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Study on Antimicrobial Activity of Chromolaena Odorata and Aerva Lanata

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ABSTRACT

Purpose: Numerous natural products from traditional medicinal plants have extensive chances for new steers to drug development because of the obtainability of chemical diverseness. Advanced modern methods are developed for the extraction, which are pointed to be the critical move in formulation of plants. These latest approaches of extraction are more effectual than the antient extraction methods for the effective development of traditional herbal remedies. For the greater extraction and study of medicinal plants, scientists prefer modern sample- preparation techniques over conventional techniques as they ensure the better-quality herbal products to the worldwide consumers. The interest in plant based therapeutic drugs and comestible plants has grown all over the World, since the increase in stipulation for chemical diverseness in investigation programs.

Design/Methodology/Approach: The study examines how different plant extract concentrations and extraction solvents affect the antimicrobial activity of several therapeutic plants. The traditional mechanical method is used for extraction. Chromolaena odorata and Aerva lanata were the medicinal plants chosen for the extraction. Different concentrations of the extracts were made such as 1%, 10% and 50%. Diethyl ether, sterile distilled water and ethanol are the solvents used for extraction. Two common techniques were used to observe whether the selected plants have any antimicrobial activity against the organisms, which were agar disc diffusion and well diffusion method. Klebsiella pneumonia, Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus Staphylococcus aureus were the microorganisms selected for this study.

Findings/Result: Among the selected extracts, aqueous extract gave more activity against infectious microbes. C. odorata showed better reaction with S. aureus, K. pneumonia and B. subtilis, were as A. lanata found well reactive against P. aeruginosa, S. aureus and K. pneumonia.

Originality/Value: This study gives us an outline about the antimicrobial activity of C. orodata and A. lanata on various infectious microbes, based on the collected data.

Paper type: Case study based research approach.

Keywords: Chromolaena odorata, Aerva lanata, antimicrobial activity, Standardized extraction.

1. INTRODUCTION

Plants are more probable to be exposed to many microbes like bacteria, virus and fungus throughout their life time. Some of them create relationships that are advantageous to plants, but many plant-associated microorganisms are diseases that interfere with plant's normal growth, development, reproduction and

yield. Plants have an intrinsic immunity that involves multiple layers of defense response to stop the spread of disease.

According to WHO (World Health Organization) data, more than 80% of the World's population still relies on traditional plant based medications and also use medicinal plants for basic health care practices. In Asia, medicinal plants have been utilized for hundreds of years, suggesting a long history of human-environment interaction. Traditional plant-based treatment contains a variety of chemicals that can treat both deadly infections and chronic health disorders [1]. People discovered hundreds of phytochemicals from plants that are safe and broadly effective in response to the side effects and microbial resistance caused by chemically synthesized medications. Several known medically advantageous properties of such phytochemicals including their anti-microbial, anti-cancer, anti-oxidant, analgesic, anti-diarrheal and even having the ability to heal wounds [2]. Yet, to prove a bioactive compound's efficacy and confirm the attributes asserted by our traditions, clinical experimentations are a must. Understanding the pharmacokinetics, bioavailability, effectiveness, safety and medication interaction of newly created biologically active compounds is aided through clinical studies [3].

Chromolaena odorata is a multi-stemmed shrub, which can reach a height of 10 meters when grown as climbing vegetations and 2.5 meters when grown in the open. It is a neotropical shrub which is widely distributed to different tropics. Distributed areas, grass lands, fallows and forest plantings all exhibits pure strand formation. As they are having efficient short and long-distance dispersal ability, they can spread rapidly. The ability of sexual reproduction is attained by 1 year. There are 70 insect- pollinated flowers at the terminal cymes. It will take almost a month to their small fruits to get matured and they weigh nearly 0.2mg only. Fruits are normally disseminated by wind since they must be released in dry, windy conditions; however, because of the tiny hooks on the fruit, animals can also help to disperse the fruits. Seed germination takes place at the beginning of the rainy season as water and light is favorable. Shoot rooting can happen once the shoot touches the ground. To ensure the survival of plants in the event of a fire, a drought or mechanical harm, through coppicing, the plant process specific underground 'organ'. The whole plant poses medicinal values and they can be used for the treatment of burns, hemorrhages, hemorrhoids, indigestion, skin disease, traumatic injury, edema, fracture and infection [4].

