MUNTINGIA CALABURA – ANTIBACTERIAL AND ANTIFUNGAL STUDY OF ISOLATED PHARMACEUTICALLY ACTIVE COMPOUNDS

Abstract

The Isolated Muntingia calabura's active fractions, which demonstrated greater antibacterial activity. Muntingia calabura stem bark extracts in methanol and ethyl acetate had the strongest antibacterial activity of all the extracts. Using silica gel column chromatography (100–200), bioassay guided fractionation of methanol and ethyl acetate extracts obtained from Muntingia calabura hard wood stem was carried out to identify the active fractions and remove the non-active fractions. Different ratios of hexane and ethyl acetate were utilized as eluent.

Keywords: Muntingia Calabura, Isolation, Antimicrobial activity

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I. INTRODUCTION

As a result of bacteria developing resistance to a number of modern antibiotic medications, health issues resulting from the chemotherapy treatment of infectious diseases increase exponentially. For this reason, there has been a great deal of research done on finding novel antimicrobial compounds from medicinal plants that can be used to limit the pathogenicity of microbes. The creation of new antimicrobial compounds with the ability to address issues caused by microbes is still of utmost interest to scientists worldwide. Research on native plants is quite powerful in terms of creating natural medications, particularly effective antibiotics [1].

Antibiotics typically show an inhibitory mechanism of the cell synthesis, preventing cells from proliferating and ultimately leading to cell death. [2]. The main causes of the emergence of bacterial resistance to various antibiotics are genetic mutations and the subsequent selection of resistant mutants that are abundant in soil, plants, animals, and humans [3]. Research has indicated a growing interest in plants as a potential source of new medications due to obstacles posed by the emergence of multi-resistant drug organisms and the negative effects of contemporary treatments [4].

Antibiotics have been a key component in the fight against infectious diseases caused by bacteria and other microbes for the past sixty years. Antimicrobial chemotherapy has significantly increased in popularity. The twentieth-century average life expectancy. Despite this, the rise of pathogenic germs that are resistant to antibiotic treatment has led to serious health issues such as septicemia, pneumonia, gonorrhea, wound infections, and ear infections in children. The ways that bacteria have evolved to withstand antibiotics are diverse. These days, over 70% of the bacterial types that cause acquired infections in hospitals have at least demonstrated resistance to some of the first-line antibiotics.

In order to have a supply of potent medications on hand, ongoing research and development of novel antibiotics has replaced the microbial evolution of resistance and financial incentives. There might be a gene pool in nature, specifically for antibiotic resistance in bacteria. The majority of bacteria that are thought to manufacture a certain antibiotic show signs of resistance to that antibiotic as well.

Reviewing the data revealed that some staphylococci strains had developed resistance to penicillin very away, following the same pattern of resistance to chloramphenicol, streptomycin, and tetracycline that had been noted in 1946. The same situation has repeatedly occurred over time: well-known bacterial pathogenic strains have developed resistance to one or more clinically used antibiotics.

A growing body of research has revealed the mechanisms underlying bacterial resistance, including the exchange of drug-resistant genes across strains and within the species. For instance, Staphylococci's antibiotic-resistant genes are carried on plasmids, which may have successfully transferred horizontal gene transfer (HGT) to other bacterial strains like Streptococcus, Bacillus, and Enterococcus in the form of DNA segments or transposons. Numerous mechanisms behind the exponential increase in antibiotic resistance have been identified. These techniques could involve changing the antibiotic's chemical

composition or rendering it inactive, which would decrease its affinity for ribosome attachment and stop the creation of proteins in bacteria.

In addition to medication resistance in bacteria, there appears to have been a rise in antifungal resistance during the last ten years [5]. There are a number of causes for this resistance, however the research on fungal resistance received very little attention [6]. The introduction of persistent immune-suppressive diseases like candidiasis etc., among other things, as well as the regular and frequently careless use of broad spectrum antibiotics in the hosting of intravenous devices are the main causes of the immune system's resistance to a wide variety of antibiotics.[7]. The need for safe, more effective, reasonably priced medications that can treat significant fungal infections has increased as a result of these advancements and the ensuing rise in fungal infections.

