products to act
against bacterial resistance. These agents can act alone or in combination with antibiotics to enhance the antibacterial activity against a wide range of bacteria (Bahman Khameneh, 2019).

Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics. In 2007 WHO estimated that 25% of available drugs are derived from plants used in folk medicine (Cushnie TP, 2011). Besides the long-established clinical use, the plant-derived compounds display good tolerance and acceptance among patients and seem like a credible source of antimicrobial compounds. Among 109 new antibacterial drugs, approved in the period 1981–2006, 69% originated from natural products (liczewski, 2019).

**ANTIMICROBIAL PROPERTIES OF MEDICINAL PLANTS** - Although there are many medicinal plants proven to have antimicrobial effect on variety of microorganisms, the current review focuses on Four main medicinal plants and their antimicrobial activity viz; **Lemon (Citrus limon)**, **Aloe Vera (Aloe barbadensis miller)**, **Green tea leaves (Camellia sinensis)**, **Curry leaves (Murraya koenigii)**.

**Lemon (Citrus limon):**- Citrus are the most important crops in the world in terms of production according to the Food and Agricultural Organisation (FAO), with 240,780 million metric tons produced in 2013. Citrus plants are grown in many countries all over the world and among the major African citrus-producing countries is Tunisia. Thus, Citrus would be considered as one of the most economically important crops in Tunisia. The genus Citrus belongs to the Rutaceae family that comprises of about 140 genera and 1300 species and, for instance, Citrus limon (Lemon) is among important species of genus Citrus. Lemons are one of the most popular acid citrus fruits. Essential oils were composed of many valuable natural products that may be described as mixtures of hydrocarbons, oxygenated compounds and nonvolatile residues. They include terpenes, sesquiterpenes, aldehydes, alcohols, esters and sterols. Citrus plants constitute one of the main sources of essential oil, which are extensively studied for their potential uses in the food industry (Anis Ben Hsouna, 2017).

The genus citrus is one of the most effective herbs in traditional medicine that belongs to the family of Rutaceae. The members of this genus are characterized by many biologically active secondary metabolites such as flavonoids, limonoids, coumarins and furocoumarins, sterols, volatile oils, organic acids, and alkaloids. Many citrus species are recognized for their medicinal, physiological, and pharmacological activities including antimicrobial, antioxidant, anticancer, anti-inflammatory, and hypoglycaemic activities (Chandak, 2018).

Citrus by-products, if utilized fully, could be major sources of phenolic compounds. The peels, in particular, are an abundant source of natural flavonoids, and contain higher amount of phenolics compared to the edible portions. Lemon is very rich in important natural compounds, including citric acid, ascorbic acid, minerals, flavonoids, and essential oils. Citric acid is a weak tricarboxylic acid that is naturally concentrated in citrus fruits. It increases the acidity of the bacterial environment making it difficult for it and microbes to
survive and most importantly to reproduce. Many plants have been evaluated not only for direct antimicrobial activity, but also as a resistance modifying agent. The resistance modifying agents are compounds which potentiate the activity of an antibiotic against resistant strain (Chandak., 2018).

A study on antibacterial activity of lemon was done and an was made to check antibacterial and antibiotic potentiation activity of lemon against drug resistant phenotypes. Simultaneously antibacterial and antibiotic potentiation activity of citric acid was also carried out. Antimicrobial activity of lemon crude juice as well as lemon peel extract was estimated against *S. aureus* and *P. aeruginosa*. The sensitivity of *S. aureus* and *P. aeruginosa* was determined by performing antibiotic sensitivity test (AST). They were found to be resistant to tested antibiotics and since *S. aureus* was resistant to oxacillin it was classified as methicillin resistant *S. aureus* (MRSA).

Antioxidant activity was determining was determined by Ferric Reducing Antioxidant Power (FRAP), it was found that the reducing power of the samples increased with increase in concentration. Both Lemon juice and Lemon peel extract showed increasing trend in reducing power with the increase in concentration. The lemon peel extract displayed highest reducing power than lemon juice. This shows that the lemon juice and lemon peel extract constituent’s possesses reducing power capabilities and can act as a potent antioxidant. The phytochemical analysis showed presence of phenols, flavonoids, steroids, reducing sugar and alkaloids in both lemon juice and lemon peel extract. Lemon juice also showed presence of amino acid. Lemon peel extract gave positive results for terpenoids and both lemon juice and lemon peel extract gave negative results for saponins and glycosides (Chandak., 2018).
Since lemon juice, lemon peel extract and citric acid showed good antibacterial activity; their individual MIC was performed and was found to be 1:4 for which inhibition zone was observed for both MRSA and resistant \( P. \) aeruginosa. To determine the potentiation \( 1:10 , 1:20 , 1:30 \). For lemon juice and crude extract, \( 1:10 \) dilution was the most effective in combination of various antibiotics. Likewise, for \( P. \) aeruginosa, \( 1:10 \) dilution of all three samples was found to be most effective in increasing inhibitory action of piperacillin but all three samples failed to change the inhibitory action of norfloxacin (Chandak., 2018).

