International Journal of Advanced Research in Engineering Technology & Science
ISSN: 2349-2819
Email: editor@ijarets.org Volume-10, Issue-7 July – 2023 www.ijarets.org

ANTIMICROBIAL ACTIVITY OF EXTRACTS OF TERMINALIA BELLERICA AND AEGLE MARMELOSE PLANTS USED IN INDIA TRADITIONAL MEDICINES TYPE OF FUNGAL AND INFECTIONS

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ABSTRACT

Several plants that are significant to India's Ayurvedic medicine system—which is used to treat intestinal disorders—were screened. The selection of medicinal plants was based on the common disorders for which the various ethnic groups used them. Aegle marmelos, Azadirachta indica, Terminalia chebula, Mangifera indica, and Ocimum sanctum are five significant medicinal plants whose extracts (aqueous and ethanol) have been studied for their comparative in vitro antibacterial potential using microbial growth inhibition assays against common human pathogenic bacteria (Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli) of clinical origin. The ethanol extract of the plant materials exhibited more antibacterial activity than their watery equivalents, and all of the plant components shown varied degrees of strain-specific inhibitory effect. In addition, T. chebula and A. marmelos exhibited the greatest antibacterial activity; T. chebula's antibacterial potential was greater than that of the other two. We chose 36 distinct medicinal plants from 30 families that are used to treat enteric infections from Melghat Forest (Amravati district) in order to test the bioactive components of T. chebula for possible application as therapeutic agents for the treatment of common bacterial infections. Antibacterial properties of extracts made in methanol, ethanol, and water

Keyword *:- Plant extracts, medicinal herbs, pathogenic microorganisms, and antibacterial properties.*

INTRODUCTION

For a wide range of illnesses, a sizable segment of the global population, particularly in poorer nations, relies on the traditional medical system. Several hundred genera are utilized medicinally, mostly as herbal preparations in various indigenous medical systems across the world. These systems are the source of extremely effective and powerful medications that have withstood the test of time, with most of them being unreplaceable by modern chemistry.

According to data from the World Health Organization, 80% of people on the planet only utilize traditional medicine, and a significant portion of these treatments employ plant extracts or their active ingredients (WHO, 1993). The indiscriminate use of antimicrobial medications has led to the development of antibiotic resistance in bacteria. As a result, treating infectious illnesses has become extremely difficult clinically. Apart from this

issue, antibiotics can also cause hypersensitivity, immunosuppression, the loss of beneficial gut and mucosal bacteria, and allergic responses in the host. As a result, new antimicrobial medications must be created in order to treat infectious infections. One method is to look for potential antibacterial qualities in native medicinal herbs. One abundant source of new antibacterial and antifungal chemotherapeutic drugs is medicinal plants. The strategic placement of bioactive phytochemicals is one approach that might be used to achieve this goal.

Numerous secondary metabolites found in plants, including tannins, terpenoids, alkaloids, and flavonoids, have been shown to have antimicrobial qualities in vitro and may offer a safer, more affordable, and effective alternative to antibiotics when treating microbial diseases. Proteins precipitate with tannin, a phenolic substance that is soluble in acetone, alcohol, and water. Despite the enormous advancements in human medicine, infectious illnesses produced by bacteria, fungi, viruses, and parasites continue to pose a serious danger to public health. Their influence on medicine and the rise of widespread drug resistance is especially significant. Multi-drug resistance bacteria are posing a danger to the therapeutic effectiveness of many of the antibiotics now in use. The resistance of bacterial and fungal pathogens to anti-microbial agents is increasing, and they have developed a variety of defensive mechanisms against these agents.