Aerva lanata is a woody, succulent or prostrate perennial plant that belongs to the Amaranthaceae family and they contain tap roots. In most of the time they start flowering in the first year itself. They are a typical weed that may be seen grows wild, practically anywhere in India's plains. Their roots are having camphor like aroma. The whole plant is edible, especially leaves. Their leaves can be used in soup or can be taken as spinach or simply a usual vegetable. Numerous research in the field of pharmacology reveals a variety of uses, including, diuretic, hypoglycemic, anti-inflammatory, anti-diabetic, antimicrobial, antiparasitic, hepatoprotective, anti-asthmatic, anti-urolithiatic and hypolipidemic qualities [5].

2. OBJECTIVES OF THE CASE STUDY

- To study antimicrobial activity of *Chromolaena odorata and Aerva lanata.*
- To compare the antimicrobial activity on different concentrates of extracts.
- To understand the variation of activity on varying the solvent for extraction.
- To analyze the extraction power of different solvents for secondary metabolites.
- To assess the effectiveness of these plants against various pathogenic microorganism.
- To compare the efficiency of both disc and well diffusion methods in determining the antimicrobial activity.

3. RELATED WORKS

Long before microbes were identified, there was wide spread belief that certain plants had healing qualities and contained chemicals that are today recognized as antimicrobial principles. Since the beginning of time, man kind have employed many herbs to cure common illness. Many of these ancient medications are still used routinely to treat various illness.

Table 1: Review on antimicrobial activity of medicinal plants

Sl. No.	Work	Focus	Reference
1	Identification of secondary plant metabolites	Use of metabolites in drugs	Farnsworth et al. (1985) [6]
2	Phytoanticipin	Antimicrobial compounds in plants	VanEtten et al. (1994) [7]
3	Biotechnological weapons against pathogens	Phytoanticipin	Bednarek P et al. (2009) [8]
4	Proposed diffusion method	Study on plant extract and essential oil	R`ios et al. (1988) [9]
5	Antimicrobial study of essential oil	Characteristics of complex mixtures	Janssen et al. (1987) [10]
6	Antimicrobial and antifungal activity of essential oil	Agar diffusion methods	Kalemba, Kunicka (2003) [11]
7	Antimicrobial activity of folk medicines	Plant extracts	Ngwendson et al. (2003) [12]
8	Study on essential oils	Antimicrobial activity	Alma et al. (2003) [13]
9	Study on plant alkaloids	Antimicrobial activity	Klausmeyer et al. (2004) [14]
10	Study on plant flavonoids	Antimicrobial activity	Sohn et al. (2004) [15]
11	Study on plant sesquiterpene lactones	Antimicrobial activity	Lin et al. (2003) [16]
12	Study on plant diterpenes	Antimicrobial activity	El-Seedi et al. (2002) [17]
13	Study on plant triterpenes	Antimicrobial activity	Katerere et al. (2003) [18]
14	Study on plant naphtoquinones	Antimicrobial activity	Machado et al. (2003) [19]
15	Activity of spices on human pathogenic bacteria and yeast	Antimicrobial activity	Arora, Kaur (1999) [20]

4. METHODOLOGY

This study consists of isolation of infectious microbes, extraction of medicinal plants using traditional mechanical method and the antimicrobial activity is studies using agar diffusion methods. The efficiency of both disc and well diffusion method is verified.

a. Collection of infectious microorganisms

Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis and *Klebsiella pneumonia* were carefully collected from Karothukuzhy Hospital, Aluva. The organisms were kept on agar slants at 40^{o}C and subcultured every two weeks. Identity of each organism were confirmed using biochemical tests.

b. Extraction of medicinal plants

The plants selected were *Chromolaena odorata* and *Aerva lanata.* The extracts were prepared in 50%, 10% and 1% concentration of different solvents like ethanol, diethyl ether and sterile distilled water, using traditional mechanical method. The content in the tube were adjusted at 60^{0}C temperature for 3hours. This treatment allows the extraction of secondary metabolites to a certain extent. After extraction, the content is filtered using muslin/ cheese cloth.

c. Preparation of filter paper disc

The disc for diffusion technique was prepared by using Whatsman No: 1 filter paper and its diameter was 6mm each. The discs were sterilized by autoclaving at 151lbs for 10minutes.

d. Diffusion method

Both disc diffusion and well diffusion methods were used in this study to compare the level of diffusion and activity in both techniques.