Table 1: Examples of method of resistance towards various types of antibiotics

Antibiotic	Method of resistance
β-lactams, Chloramphenicol, Aminoglycosides	Enzymatic alteration or breakage to render an antibiotic compound inactive
Lincomycin, β-lactams, Erythromycin,	Lowers or eliminates the antibiotic's affinity to the target cell
Chloramphenicol	Less absorption into the cell
Tetracycline	Cellular efflux that is active
Trimethoprim , Sulfonamides	Metabolic bypass of repressed response
Sulfonamides, Trimethoprim	Target overproduction of antibiotics (titration)

Worldwide approximately, 130 drugs extracted from higher plants or modified further synthetically are currently in use[8]. Clinically, important pathogens fetch the interest of scientists to carry on research and development programs for the innovation of newer broad spectrum antimicrobial agents due to its multi drug resistant property[9]. Some, other factors which can be considered for the discovery of new drugs are the less availability and high cost of new generation antibiotics necessitates which were seek for the substances from alternative medicine that are claimed antimicrobial activity.

Numerous studies on the screening of medicinal plants for antibacterial properties have been published. [10]. Folk remedies from Nigeria and the plants that make them up are said to have antibacterial properties [11]. Moskalenko [12] presumably tested ethnomedicinal plants from the Far East to determine their antibacterial efficacy. In addition, a number of preparations from medicinal plants that have demonstrated strong antibacterial action have also been documented in various traditional literatures. [13].

The medicinal herbs of Indonesia exhibit antibacterial properties against several tested microbial species. Certain Turkish medicinal plants have reportedly shown notable antibacterial activity against various strains and types of Streptococcus species [14].

II. MATERIALS AND METHODS

1. Antimicrobial Studies

- **Preparation of Sample/Test Solution for Antimicrobial Activity:** Each isolated component from various plant parts was synthesized at a concentration of 200 mg/ml in DMSO, which had no effect on the proliferation of microorganisms.

- **Microorganisms Used:** The antibacterial properties of isolated chemicals and plant extracts were tested using the following strains of Gram-positive and Gram-negative microorganisms. These cultural traditions were acquired from many origins and kept up to date through appropriate media.

2. **Bacterial Cultures/ Test Organisms:** A portion of the cultures are received from the Department of Microbiology at Kakatiya University in Warangal, Telangana, India, and the Microbial Type Culture Collection Center at the Institute of Microbial Technology (IMETCH, Chandigrah).

   Micrococus luteus KUCC 09
   Bacillus cereus KUCC 23
   Bacillus subtilis KUCC 17
   Escherichia coli KUCC 03

   - **Human Pathogens:**
     Klebsiella pneumoniae KUCC 11
     Proteus vulgaris KUCC 21
     Salmonella paratyphi A KUCC 18
     Staphylococcus aureus ATCC

     It was from Kakatiya Medical College (KMC), in Warangal, Telangana, India, where these bacterial samples were acquired.

   - **Fungal Species:** The antifungal activity of the isolated compounds was evaluated against the following dermatophytes and fungus species. These were acquired from the Fungal Culture Collection Laboratory, Department of Microbiology, Kakatiya University, Warangal, Telangana, and Kakatiya Medical College, Warangal, Telangana.

     Candida albicns KUCC 23
     Microsporum gypscum KUCC 31
     Aspergillus fumigates KUCC 25
     Aspergillus flavus KUCC 24
     Aspergillus niger KUCC 29

3. **Media Used for the Assay:** Various specialized growth media were employed to cultivate the microorganisms.

