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Research Article

A Study on Antimicrobial Activity of Endophytic Fungi from *Garcinia mangostana* L.

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Abstract

Mangosteen (*Garcinia mangostana* L) is a tropical fruit plant which is native to Asia, Australia, Africa and Polynesia which is endowed with potentially beneficial properties and so called as super-fruit. Mangosteen plant is widely used as antibacterial, anti-viral, anti-fungal, anti-inflammatory, antioxidant, anti-allergic and anti-malarial agent, hence it posses those properties. It is an ideal source of endophytic fungi. Endophytes are the microorganisms which form symbiotic association with the host plant. They are often bacteria and fungi that within the plants without causing apparent harm to the host plant. Endophytic fungi have proven to be a source of secondary metabolites and several extracellular enzymes such as amylase, lipase, and protease. Fungal endophytes residing within these plants could also produce metabolites similar to or with more activity than that of their respective host do. These fungal enzymes as well as fungal biomass can be used for detoxification or in the bioremediation of industrial and agricultural waste and other polluting compounds. The production of enzymes from endophytic fungi for commercial use is an unexplored field. In the present work nine different types of endophytic fungi were isolated. Isolation and study of cultural characters of endophytic fungi were done using sabouraud dextrose agar, potato dextrose agar and malt extract agar and microscopic study has done using lactophenol cotton blue staining technique. By using Mueller Hinton agar medium antimicrobial activity checked, ENF1, ENF2, ENF3, ENF4 and ENF7 showed antimicrobial activity.

Keywords: *Mangosteen; Endophytic Fungi; Antimicrobial Activity; Fungal Isolate; Antimicrobial Activity*

Abbreviations

%: Percentage; *et al*: et allia; g: Gram; ml: Milliliter; SDA: Sabouraud Dextrose Agar; MEA: Malt Extract Agar; PDA: Potato Dextrose Agar; LPCB: Lactophenol Cotton Blue; MHA: Mueller Hinton Agar; ENF 1: Endophytic Fungi 1; ENF 2: Endophytic Fungi 2; ENF 3: Endophytic Fungi 3; ENF 4: Endophytic Fungi 4; ENF 5: Endophytic Fungi 5; ENF 6: Endophytic Fungi 6; ENF 7: Endophytic Fungi 7; ENF 8: Endophytic Fungi 8; ENF 9: Endophytic Fungi 9; cm: Centimetre; sps: Species; °C: Degree Celsius; °F: Degree Fahrenheit; α: Alpha; µl: Microlitre; µg: Microgram; min: Minute; s: Second

Introduction

Endophytes are microorganisms that are present in living tissues of various plants, establishing mutual relationship without causing any symptoms of disease [7]. The word endophyte means 'in the plant'. Endophytic microbes such as bacteria and fungi are known to be able to associate with plant tissue. Endophytic fungi grow in a very intimate interaction with their host plant cells. This growth pattern indicates that fungal hyphae are substantially attached to plant's (host) cell wall, but don't invade plant cells. Endophytic fungal hyphae appear to be grown within the intercellular spaces of the plant tissue, with a growth rate as same as their host. One or more endophytic organisms are found in nearly every ter-

restrial plant. Some endophytes may enhance host growth, nutrient acquisition and improve plants ability to tolerate abiotic stress such as drought and decrease biotic stress by enhancing plant resistance to insects, pathogens and herbivores as they produce secondary metabolites and they are potential resource for various kinds of secondary metabolites which have potential bioactive properties. Some of the bioactive compounds produced by endophytes inhabit in various plant species can be used in agricultural, pharmaceutical and food industries since they have been reported to have antimicrobial activities and can also act as enzymes [5].

In general, the outcome of this interaction relies on the environmental factors as well as genotype of both the host plant and the interacting microorganism. The ability of endophytes to enter and thrive in host tissue makes them unique, showing multidimensional interaction within the host. Several activities of host have been influenced by the presence of endophytes. Endophytic fungi also have the ability to produce the same active compounds produced by the host plants. Because of that, endophytic microbes can be used in search for new source of antimicrobial drugs.