4.4.1 Agar well diffusion method

Lawn culture of organisms were prepared by using sterile swabs soaked in peptone water or nutrient broth culture of the organism on a dry nutrient agar plate. 4mm wells were punched out of the dry agar using a well puncture and 10µl of extracts of both the plants in different concentration were added.

4.4.2 Agar disc diffusion method

Each organism was inoculated to peptone water and Lawn cultures were made by soaking a sterile swab in the inoculated peptone water and spread evenly over the dry petri plates with nutrient agar. All of the extracts were impregnated in various quantities on sterile filter paper discs. These discs were finally placed on the inoculated nutrient agar plated with even spacing.

5. ANALYSIS OF ANTIMICOBIAL ACTIVITY OF *Chromolaena odorata*

The extract of *C. odorata* showed antimicrobial activity against S. aureus, K. pneumonia and *B. subtilis*. The extract in sterile distilled water showed better activity that other solvents, against certain organisms varying in the level of activity among different concentrates and methods. Additionally, it was shown that the action against the organism improved with increasing extract concentration. Both diffusion methods revealed essentially comparable responses.

Table 2: Analysis of antimicrobial activity of *C. odorata*

Solvent	Bacteria	Concentrate	Zone of inhibition (cm)	
			Disc diffusion	Well diffusion
Sterile	P. aeruginosa	50%	-	-
		10%	-	-
		1%	-	-
	S. aureus	50%	1.9	-
		10%	-	2.1

distilled water		1%	-	-
	K. pneumoniae	50%	2.1	1
		10%	-	-
		1%	2.2	1.8
	B. subtilis	50%	-	-
		10%	-	-
		1%	-	-
Diethyl ether	P. aeruginosa	50%	-	-
		10%	-	-
		1%	-	-
	S. aureus	50%	-	-
		10%	-	-
		1%	-	-
	K. pneumoniae	50%	-	-
		10%	-	-
		1%	-	-
	B. subtilis	50%	-	-
		10%	-	-
		1%	-	-
Ethanol	P. aeruginosa	50%	-	-
		10%	-	-
		1%	-	-
	S. aureus	50%	-	-
		10%	2.5	1
		1%	-	-
	K. pneumoniae	50%	-	-
		10%	-	-
		1%	-	-
	B. subtilis	50%	2.1	1
		10%	-	-
		1%	-	-

6. ANALYSIS OF ANTIMICROBIAL ACTIVITY OF *Aerva lanata*

The extract of *A. lanata showed activity against P. aeruginosa, S. aureus* and *K. pneumonia.* All the three extracts made from different solvents showed well defined results for certain organisms, but the activity in aqueous extract was giving better result. The outcomes from the two diffusion techniques did not significantly differ.

Table 3: Analysis of antimicrobial activity of *A. lanata*

Solvent	Bacteria	Concentrate	Zone of inhibition (cm)	
			Disc diffusion	Well diffusion
	P. aeruginosa	50%	-	-
		10%	-	-

Sterile distilled water		1%	-	-
	S. aureus	50%	-	-
		10%	-	-
		1%	2.3	2.5
	K. pneumoniae	50%	1.6	1.5
		10%	2.5	2
		1%	-	-
	B. subtilis	50%	-	-
		10%	-	-
		1%	-	-
Diethyl ether	P. aeruginosa	50%	1.9	2.9
		10%	1.2	3.3
		1%	-	0.9
	S. aureus	50%	-	-
		10%	-	-
		1%	-	-
	K. pneumoniae	50%	-	-
		10%	-	-
		1%	-	-
	B. subtilis	50%	-	-
		10%	-	-
		1%	-	-
Ethanol	P. aeruginosa	50%	-	-
		10%	-	-
		1%	-	-
	S. aureus	50%	3.5	2.3
		10%	2.5	2.1
		1%	-	-
	K. pneumoniae	50%	-	-
		10%	-	-
		1%	-	-
	B. subtilis	50%	-	-
		10%	-	-
		1%	-	-

7. COMPARISON OF EFFICIENCY OF DIFFUSION METHODS

In this investigation, there was no discernible difference between the effectiveness of the disc and well diffusion methods. The activities shown in both the methods were almost similar.