- **Media for Bacterial Cultures**

  - **Nutrient Agar (NA)**

Peptone	5.00g
Beef extract	3.00g
Sodium chloride	5.00g
Agar	20.00g
Distilled water	1000 ml

  - **Nutient Broth (NB)**

Peptone	5.00g
Beef extract	3.00g
Sodium chloride	5.00g
Distilled water	1000 ml

  - **Double Strength Nutrient Agar (DsNA)**

Peptone	10.00g
Beef extract	10.00g
Sodium chloride	5.00g
Agar	20.00g
Distilled water	1000 ml

- **Media for Fungal Cultures**

  - **Sabourad's Dextrose Agar Medium** (SDA)

Peptone	10.00g
Dextrose	40.00g
Agar	20.00g
Distilled water	1000 ml
pH	6.8

  - **Asthana and Hawker's Medium** (AH)

Glucose	5.00g
KNO_3	3.50g
KH_2po_4	1.75g
$Mgso_4$	0.75g
Agar	20.00g
Distilled water	1000 ml

    A medium without agar was employed as broth medium.

III. CULTIVATION TECHNIQUES

1. **Slant Preparation:** After 10 ml of aliquots of molten material were dispensed into 30 ml test tubes and sanitized, agar slants were created. Test tubes were held at a 30 degree angle and given time to settle.

2. **Plate Preparation:** Twenty milliliter aliquots of sterile molten media were sterilised petridishes using sterile method. The plates were utilized for the assay after they solidified. set up

3. **Sub- Culturing:** By moving a loopful of inoculums from culture slants to recently prepared agar slants, subcultures were created. The ideal circumstances for incubation were met with these. Fungi: Asthana and Hawker's medium, room temperature, 5-7 days; Bacteria: double strength nutritional agar, 37^0C for 24 hours; Dermatophytes: Sabourad's Dextrose Agar media, 22^0C for 5-7 days in darkness.

IV. ANTIBACTERIAL ACTIVITY

1. **Preparation of Inoculums:** One to five well separated colonies were placed in five milliliters of nutritional broth. For 24 hours, the broth cultures are incubated at 37^0C until a faint, perceptible turbidity appears. Half of the MC Farland standard is achieved by adjusting the turbidity of broth cultures (1x108 to 5x108 cfu/ml). This served as the assay's starting culture.

2. **Antimicrobial Assay by Well Diffusion Method:** The well diffusion method or the agar cup plate technique were employed to carry out the antimicrobial assay.

3. **Agar Cup Plate Method:** Using a sterilized glass spreader, a standardized value of 1 to 2 x 107 cfu/ml, or 0.5 MC Farland standard of bacterial culture, was applied to the surface of a nutrient agar media plate. With a sterile cork borer, the 8mm wells were bored. Isolated compounds containing 50, 100, and 150 µg/mL were added to the wells. For roughly twenty-four hours, the plates were incubated aerobically at 37^0C. The inhibitory zones were measured with a ruler. Comparisons were made between the outcomes and the positive and negative control wells, which contained 10 mg/ml of streptomycin and just DMSO, respectively.

V. ANTIFUNGAL ACTIVITY

1. **Preparation of Spore Suspension:** Spores were taken from the fresh cultures and placed in a test tube with sterile distilled water (fungi) and dextrose broth (dermatophytes) from Sabourad. Using the spore suspension that was therefore produced, antifungal activity was evaluated.

2. **Antifungal Assay:** The agar well diffusion method was used to screen the isolated compounds for their antifungal properties. Test organisms were added to the nutrient agar medium plates for inoculation. After letting the plates set, sterile cork borer holes measuring 7 mm were punched into them. Next, 0.05 ml to 50 µl of the test compounds are added to the wells. For 72 hours, plates were incubated at 30^0C. Measurements and records were made of the inhibitory zones.

3. **Evaluation of Antimicrobial Activity:** Studies on the antibacterial (Table 1.2) and antifungal (Table 1.3) properties of isolated compounds from Muntingia calabura extracts have been focused on in relation to the bacterial and fungal resistance displayed by human pathogenic organisms.