Many medicinal plants are being screened for possible antibacterial action due to the pathogenic microbial infectious pathogens' increased antibiotic resistance and chemotherapeutic failure. Contrary to synthetic drugs, natural products derived from higher plants may provide a new source of antimicrobial agents with potentially novel mechanisms of action Antimicrobials derived from plants also have a great deal of therapeutic potential to treat a variety of infectious diseases Antibiotics also occasionally cause hypersensitivity, immunosuppression, allergic reactions, and the loss of beneficial gut and mucosal microorganisms in addition to this issue Thus, the development of substitute antibacterial medications is imperative. the majority of plants have one or more of the following therapeutic qualities: they are sedative, laxative, diuretic, cardiotonic, antibacterial, antifungal, antiviral, and others. Plants can have toxic effects because of active ingredients in their root, shoot, bark, fruit, and other components, as well as occasionally across the entire plant. Alkaloids, glycosides, saponins, essential oils, mucilage, tannins, bitter principles, etc. are the primary categories of active ingredients. These substances are referred to as antimicrobials since they inhibit or eliminate bacteria.

Plant extracts are used extensively in traditional medicines. These plant extracts are typically extracted using several types of solvent. Antimicrobials are such extracts that inhibit and/or eliminate microorganisms. These antibacterial compounds may be present in varying degrees in different types of medicinal plants.. Numerous studies conducted in the United States, the United Kingdom, and other nations have demonstrated that higher plants may also provide antibiotics. Numerous physiologically active substances present in plants have antibacterial qualities. The number of plants with antibacterial activity that are being evaluated globally for a variety of ailments has increased recently. The goal of the current study is to assess the antibacterial properties of many medicinal plants that are used to treat various infectious disorders. also practice other forms of medicine based on their experiences.

This can be explained by things like the scarcity of medical professionals, physicians, drugs, and road infrastructure, as well as the high cost of these treatments. It is estimated that between 75 and 80 percent of Nepal's rural residents employ these traditional treatments While herbal medicine is widely used in Nepal, only few species have undergone biological activity screening. Therefore, it seems sense to look into the potential uses of Nepalese medicinal herbs further.

However, the first stage in the drug development process is always proving activity in a bioassay In Nepal, a wide variety of therapeutic herbs have long been recognized. Nepal-Himalaya was described as a hallowed paradise of strong medicinal and aromatic plants during the Vedic era, when the history of medicine and medicinal plants in the nation began. These centuries-old medical systems are being more and more acknowledged for their roles that are complementary to, and occasionally better than, those of western medical systems. Ayurvedic medicine is not the only useful information held by humanity; Nepalese villages also practice other forms of medicine based on their experiences.

OBJECTIVES

1. An assessment of various medicinal plants' antibacterial properties.
2. to investigate the antibacterial properties of plant extracts used in medications, such as those from Terminalia bellerica and Aegle marmelose.
3. to investigate how some plants used in Indian traditional medicine might fight infections by evaluating their antibacterial potential

RESEARCH METHODOLOGY

Gathering, removing, and combining plant materials: A botanist recognized and verified newly matured plant materials, which included leaves from O. sanctum, A. indica, A. marmelos, T. chebula, and M. indica, that were gathered from various locations in West Bengal. Each plant's air-dried powdered leaves or fruits were extracted using ethanol and water in a Soxhlet equipment for a whole day. The crude extracts were dried at room temperature in a constant air current after the solvents were eliminated in a rotary evaporator. For microbiological investigation, the dried extracts of the plant components were then kept in airtight jars at 4oC. Each plant material's ethanolic extract was dissolved in 5% dimethylsulfoxide (DMSO) and its aqueous extract was dissolved in distilled water to produce an extract that had a final concentration of 100 mg/ml.

Microorganisms

In this investigation, reference standard strains as well as clinical isolates were employed. Type strains of Staphylococcus aureus (ATCC 6538P), Pseudomonas aeruginosa (ATCC 9027), and Escherichia coli (ATCC 8739) were obtained from the National Collection of Industrial Microorganisms, Pune, India, and were kept in nutrient agar slants. Clinical isolates of these three bacteria were obtained from the Department of Bacteriology and Serology, Calcutta School of Tropical Medicine, Kolkata. Test for susceptibility After being injected into Mueller Hinton Broth (Oxoid) medium, the examined bacterial strains were incubated for three to six hours at 35 degrees Celsius in a shaker water bath, or until the culture reached a turbidity of 0.5 McFarland units. 5 × 105 cfu/ml was the final inoculum size that was modified (NCCLS 1993).