However, there was a minor variation in the inhibition zone's size and clarity. Disc diffusion method shown more rapid result as compared to the other method. The variation in diffusion might be the result of some outside forces that are having an impact on the activity. Ehen the disc diffusion technique was compared to well diffusion, the antibacterial activity of both medicinal herb was shown to be more firmly

supported. The comparative efficiency is as follows.

Table 4: Comparison of efficiency of diffusion methods (*C. odorata*)

Solvent	Organism	Activity	
		Disc	Well
Sterile distilled water	P. aeruginosa	×	×
	S. aureus	√	×
	K. pneumoniae	√	√
	B. subtilis	×	×
Diethyl ether	P. aeruginosa	×	×
	S. aureus	×	×
	K. pneumoniae	×	×
	B. subtilis	×	×
Ethanol	P. aeruginosa	×	×
	S. aureus	√	√
	K. pneumoniae	×	×
	B. subtilis	√	√

Table 5: Comparison of efficiency of diffusion methods (*A. lanata*)

Solvent	Organism	Activity	
		Disc	Well
Sterile distilled water	P. aeruginosa	×	×
	S. aureus	√	√
	K. pneumoniae	√	√
	B. subtilis	×	×
Diethyl ether	P. aeruginosa	√	√
	S. aureus	×	×
	K. pneumoniae	×	×
	B. subtilis	×	×
Ethanol	P. aeruginosa	×	×
	S. aureus	√	√
	K. pneumoniae	×	×
	B. subtilis	×	×

8. FACTORS AFFECTING THE SIZE OF INHIBITION ZONE

In both the diffusion methods, the size of inhibition zone can be affected by many external factors. Some of them are:

- The bacterial concentration of inoculum
- The type of test medium utilized for the procedure
- Difference in the biochemical structure of the compounds extracted
- pH of both the medium and the extracts
- Subjective error in finding the edge of zone
- Depth of the selected medium

- Metabolic activity of bacteria
- Length and condition of the incubation
- Presence of other compounds [21]

9. CLINICAL MANIFESTATION OF SELECTED ORGANISM

Staphylococcus aureus are found in human respiratory system and on the skin surfaces. *S. aureus* may cause a variety of respiratory illnesses as well as food poisoning, but it can also cause moderate skin infections such as pimples, impetigo, boils, cellulitis folliculitis, carbuncles, scaling skin syndrome and abscesses. Sometimes they may even cause life-threatening issues like pneumonia, osteomyelitis, meningitis, endocarditis, bacteremia, toxic shock syndrome and sepsis.

Bacillus subtilis is commonly found bacteria in soil, more evidence suggests the presence in normal gut commensal in humans. They rarely cause food poisoning.

Pseudomonas aeruginosa are common disease-causing bacteria in animals and humans. In animals, they may cause generalized inflammation and sepsis in those with reduced immunity or having infect damaged tissues. If colonization occurs in any critical body organs, mainly lungs, kidney, liver, urinary tract, then the result will be fatal.

Klebsiella pneumonia has been discovered in the typical flora of the mouth, skin and gut. In addition to pneumonia, urinary tract infection, thrombophlebitis, cholecystitis, meningitis, inflammation and hemorrhage in the human lungs and even cell death that occasionally result in a thick, bloody, mucoid sputum. They can also cause bacteremia and septicemia.

10. CONCLUSION

Based on the study conducted on two different medicinal plants, we have reached the following conclusions:

- Both the selected plants showed visible antimicrobial activity for the selected organisms.
- Extraction performed with sterile distilled water proved to be more active in solubilizing the secondary metabolites, providing better support for highlighting the antimicrobial activity of medicinal plants.
- A better supportive result was showed on disc diffusion method when compared to well diffusion method.
- The current study indicates that *C. odorata* and *A. lanata* are potent inhibitors of pathogens like P. aeruginosa, *B. subtilis*, *S. aureus* and *K. pneumonia.*
- Both aqueous and ethanolic extracts and to a certain extent ether extract, can be used as a mean for treating the disease associated with the organisms taken for the study.
- Both the plants can be included in the list of herbal medicines due to their high therapeutic purpose and can therefore use as a very safe alternative medicine.

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