In the current scenario the occurrence of number of infectious disease is very high, for example as a result of un-proper handling of drinking water system, like underground piping etc can accounts for the occurrence of coliform in water. *E.coli* can lead, mild to serious infections of the digestive tract. One of the serious infections caused by *E.coli* is diarrhea due to inflammatory processes and cytotoxin invasion in colonic manifestations dysentery syndrome with diarrhea with mucus and blood. Disease caused by such infection is generally treated with synthetic drugs. Antibiotics are synthetic drugs used to treat infectious diseases. However, improper use of antibiotics will hasten the development of resistant germs that cause infections. So it is necessary to find an alternative safer treatment with mild side effects. Therefore it is believed that search for novel compounds should be directed towards plants that commonly serve indigenous population for medicinal purpose as they are expected to harbour novel endophytes that may produce unique metabolites with diversified applications [7].

Garcinia mangostana L belongs to family Clusiaceae, commonly known as Mangosteen. It is a tropical fruit plant which is native to Asia, Australia, Africa and Polynesia which is endowed with potentially beneficial properties and so called as super-fruit [4]. Mangosteen is generally grown in consistently warm conditions, as exposure to temperatures below 0°C (32°F) for prolonged periods will usually kill a mature plant. They are known to recover from brief cold spells rather well, often with damage only to young growth. Experienced horticulturists have grown this species outdoors, and brought them to fruit in extreme south Florida. The tree grows from 6 to 25metres tall. The juvenile mangosteen fruit, which does not require fertilization. First appears as pale green or almost white. As the fruit enlarges over the next two to three months, the color of exocarp deepens to darker green. During this period, the fruit increases in size until its exocarp became 6–8cm in outside diameter, remaining hard until a final, abrupt ripening stage. The subsurface chemistry of the mangosteen exocarp comprises an array of polyphenols, including xanthones and tannins that assure astringency which discourages infestation by insects, fungi, plant viruses, bacteria and animal predation while the fruit is immature [2].

The entire parts of mangosteen tree such as, hull, bark, fruit and leaves have been used as traditional medicine. It is used as an antibacterial agent since old-age times. Mangosteen plant's leaves and bark have been used in oral care in some African countries, as chew sticks and an astringent [4]. In Asian countries mangosteen has been used in traditional medicine for treatment of various diseases such as skin infection, dysentery, diarrhea, and cholera [6]. Previous phyto-chemical studies reported that mangosteen contains secondary metabolites [3]. *Garcinia mangostana* has recently received a lot of attention because of its medicinal value as well as its potential bioactive properties. It has been reported that fungal endophytes residing within part of plants could also produce metabolites similar to or with more activity than that of their respective hosts [7].

Medicinal plants are considered pharmaceutical agents since it is important for the development of drugs. According to WHO, herbal medicine and traditional medicine are utilized by around 80% of the world's population in developing nations, for primary health care. Various research works have shown that, fungi have the ability to produce industrial enzymes, antimicrobial agents. Several studies indicate that the extracellular enzymes such as amylase, pectinase, cellulase and protease are produced by endophytic fungi as a strategy for the resistance to infections and getting sustenance from the host [11].

The current study aims to isolate and identify fungal endophytes from leaves of *G.mangostana* to study their diversity and to

detect their antimicrobial activity against human pathogens. This can be considered as a new source of antimicrobial agents that can be used in the industrial as well as pharmaceutical applications.

Materials and Methods

Isolation and characterization of endophytic fungi from mangosteen leaves

Plant material

Healthy, disease free leaves of mature Garcinia mangostana were collected from different localities of Ernakulam district.

Sample collection

Fresh and healthy leaves and fruits were plucked by hand from actively growing mangosteen tree from different localities of Ernakulam district. Later, put it in sterile zip lock plastic bags and brought to the laboratory on the same day for the isolation of fungal endophytes.