Susceptibility tests

Were carried out using a modified agar-well diffusion method Using a sterile glass rod spreader, a 1.0 ml amount of the standard suspension of every test bacterial strain was equally distributed over Mueller Hinton Agar, and the plates were left to dry at room temperature. The reconstituted extract (both aqueous and ethanol) was then added to 100 µl volumes of 100 mg/ml of agar that had been drilled with a 6-mm diameter well. The plates were incubated at 37oC for 24 hours after being kept at room temperature for two hours. The diameter of the inhibition zone (IZD) was calculated to the closest millimeter (mm). The experimental positive control

was gentamicin (8 µg/ml) from Nicholas, India, while the negative control was 5% DMSO. For every bacterial strain under evaluation, the tests were run three times, and the findings were ultimately reported as an arithmetic average. The only extracts that underwent additional evaluation for their minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), and total activity determination against the clinical isolates were those that demonstrated a strong inhibitory zone diameter (IZD = 12 mm or more) and a wider spectrum of antibacterial activity (Baur et al., 1966). The IZD values of T. chebula and A. marmelos shown a similar inhibitory effect against both type strains and clinical isolates. Therefore, the extracts' MIC, MBC, and total activity values were solely evaluated against the clinical isolates.

Determination of minimal inhibitory concentration (MIC)

The macrobroth dilution assay method was used to estimate the minimal inhibitory concentration values of the ethanol extract of the plant materials of T. chebula and A. marmelos against each of the bacterial test strains (NCCLS 1993). Mueller Hinton Broth was used as a diluent to make two-fold serial dilutions of the ethanol extracts of T. chebula and A. marmelos (0.1-100 mg/ml) in tubes. Test organisms were seeded into duplicate tubes of each dilution at the standard concentration (5 × 105 cfu/ml). Gentamicin (Nicholas, India) at two-fold serial dilution (0.125-512 µg/ml) served as the experimental positive control. For a whole day, the tubes were incubated at 37 °C. The minimum inhibitory concentration (MIC) was determined by taking the lowest concentration of the extract that did not exhibit any discernible growth.

Determination of minimal bactericidal concentration (mbc)

By aspirating 0.1 ml of culture media from each tube (in the macrobroth MIC experiment) that did not appear to be growing and subculturing it on fresh MHA, the minimal bactericidal concentration (MBC) was ascertained. For a whole day, the MHA plates were incubated at 37°C. On the MHA sub-culture, the MBC was found to be the least concentrated and to be displaying no discernible increase. The MICindex (MBC/MIC) values for T. chebula and A. marmelos against each strain assessed were calculated using the MIC and MBC values that were obtained. Calculating the overall activity In order to compare the antibacterial potency of A. marmelos and T. chebula extracts quantitatively, the total activity (Ta) of the ethanol extracts of both strains was measured from the per gram extracted material and MIC values against each test strain using the following mathematical equation (Eloff 2004).

Ta (ml) is the amount taken out of 1 g (mg) of MIC(mg ml-1).

The units were converted to milliliters (ml) and represented the extent to which the active extracts in a single gram of plant material could be diluted without compromising the test organisms' ability to thrive (Eloff 2004).

RESULT

Table 1 demonstrated that while the ethanol and aqueous extracts of A. indica showed strong (IZD = 18 mm and 20 mm) inhibitory activity against S. aureus, they were unable to demonstrate any encouraging (IZD = 8 mm or more) inhibitory action against P. aeruginosa and E. coli, two Gram-negative bacteria. The test strains were all subjected to moderate (IZD = 9 mm-11 mm) inhibitory activity by the ethanol extract of O. sanctum and M. indica. However, against every strain examined, their aqueous extracts were unable to demonstrate any encouraging inhibitory action (IZD = 8 mm or greater). T. chebula and A. marmelos' water extracts had an intermediate (IZD = 8 mm to 10 mm) inhibitory activity against all of the strains examined, while their ethanol

extracts demonstrated a high (IZD = 15 mm to 23 mm) inhibitory action. The plant extracts' gentamicin-related percentage of inhibition against the test strains varied from 19 to 100%.