FIGURE 2 – Phytochemical analysis of lemon juice and lemon peel extract

FIGURE 3 - Antibiotic-potentiation activity of lemon juice, peel extract and citric acid against \( P. \) aeruginosa
Aloevera (*Aloe barbadensis miller*) :- Aloe vera (Aloe barbadensis miller) is a plant, which belongs to the family of Liliaceae and is mostly succulent with a whorl of elongated, pointed leaves. The name is derived from the Arabic word ‘alloeh’ which means ‘bitter’, referring to the taste of the liquid contained in the leaves. Aloe that is believed to have originated in the Sudan. Aloe vera grows in arid climates and is widely distributed in Africa, India and other arid areas. The species is frequently cited as being used in herbal medicine. Aloe vera is a perennial, drought resisting, succulent plant. It has stiff green, lance-shaped leaves containing clear gel in a central mucilaginous pulp. Its thick leaves contain the water supply for the plant to survive long periods of drought. The leaves have a high capacity of retaining water also in very warm dry climates and it can survive very harsh circumstances. When a leaf is cut, an orange-yellow sap drips from the open end. When the green skin of a leaf is removed a clear mucilaginous substances appears that contains fibres, water and the ingredient to retain the water in the leaf. The gel contains 99.3% of water, the remaining 0.7% is made up of solids with carbohydrates constituting for a large components. Concentrated extracts of Aloe leaves are used as laxative and as a haemorrhoid treatment. Aloe gel can help to stimulate the body’s immune system (Kedarnath, 2013).

Aloe vera (*A. barbadensis Miller L.*) is most biologically active among 400 species. The genus Aloe belonging to family Alliaceae is a succulent plant of 80-100cm in height which matures in 4-6 years and survives for nearly 50 years under favorable conditions. The plant is native to southern and eastern Africa along the upper Nile in the Sudan, and it was subsequently introduced into northern Africa and naturalized in the Mediterranean region and other countries across the globe (Saket Kumar, 2017).

The antimicrobial activity of *aloevera* extract was tested against pathogenic bacteria like *Staphylococcus aureus*, *Klebsiella pneumonia* and *E.coli* and fungi like *Aspergillus niger* and *Candida*. Aloe vera revealed to evaluate antimicrobial activities of various extracts of *Aloe vera*. The successive leaf extracts using petroleum ether, chloroform and methanol of *Alovera* were tested for their antimicrobial efficiency against pathogenic bacteria and fungi (*Staphylococcus aureus*, *Klebsiella pneumonia*, *E.coli*) and fungi like (*Aspergillus Nige*, *Candida*) at a dose 1: 20mg/ml and 2:40mg/ml.

The standard drugs used for comparison were Streptomycin and Fluconazole against bacteria and fungi. Among the extracted tested for their antibacterial activity, the leaf extracts showed moderate to high activity against both gram positive and gram negative bacteria. The extracts using petroleum ether, chloroform, and methanol of *Aloe vera* showed active antimicrobial activity against *Staphylococcus aureus*, *Klebsiella pneumonia*, *E.coli* and, and antifungal activity against *Candida and Aspergilus niger* (Kedarnath, 2013).

The extracts of petroleum ether at the dose level of 20mg/ml showed the inhibition zone of *Staphylococcus aureus* (15mm), *Klebsiella* (19mm), *E.coli* (14mm), (Fig. 1) whereas the extracts of petroleum ether at the dose level of 40mg/ml showed the inhibition zone of *Staphylococcus aureus* (20mm), *Klebsiella* (20mm), *E.coli* (10mm).
Chloroform extract has shown a high zone of inhibition in Klebsiella pneumonia, Escherichia coli and Aspergillus Niger but moderate zone of inhibition in Staphylococcus aureus and Candida. Methanol extract as shown high zone of inhibition in Staphylococcus aureus, Klebsiella pneumoniae and Candida but moderate zone in Escherichia coli and Aspergillus Niger. When compared the zone of inhibition with the standard drugs like streptomycin and flucanozole. The plant extracts have shown almost equal to the standard drug. The above parameter supports the strong scientific basis for the use of these plants in traditional treatment of microbial diseases (Kedarnath, 2013).

FIGURE 4 - Antimicrobial Activity of Leaf Extract of Aloe vera in 20 mg and 40mg Concentration
The results lend credence to the folkloric use, if this plant in treating microbial infection and shows that *Aloe vera* could be exploited for new potent antimicrobial agents (Kedarnath, 2013).