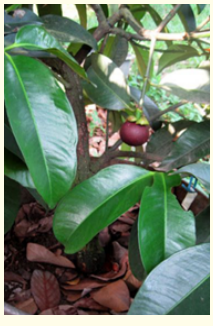

Figure 1: *Garcinia mangostana* L.

Isolation of endophytic fungi: [7]

Materials:

- Distilled water
- 70%ethanol
- 5.25% sodium hypochlorite solution
- Sterile filter paper
- Sterile forceps
- Sterile scalpel blade with holder
- Chloramphenicol
- Sterile Petri-dishes
- Sabaroud dextrose agar (SDA)
- Potato dextrose agar (PDA)
- Malt extract agar (MEA)
- Sterile tweezer

Methodology

Fresh leaves and fruits were washed thoroughly under running tap water in-order to remove adhering dirt particles. The outer surface of both leaves and fruits were initially sterilized by immersing it in 70% ethanol for 1min, then dip in 5.25%sodium hypochlorite solution for 5min, followed by dipping again in freshly prepared 70%ethanol for 30s. Finally it is taken and washed by dipping in sterile distilled water for 3s to 5s for three times. After surface sterilization, the samples were dried on sterile filter paper and each plant sample was cut with a sterile blade into 1cm square segments. Each sample was then placed onto Sabouraud dextrose agar (SDA), Potato dextrose agar (PDA) and Malt extract agar (MEA) (supplemented with chloramphenicol (50µg/ml) in-order to prevent bacterial growth) by exposing their inner tissue surface, using tweezer. Each Petri dish was placed with 2-3 slices of samples and controls are maintained to ensure the efficiency of surface sterilization, were incubated at 28°C for 5-6 days. Following the incubation, different fungal strains emerged from each sample and individual strains were isolated. Later slide culture was done to get pure endophytic fungal strains with uniform colony. After purification, the endophytic fungal isolates were transferred separately into SDA slants in test tubes as well as sterile SDA plates for stock culture and stored at 4°C for further examinations.

Slide culture technique for pure culture: [12]

Materials:

- SDA plate
- Slide culture apparatus

Methodology

The endophytic fungi obtained were made pure by slide culture technique.

Characterization and identification of isolated endophytic fungi [5]

Characterization and identification of endophytic fungal isolates were done by macroscopic and microscopic study.

Microscopic characterization (Lactophenol cotton blue staining): [9]

Methodology:

Microscopic examination of fungi was done using lactophenol cotton blue (LPCB) staining technique. Lactophenol cotton blue is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi. LPCB stain has 3 principle components which includes, Phenol (Kills live organism), Lactic acid (preserves fungal structures) and cotton blue (stains the chitin and cellulose of fungal cell wall intensely blue).

Macroscopic examination: [5]

The pure growth obtained after slide culture, ploughed on SDA for macroscopic study.

Preparation of fungal extract for doing antimicrobial assay [3]

Materials:

- Sterile scalpel blade
- 500ml conical flask
- Sterile potato dextrose broth media
- Sterilized spatula
- Whatsmann no:1 filter-paper
- Ethyl acetate
- Separating funnel
- Sterilized flask

Methodology

Endophytic fungal metabolites were screened for secondary metabolites. Endophytic fungal mycelia inoculated into 250ml of sterile sabouraud dextrose broth and incubation at room temperature for three weeks in rotary shaker. After three weeks of incubations, cultures of endophytic fungi were filtered using whatsmann no:1 filter paper to remove mycelial mats. Fungal metabolites were extracted by solvent extraction procedures using ethyl acetate as organic solvent. Equal volumes of metabolites filtrate and ethyl acetate were measured and placed into separating funnel; the mixture was shaken vigorously for 10min and then left to stand to allow cell masses to get separated from the solution. The aqueous solution was discarded and the organic solution was collected and kept in a sterile flask and metabolites were extracted by removing excess solvent using rotary shaker. The fungal crude extracts obtained were stored at 4°C for further assays.