The plant extracts had a similar inhibitory effect on the standard type bacteria as they did on the clinical isolates. The MIC values for A. marmelos and T. chebula against the test strains varied from 3.12 to 6.25 mg/ml and 1.56 to 3.12 mg/ml, respectively, as Table 2 demonstrated. The range of MBC values for A. marmelos was 12.50 to 50.0 mg/ml, while for T. chebula it was 1.56 to 6.25 mg/ml. T. chebula had MICindex values of 1 to 2, while A. marmelos had values of 4 to 8. Total activity levels for A. marmelos and T. chebula against the test strains varied from 107.5 to 215.0 ml and 154.7 to 309.4 ml, respectively. Gentamicin's MIC, MBC, and MICindex values against the test strains were 2–8, 4–32, and 2–4 µg/ml, correspondingly.

Table 1: Inhibitory zone diameter of medicinal plant extracts against pathogenic bacteria S. aureus, P. aeruginosa, and E. coli indicates their antibacterial activity

Treatment	Microorganisms					
	S. aureus (l.c.i.) / *S. aureus*(ATCC 6538P)		*P. aeruginosa* (l.c.i.)/ *P. aeruginosa* (ATCC 9027)		*E. coli* (l.c.i.)/ *E. coli* (ATCC 8739)	
	Inhibitory Zone Diameter (IZD) (mm)					
	Aqueous	EtOH	Aqueous	EtOH	Aqueous	EtOH
T. chebula	10^b / 9^b (47/ 42)	20^a / 21^a (95/ 100)	9^b /9^b (39/39)	21^a / 20^a (91/ 86)	10^b /10^b (41/41)	23^a /24^a (95/ 100)
A. marmelos	9^b/9^b (42/42)	17^a/16^a (80/76)	8^b/8^b (34/34)	15^a/14^a (65/60)	10^b/9^b (41/ 37)	18^a /19^a (75/ 79)
M. indica	6^c /5^c (28/23)	11^a /11^a (52/52)	7^c/6^c (30/26)	11^b/12^b (47/52)	7^c/ 7^c (29/29)	10^b/10^b (41/41)
O. sanctum	4^c/4^c (19/19)	10^b /9^b (47/42)	7^c/ 7^c (30/30)	9^b /10^b (39/43)	6^c /5^c (25/20)	9^b /8^b (37/33)
A. indica	18^a/19^a (85/90)	20^a/21^a (95/100)	5^c /4^c (21/17)	6^c/6^c (26/26)	6^c /5^c (25/20)	5^c/6^c (20/25)
Gentamicin (Nicholas, India)	21^a		23^a		24^a	

Dmso

The average of three separate experiments is given. (-): Lack of restraint. A delicate, b in between, and c resistant The values included in parenthesis represent the percentage of inhibition in relation to the standard antibiotic Gentamicin.

Table 2 lists the ethanolic extracts of T. chebula and A. marmelos's minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), MICindex, and total activity values against the test pathogens.

Treatment	Microorganisms	MIC (mg/ml)	MBC (mg/ml)	MIC_{index}	Total Activity (ml)
T. chebula	*S. aureus*	1.56	1.56	1.0	482.6/1.56=309.4
	P. aeruginosa	3.12	6.25	2.0	482.6/3.12=154.7
	E. coli	3.12	6.25	2.0	482.6/3.12=154.7
A. marmelos	*S. aureus*	3.12	12.50	4.0	670.8/ 3.12=215.0
	P. aeruginosa	6.25	50.00	8.0	670.8/6.25 =107.5
	E. coli	6.25	50.00	8.0	670.8/6.25=107.5
Gentamicin (Nicholas, India)	*S. aureus*	2.00*	4.00*	2.0	-
	P. aeruginosa	8.00*	32.00*	4.0	-
	E. coli	8.00*	32.00*	4.0	-