**Green tea leaves** (*Camellia sinensis*):- Tea originated in China, possibly as long ago as 2700 BC. Drinking water, boiled for reasons of hygiene, was made more palatable by the addition of leaves from the tea plant. In modern times, tea, in one form or another, is, with the exception of water, the world's most widely consumed beverage; more than two billion cups are drunk daily. (Pankaj K. Sahu, 2013).

The word ‘tea’ has been used to describe the shrub *Camellia sinensis*; the fresh leaves of this shrub picked as ‘two and a bud’ for processing (also termed ‘flush’); the processed flush (macerated and heat-dried in the case of green tea); and the beverage made by infusing the processed leaves in boiling water. These multiple definitions may give rise to confusion, especially as there is an additional use of the term ‘tea’, namely as an aqueous infusion of any herb (thus ‘mint tea’, ‘rooibos tea’, etc.) in the sense of the French word tisane. More recently, the discovery of antibacterial activity in the essential oil of the tea tree (*Melaleuca alternifolia* which is not related to *C. sinensis*) has added further potential for confusion. The material presented in this review is concerned predominantly with work undertaken with aqueous infusions of processed green tea leaves or components thereof. It should be noted that black tea, derived by fermentation of flush before heat drying, contains many of the same pharmacologically active components as green tea, but at lower concentrations. Black tea has, in addition, higher molecular weight oxidation products, such as the the flavins and theaerubigens, which are not found in green tea (Peter W. Taylor, 2005).

A study showed the antimicrobial activity of green tea extract by standard percolation and infusion method. The prepared extract was tested on the standard American Type Culture Collection (ATCC) strains of the *E. coli* 25922, *S. aureus* 25923, and *P. aeruginosa* 27853. The aqueous extract of the green tea was evaluated against the standard strains of *E. coli* 25922, *S. aureus* 25923, and *P. aeruginosa* 27853 as a representative...
strain for various *in vitro* antimicrobial activity parameters. There was no activity against any concentration for *E. coli* 25922 and *S. aureus* 25923 with no ZoI on MHA, whereas *P. aeruginosa* 27853 showed good activity with ZoI ranging from 16 to 9 mm for 200–12.5 µg/ml, whereas no activity was seen for 6.25 µg/ml. Being effective against the ATCC strain of *P. aeruginosa* 27853 (PA), the percolated green tea extract was further tested against the clinical isolates of nonfermenters isolated in bacteriology laboratory attached to a tertiary care hospital. All the nonfermenters isolated and found resistant to any of the standard primary drugs as advocated by CLSI were further tested for enhancement or accentuation of activity indicated by the increase in ZoI by addition of the extract (Peter W. Taylor, 2005).

**Curry leaves (*Murraya koenigii)*:** *Murraya koenigii* (L.) Spreng or its common name curry leaf tree is a small strong smelling perennial shrub commonly found in forests as undergrowth. It was originally cultivated in India for its aromatic leaves and for ornament is normally used for natural flavoring in curries and sauces. Originated in Tarai regions of Uttar Pradesh, India. It is now widely found in all parts of India and it adorns every house yard of southern India and also it is now cultivated and distributes throughout the world. The plant is used in Indian system of medicine to treat various ailments. Parts of the plant have been used as raw material for the traditional medicine formulation in India. This plant is known to be the richest source of carbazole alkaloids. It has been reported by authors that carbazole alkaloids present in *M. koenigii* (L.) Spreng and display various biological activities such as anti-tumor, anti-oxidative, anti-mutagenic and anti-inflammatory activities. *M. koenigii* leaves and roots can be used to cure piles and allay heat of the body, thirst, inflammation and itching. The aromatic leaves, which retains their flavor and other qualities even after drying, are slightly bitter, acrid, cooling, weakly acidic in tastes and are considered as a tonic, anthelmintic, analgesic, digestive, appetizing and are widely used in Indian cookery for flavoring food stuffs (Mini Priya Rajendran, 2014).
A study tested the antifungal activity of aqueous and organic extract and their respective dilutions from a medicinal plant (Murraya koenigii) against four fungi species namely: Aspergillus niger, Penicillium camemberti, Candida albicans, Penicillium funiculosum. According to the results obtained from the extraction of ethanol, distilled water and hot water, ethanol has a greater inhibitory value. In this regard, organic extract proved to have more activity against the various fungi species used than aqueous extracts. Thus it can be concluded that organic extract was more efficient in the extraction of water soluble biomolecules with antifungal activity. The aqueous extracts that demonstrated the least activity against the fungi species was cold water extract, this could be explained by the fact that when plant materials are ground and placed in cold water, some phenolases and hydrolases are released and could have modulating effects on the activity of the compounds in the extracts (Mini Priya Rajendran, 2014).

