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Assessing the Antimicrobial Potential of Plant Extracts: A Comprehensive Literature Review

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ABSTRACT: Since ancient times, humans have treated illnesses and infections with the medicinal properties of plants—often in an empirical way. More and more during the last few decades, scientific research has focused on discovering new plant medicines to treat microbial diseases. They are extensively used because they contain phytochemicals that allow them to act as antibacterial substances; however, further studies are ongoing to assess and understand the effectiveness of these extracts against various diseases. The rising problem of antimicrobial resistance (AMR) emphasizes the need for new antimicrobial agents. The effectiveness of conventional antibiotics has decreased due to the rapid emergence of resistant strains among pathogens causing illnesses worldwide. This necessitates that novel approaches be adopted in order for us to combat this global concern, and one such avenue could be through plant extract-based therapies. Thus, this paper presents an overview of literature concerning recent studies on antibacterial activity.

KEYWORDS: Antimicrobial activity, Plant extracts, phytochemicals, methodologies, invivo methods, invitro methods

I. INTRODUCTION

Antimicrobial agents are materials that either kill or inhibit the multiplication of microorganisms, including bacteria, viruses, fungus and parasites. Since their discovery in the early 20th century, antibiotics have been widely used in medicine for treatment of infections caused by bacteria. However due to overuse there has been an increase in antimicrobial resistance which requires new drug development and innovative approaches towards treatment of infectious diseases.[1]

Secondary metabolites also known as phytochemicals obtained from either utilization of plants or from their extracts have been shown to exhibit some level antibacterial action both when they are used alone or mixed with one another. The antimicrobial effectiveness of extracts obtained from a number of different plant species has been evaluated using various methods including agar diffusion and micro dilution assays.[2]Among microorganisms, notable inhibition was observed for herbal extracts like Ocimum sanctum with alcoholic extracts as the most effective.[3] There was a strong antibacterial activity seen in native temperate plants like Melaleuca and Eucalyptus species on pathogens such asStaphylococcus aureusand Pseudomonas aeruginosa. [4] Likewise, plant extracts from Taraxacum officinale showed varying inhibition levels against clinically significant bacteria including Klebsiella pneumoniae and Enterococcus faecium which are resistant to vancomycin. This shows a possibility of using plant extracts as alternatives to antibiotics in treatment of certain diseases especially those caused by antibiotic resistant bacteria.[5]Research conducted up until now shows that several bioactive compounds such as flavonoids, terpenoids and phenolics among others exist in plant extracts. These substances exhibit strong antibacterial properties as they cause rupture of microbial membranes, inhibit essential enzymes or interfere with metabolism of microbial cells. The potential chemotherapeutic value of phytochemical products and plant extracts have been demonstrated in vitro and in vivo as Resistant MicrobialAgents to bring back the effectiveness of antibiotics against

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resistant pathogenic bacteria. The increase in number of microbes that resist traditional antibiotics has led to the search for other ways to control them especially those derived from plants. It is traditional medicine makes use of plant extracts for their antibacterial and other therapeutic qualities.[6] This review explores the current state of research on antimicrobial activity of plant extracts by discussing key themes, methods, findings etc.

PLANTS CONTAINING ANTIMICROBIAL ACTIVITY

Common name	Scientific name	Class	Target organism
Aloe[7]	Aloe barbadensis, Aloe vera	Complex mixture	Corynebacterium, Salmonella, Streptococcus,Staphylococcus aureus
Ashwagandha	Withania somniferum	Lactone	Bacteria, fungi
Basil [8]	Ocimumbasilicum	Terpenoids	Salmonella, bacteria
Black pepper[9]	Piper nigrum	Alkaloid	Fungi, Lactobacillus, Micrococcus, E. coli, E. faecalis
Clove	Syzygium aromaticum	Terpenoids	General
Coca	Erythroxylum coca	Alkaloid	Gram-negative and -positive cocci
Eucalyptus	Eucalyptus globulus	Polyphenol	Bacteria, viruses
Garlic[10]	Allium sativum	Sulfoxide	General
Green tea[11]	Camellia sinensis	Flavonoid	General
Henna	Lawsonia inermis	Phenolic	S. aureus
Licorice	Glycyrrhiza glabra	Phenolic alcohol	S. aureus, M. tuberculosis
Olive oil[12]	Olea europaea	Aldehyde	General
Papaya[13]	Carica papaya	Terpenoids,alkaloid,organic acid	general
Periwinkle	Vinca minor	alkaloid	General
Rosemary	Rosmarinus officinalis	Terpenoid	General
Senna	Cassia angustifolia	Anthraquinone	General
Thyme	Thymus vulgaris	Terpenoid	Viruses, bacteria, fungi
Turmeric[14]	Curcuma longa	Terpenoid	Bacteria, protozoa