*µg/ml

DISCUSSION

Because S. aureus produces a range of suppurative (pusforming) infections and toxinoses in humans, it is clinically significant. In addition to more serious infections including pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections, it can cause superficial skin lesions like boils, styes, and furunculosis. Hospital acquired infections are mostly caused by S. aureus. surgical wound infections and infections brought on by indwelling medical devices are both considered nosocomial infections. Superantigens released into the bloodstream by S. aureus produce toxic shock syndrome and food poisoning (Michael et al. 1999). Because P. aeruginosa is an etiology of several diseases, including septic burns and wounds, conjunctivitis, endocarditis, meningitis, and urinary tract infections, it has significant clinical implications. Because of its well-known resistance to the majority of antimicrobial agents, it is noteworthy that it is used as a reference species in susceptibility testing (Ho et al. 1998). Comparably, even though E. Coli is often a gut commensal, it has gained clinical relevance due to the identification of many strains that cause diarrhea and have unique virulent characteristics. It is also a significant bacterium in urinary tract infections (UTIs) (Johnson 1991).

The results of this investigation showed that ethanol was an appropriate solvent for the extraction of bioactive plant materials, and that all plant components displayed strain-specific antibacterial activity. The bactericidal activity is categorized as (i) resistant if the zone of inhibition is less than 8 mm, (ii) intermediate if it is between 8 and 11 mm, and (iii) sensitive if it is 12 mm or greater, using Baur et al. (1966) categorization. The current results showed that every test strain was sensitive to the ethanol extract of T. chebula and A. marmelos based on this categorization. M. indica and O. sanctum showed intermediate efficacy, whereas A. indica's ethanol and aqueous extracts were solely effective against Gram-positive S. aureus. Both the ethanol extracts of T. chebula and A. marmelos demonstrated a strong wider spectrum of antibacterial activity (60-100%) against all the test strains when the potency of the aqueous and ethanol extracts of each plant material was compared with gentamicin, a reference standard antibiotic. Despite the fact that pure antibiotic substances like gentamicin have been shown to have higher antimicrobial activity than crude plant preparations (Ebi and Ofoefule 1997; Ibrahim et al. 1997), the ethanol extract of T. chebula demonstrated superior antibacterial potential in this study due to its wider spectrum of antibacterial properties, high bactericidal activity, low MICindex (1.0-2.0), high total activity, and observed against all test strains. T. chebula was found to contain 24–32% tannin, according to chemical analysis (Chung et al. 1988).

Gallic acid, corilagin, chebulagic acid, and chebulinic acid were the main ingredients of this tannin. There were eighteen amino acids in it. Additionally, there was sennoside nature, resin, and the purgative principle of anthroquinone (http://www.holistic-herbal.com/terminalia -chebula -1.html). Because T. chebula's ethanolic

and aqueous extracts both had antibacterial properties. It was assumed that T. chebula's high tannin content may be the cause of its antibacterial action. Consequently, T. chebula has been demonstrated in this work to possess broad-spectrum antibacterial activity, suggesting that it may be a more effective antibacterial agent than other plant materials. Its antibacterial properties may be attributed to the high tannin content found in it. This highlights the value of using an ethnomedical approach as a possible source of bioactive compounds to treat infectious disorders brought on by these harmful microbes.

CONCLUSION

New antimicrobials from plant extracts have been discovered via scientific study of medicinal plants. The study found that the broad spectrum antimicrobial properties and strong antimicrobial activity of the crude extracts of Woodfordia fructicosa, Sphaeranthus indicus, Butea monosperma, Acacia leucopholia, and Maytenus emerginata support their traditional use as a treatment for enteric bacterial infections. This likely explains why the native people have been using these herbs for generations to treat various illnesses.

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