Comparison of the growth inhibition of various extracts and their respective dilutions shows a strong dependent effect on extract concentrations. These results revealed that antifungal activity of various extracts was enhanced by increasing the concentration of the extracts, thus the inhibition activity of the extracts was concentration dependent (Mini Priya Rajendran, 2014).

Antiviral activity:- A.C. Kudi, S.H. Myint studied antiviral activity of 17 plant extracts tested against astrovirus, poliovirus 1, herpes simplex virus 1, equine herpes simplex virus, bovine parvovirus and canine parvovirus four of the plant extracts inhibited all the human viruses and two all the animal viruses. A total of six plant extracts inhibited astrovirus and poliovirus. Two of the extracts; Anacardium occidentale
(Anacardiaceae) and Sterculia setigera (Sterculiaceae), were found to inhibit both human and animal viruses used in the study. Most of the plants have partial activity at the lowest concentration of 100 mg/100 ml (dilution factor of three) but full activity at 400 mg/100 ml (neat) against some of the viruses. Examples are Anogeissus schimperi against Herpes simplex virus, Guiera senegalensis against human viruses and one of the animal virus (EHSV), Bauhinia thonningii against parvovirus, Boswelia dalzielii against the animal viruses, Detarium senegalensis against all the viruses and Dichrostachys glomerata against parvovirus. Some of the plant extracts were found to be cytotoxic at the neat concentration (400 mg/100 ml) but not at twofold dilution (A.C. Kudi, 1999).

INTERACTION BETWEEN NATURAL PRODUCTS AND ANTIMICROBIAL DRUGS (SYNERGISM):

In addition to the antimicrobial action of plant extracts and essential oils, a synergism between conventional antimicrobial drugs and products obtained from medicinal plants has also been reported. Possible interactions among medications are frequently observed, which has motivated researchers to test such possibilities. However, it must be emphasized that interactions between synthetic and natural drugs depend on several factors including pharmacokinetics and employed doses, since combinations confirmed in vitro may not have the same effect on humans (Silva NCC, 2010).

Synergism between the essentials oils of cinnamon (C. zeylanicum), lemon grass (C. citratus), peppermint (M. piperita), ginger (Z. officinale), clove (C. aromaticus) and rosemary (R. officinalis) and eight antimicrobial drugs (chloramphenicol, gentamicin, cefepime, tetracycline, sulfazotrim, cephalothin, ciprofloxacin and rifampicin) was tested by the Kirby and Bauer method against strains of S. aureus and E. coli. The highest rates of synergism were between lemon grass and the eight drugs tested followed by mint and seven drugs (except cefepime against S. aureus). Against E. coli, only rosemary associated with three drugs and lemon grass with two antimicrobials revealed synergism (Zago JAA, 2009).

Thus, studies on the interactions between natural products and antimicrobial drugs have also multiplied in recent years, indicating the importance of elucidating those types of interactions, which can be either favorable, such as in synergism, or harmful, as in antagonism. However, such associations, even if beneficial, will not necessarily be used in therapy against infectious diseases, since further studies are still required, especially in vivo studies and research on the toxicity of these products to humans.
CONCLUSION - There is a promising future of medicinal plants as there are about half million plants around the world, and most of them are not investigated yet for their medical activities and their hidden potential of medical activities could be decisive in the treatment of present and future studies. In the development of human culture medicinal plants have played an essential role, for example religions and different ceremonies. Among the variety of modern medicines, many of them are produced indirectly from medicinal plants, for example aspirin. Many food crops have medicinal effects, for example garlic. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. The medicinal effects of plants are due to secondary metabolite production of the plants. Keeping this in consideration there have been increased waves of interest in the field of research in natural product chemistry. This interest can be due to several factors, including therapeutic needs, the remarkable diversity of both chemical structure and biological activities of naturally occurring secondary metabolites, the utility of novel bioactive natural compounds as biochemical probes, the development of novel and sensitive techniques to detect biologically active natural products, improved techniques to isolate, purify, and structurally characterize these active constituents, and advances in solving the demand for supply of complex natural products. The importance of traditional medicine has also recognized by World Health Organization (WHO) and has created strategies, guidelines and standards for botanical medicines. For the cultivation, processing of medicinal plants and the manufacture of herbal medicines agro-industrial technologies need to be applied. Medicinal plants are resources of new drugs and many of the modern medicines are produced indirectly from plants (Refaz Ahmad Dar, 2017).

From the above review, it can be concluded that medicinal plants have been playing an essential role in the development of human culture. As a source of medicine, Medicinal plants have always been at forefront virtually all cultures of civilizations. Medicinal plants and medicinal plants extract indeed have a great impact and can serve as alternatives of antibiotics.

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