Table 1; plants containing antimicrobial activity with their scientific name, class and target organism [15]

PHYTOCHEMICALS FOR ANTIMICROBIAL ACTIVITY

Class	Subclass	Example(s)	Mechanism
Phenolics	Simple phenols	Catechol	Substrate deprivation
		Epicatechin	Membrane disruption
	Phenolic acids	Cinnamic acid	
	Quinones	Hypericin	Bind to adhesins, complex with cell wall, inactivate enzymes
	Flavonoids	Chrysin	Bind to adhesins
	Flavones	Abyssinone	Complex with cell wall
			Inactivate enzymes

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			Inhibit HIV reverse transcriptase
	Tannins	Ellagitannin	Bind to proteins
			Bind to adhesins
			Enzyme inhibition
			Substrate deprivation
			Complex with cell wall
			Membrane disruption
			Metal ion complexation
	Coumarins	Warfarin	Interaction with eucaryotic DNA (antiviral activity)
Terpenoids, essential oils		Capsaicin	Membrane disruption
Alkaloids		Berberine	Intercalate into cell wall and/or DNA
		Piperine	
Lectins and polypeptides		Mannose-specific agglutinin	Block viral fusion or adsorption
		Fabatin	Form disulfide bridges

Table 2:phytochemicals for antimicrobial activity with their subclasses, examples, and mechanism of action[15]

SOLVENTS USED FOR THE EXTRACTION OF PLANTS

SOLVENTS	POLARITY
n-Hexane	0.009
Diethyl ether	0.117
Ethyl acetate	0.228
Chloroform	0.259
Acetone	0.355
n-Butane	0.586
Ethanol	0.654
Methanol	0.762
Water	1.000

Table 3:Solvents used for extraction of plants with their polarity[16]

EVALUATION OF ANTIMICROBIAL ACTIVITY

INVITRO EVALUATION METHODS

1. Agar Disk Diffusion method.

The agar disk diffusion method is a conventional technique for finding out if an antibiotic is ineffective. There are antimicrobial disks placed on agar contaminated plates and then measuring the zone of inhibition is performed to qualitatively assess the effectiveness of this approach.[17]

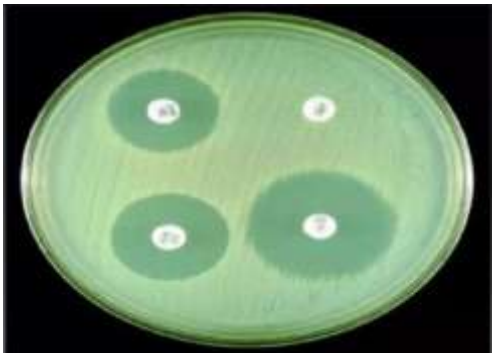

Figure 1:Agar disk diffusion method using antibiotics

2. Diluting Broth method:

Antimicrobial drug concentrations can be determined by the broth dilution method, which

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leads to levels of the drug that prevent microorganism's growth in vitro. In order for this assay to work, a series or stepwise dilutions of the agent must be prepared in a suitable broth medium. [18]

Minimum inhibitory concentration:

MICs are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum Bactericidal Concentrations (MBC) are defined as the minimum inhibitory concentration (MIC) required to completely prevent post-antibiotic growth of a pathogenic organism irrespective of its passage onto antibiotic-free media.