Table 2: Antimicrobial properties of flavones derived from extracts of Muntingia calabura

Bacteria	Compound-1			Compound-2			Compound-3			Compound-4			Streptomycin		
	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150
Gram negative															
Klebsiella pneumonia	08	14	18	07	12	15	06	11	14	07	10	15	10	12	24
Proteus vulgaris	10	18	25	10	16	20	08	12	15	10	14	19	12	13	26
Salmeonella paratyphi	--	--		--	--		--	--	--	--	--	--	08	10	22
Escherichia coli	12	19	26	10	15	22	07	11	14	08	13	17	12	14	28
Gram positive															
Micrococcus luteus	10	16	20	08	13	17	06	10	13	07	12	16	09	12	23
Bacillus subtilis	08	15	22	08	12	18	06	11	14	08	13	16	10	13	25
Bacillus cereus	07	15	20	08	13	17	09	11	13	10	12	15	10	13	20
Staphylococcus aureus	08	14	19	07	12	15	07	10	12	08	11	14	09	12	22

Compound concentrations in µg/mL, Compound-1: 5,8-dihydroxy-6,7,4′trimethoxy flavones**, Compound-2**: 6,4′ dihydroxy 3′ propen chalcone, **Compound-3:** 7-(alloxy)-2-Phenyl-4H-Chro men-4one, **Compound-4:** 7-hydroxy-4-oxo-2-phenyl-4H-chromen-8-carbaldehyde

Table 3: Antifungal activity of flavones derived from extracts of Muntingia calabura

Fungi	Compound-1			Compound-2			Compound-3			Compound-4			Nystatin		
	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150
Dermatophytes															
Candida albicans	03	08	13	02	05	07	01	02	02	07	09	12	10	14	21
Microsporum gypscum	02	05	09	02	04	07	02	02	04	05	08	10	09	13	18
Aspergillus fumigatus	--	--		--	--		--	--	--	--	--	--	08	13	16
Aspergillus flavus	05	09	14	04	07	11	03	05	07	04	07	10	09	10	24
Aspergillus niger	04	07	12	06	08	11	04	05	07	05	08	11	07	12	26

Compound concentrations in µg/mL, Compound-1: 5,8-dihydroxy-6,7,4′trimethoxy flavones**, Compound-2**: 6,4′ dihydroxy 3′ propen chalcone, **Compound-3:** 7-(alloxy)-2-Phenyl-4H-Chromen-4one, **Compound-4:** 7-hydroxy-4-oxo-2-phenyl-4H-chromen-8-carbaldehyde

VI. RESULTS AND DISCUSSION

The compounds' antibacterial activity that was discovered after isolation. None of the substances were able to stop Salmonella typhi from growing. Out of all the compounds that were evaluated, compound 1 exhibited the highest level of antibacterial activity, followed by compound 2. Compounds 1 and 2 exhibited the maximum zone of inhibitions (25, 26, and 20–22) at 150 µg/mL when applied to Gram-positive strains of Proteus valgaris and E. coli, respectively. However, compounds 1 and 2 also prevented Bacillus subtilis, Bacillus cereus, and Micrococus leutus from growing. Compounds 1 and 2 have a high zone of inhibition against Bacillus subtilis, Bacillus cereus, and Micrococcus leutus of 20, 22, 20, and 17, 18, 17, respectively, at 150 µg/mL. The results are shown in table 1.2. It was also discovered that compounds 1 and 2 were connected to the hyperfungal response. The results are shown in table 1.3. The results of the antifungal and antibacterial activity were compared with the established benchmarks of nystatin at 10 µg/mL and streptomycin.

All of the chemicals demonstrated antibacterial action in a concentrated, dependent manner, in the end. All of the substances are required for additional research, including clinical trials, in order to be used as antibacterial and antifungal medications.

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