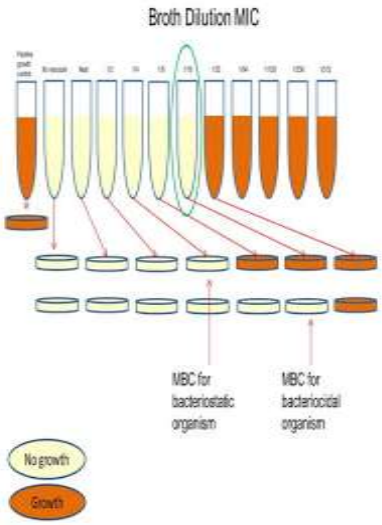

Figure 2:Broth dilution method used to evaluate the minimum inhibitory concentration (MIC) of antimicrobial agent

3. Cross streak method:

The cross-streak method tests the antimicrobial activity by streaking a pathogen across a line of an agar plate in contact with a test substance. It measures the inhibition of growth and can reveal both bactericidal and bacteriostatic effects.[19][20]

Figure 3: Cross steak method for evaluating antimicrobial activity

4. TLC, or thin-layer chromatography:

TLC allows for the separation and identification of different compounds within mixtures such as antimicrobial agents. When combined with bioautography, it can also demonstrate areas where microbes have been inhibited from growing, therefore exhibiting antimicrobial activities.[19][21]

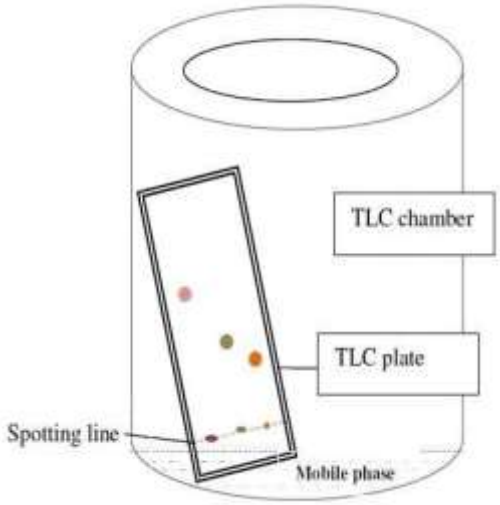

Figure 4: Thin layer chromatography method for the evaluation of antimicrobial activity

5. Agar well diffusion

The method known as agar well diffusion has become common for assessment of the effectiveness of plant or microbial extracts antibiotic against different bacteria. Using this method, we spread a certain amount of microbial inoculum on an agar plate, which is similar to the disk-diffusion technique. The next step involves

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making a well of 6 to 8 millimeters in diameter and placing some volume (20-100 µL) containing the antimicrobial agent or extract. In proper conditions, the substance spreads out within the agar and stops growth of the organism being tested. [19] [22]

Figure 5: Agar well diffusion method for the evaluation of antimicrobial activity

6. Antimicrobial gradient method

The sterile gradient technique or Etest is another name for it, uses the concepts of dilution and diffusion to determine the Minimum Inhibitory Concentration (MIC) of antimicrobial agents. Bacteria are put in agar first and then the gradient strip of the antimicrobial agent is placed. The point where the strip and the region of growth inhibition meet is the point where the MIC is found out. The company sells Etest strips at a cost of $2 to $3 each. Therefore, the technology is not only affordable but also frequently applied in hospital settings, and it is inexpensive in the case of the tests involving various compounds that should be detected.[19][23]

Figure 6: E-TEST for the evaluation of antimicrobial activity

7. ATP bioluminescence assay

The bioluminescence assay performed by the fungi and the cells to quantify ATP will show the healthy conditions of the cells. Without the help of which we would not be able even to move the ball mill is inoperable if the containment is breached. It is ATP being the meters that are constant in every cell that might fill in the number of microorganisms which are the inhabitants of the given cell. In this assay, samples of luminescence are used to express the amount of emitted light that the d-luciferin and ATP produce in the company of luciferase. The teens use this method to get a numerical picture of their cell numbers with light production being a means of cells having responded to some physical milieu. Some of the applications involve the following: Evaluation of Gam Mite, biofilm effect assessment, and testing for antimicrobial activity against diverse pathogens. One of the main benefits is the speed of the tests; some of them can give results in 3 to 5 days which is much faster than traditional methods that take one or some weeks before showing any outcomes.[19][24][25]

8. Time kill test

Time-kill test is a necessary approach to understand the potency of a specific agent against fungi or bacteria. It gives an actual picture of the drug's kinetics under conditions determining either its relation to time or concentration. This sample was standardized in the United States and given a general growth condition working for several organisms by Clinical and Laboratory Standard Institute (CLSI) document M26-A. The addition of different concentration levels of antibiotics to broth that is later incubated for a selected time and the subsequent observation of these culture visually are the activities involved in this assay. The reference sample is a growth control to be used as a comparison. The assay protocol, which is expected to include the measurement of the percentage of dead cells as in the case where a control is used, will have a duration of 6 hours after the treatment or 24 hours when 90% cell death takes place. This method has contributed to research aimed at the identification of antifungal substances and the evaluation of interactions between drugs.[19][26]

INVIVO EVALUATION METHOD

1. Mouse ascending UTI model

Recurring UTIs are caused by E.coli in women as the predominant reason to the rest of the strain. Whereas male mice can be specified for

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male UTIs research, female mice are most frequently used in studying these infections. Bacteria are cultivated in order to get the strains with more virulence factors, and in some cases, the liquid cultures on solid medium are prepared in order to have the bacterial inoculum. An intraurethral or intravesical method is used to induce urinary tract infections (UTIs) in mice, targeting the bladder while preventing the kidney infection directly. The quantity of bacteria and the extent of kidney and bladder inflammation are directly linked. Experimental or control group data joins forces to calculate the balance of host traits against the contributing of external bacteria forms by affecting infections. [27]

2. Rabbit skin burn infection model

The infection of burn wounds largely causes mortalities among burn victims, especially by means of the multi-drug resistant bacterium known as Pseudomonas aeruginosa. For this reason, there is a need for alternative therapies as traditional antibiotics are not generally sufficient to help in this case. One such interesting strategy would involve using probiotic bacteria, such as Lactobacillus spp., which have been found to possess antibacterial properties against wound infections and promote wound healing. This is particularly important during the development of new medicines because while traditional mammalian models are expensive complicated, and face ethical challenges in vivo models would be necessary. On the other hand, the Galleria mellonella (wax moth larva) model presents a less irritating and expensive research option that can easily be taken care of at home. The present study exhibited that several Lactobacillus spp. could curtail the effects of Pseudomonas aeruginosa infections and improve survival chances for victims with burn wounds hence proving that the Galleria mellonella model is instrumental for probiotic screening. [28]

3. Mouse systemic infection model

Animal models are critical for understanding pathophysiology of infections, testing new systemic antifungal agents, and evaluating alternate approaches like immune modulation. The well-developed and reliable mouse model of intravenous infection-induced systemic candidiasis is commonly used. However, this model is composed of immunologically naïve mice to reproduce that there are no local infection processes; hence it is unrepresentative of the natural infectious process in which fungi bypass mucosal barriers as people do through development of adaptive immunity against mucosal colonization. To address this problem, mouse models characterized by systemic infections including those due to Candida and intestinal colonization have been developed providing novel insights into host-fungi co-existence plus immune response to foreign particles. The major findings and unresolved issues are discussed in this review highlighting their relevance to translating results from mice studies into clinical settings. [29]

II. CONCLUSION

To summarize everything, we can say that the evaluation of the antibacterial properties found in the various plants presents a promise for achieving an alternative means of obtaining sustainable and natural antibiotics. However, there are some challenges that researchers must overcome in order to make full use of their medical benefits. Extraction methods, assay procedures and quality control systems should be standardized so that results obtained from different studies are repeatable and reliable. Besides this, it is important to know how these phytochemicals work if they have to be improved on their safety and efficacy against microorganisms.

For the way forward then, it is important to have multidisciplinary collaboration among pharmacologists, microbiologists, physicians and botanists. These partnerships facilitate identification, isolation and characterization of new bioactive plant compounds paving the way for more development of new antimicrobial agents. Moreover, more investigations on combined actions of phytochemicals with synthetic antibiotics would lead us towards combination therapies which should enhance treatment outcomes while curbing resistance rates.

In conclusion therefore although there exist some mountains still left unchartered; searching into herbal extracts for anti-microbial properties holds great hopes for providing us with more choices when it comes to effectively managing infectious diseases.

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