Antimicrobial assay [8,10]

Gram positive bacteria, *Staphylococcus aureus*, gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and a yeast *Candida albicans* collected from the Department of Microbiology, Presentation College Of Applied Sciences, Puthenvelikara, were used for antibiogram.

Screening of antimicrobial activity [8]

Crude extracts of the isolated endophytic fungi were screened for their antimicrobial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Candida albicans* using agar well diffusion method. Four wells were made in each petri-dish using well puncture and a concentration of 20µl, 30µl, 40µl and 50µl of fungal extract were loaded in each of the wells by using micropipette. Positive (chloromphenicol) and negative control disc kept along each batch. The zones of inhibition were measured after 24 hours of incubation at 37°C. The test were performed in triplicates and the result has been presented as mean value (± standard deviation).

Statistical analysis

Antimicrobial activity of the isolated endophytes in four concentrations were done against 5 pathogenic organisms, zone of inhibition was measured. For each organism, three replicate trials were conducted. The zone of inhibition was calculated as a mean of three replicates. Data was expressed as mean ±SE. Statistical analysis was done by ANOVA using the statistical package INSTAT and means were compared by Turkey-Kramer Multiple Comparisons Test.

Result and Discussion

Isolation of fungal endophytes [7]

After 3 days of incubation, growth obtained on SDA, PDA and MEA plates inoculated with leaf samples. Absence of growth on control plates showed the efficiency of surface sterilization while the growth on test plates showed confirmation of the presence of endophytes.

Plate 1: Endophytic fungi 1 (ENF1) (Top View).

Plate 2: Endophyticfungi1 (ENF1) (Bottom View).

Plate 3: Endophytic fungi 2 (ENF2) (Top View).

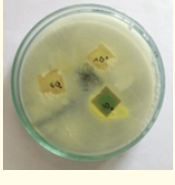

Plate 4: Endophytic fungi 2 (ENF2) (Bottom View).

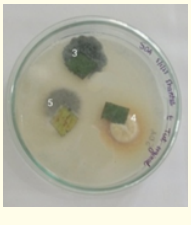

Plate 5: Endophytic fungi 3 (ENF3), Endophytic fungi 4 (ENF4) and Endophytic fungi 5 (ENF5) (Top View).

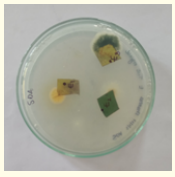

Plate 6: Endophytic fungi 3 (ENF3), Endophytic fungi 4 (ENF4) and Endophytic fungi 5 (ENF5) (Bottom View).

Plate 7: Endophytic fungi 6 (ENF6) (Top View).

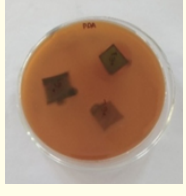

Plate 8: Endophytic fungi6 (ENF6) (Bottom View).

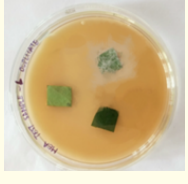

Plate 9: Endophytic fungi 7 (ENF7) (Top View).

Plate 10: Endophytic fungi 7 (ENF7) (Bottom View).

Plate 11: Endophytic fungi 8 (ENF8) (Top View).

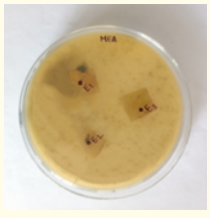

Plate 12: Endophytic fungi 8 (ENF8) (Bottom View).

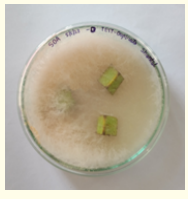

Plate 13: Endophytic fungi 9 (ENF9) (Top View).

Plate 14: Endophytic fungi 9 (ENF9) (Bottom View).

During this study 9 fungal isolates were recovered from *G. mangostana* leaves and named them as ENF1, ENF2, ENF3, ENF4, ENF5, ENF6, ENF7, ENF8 and ENF9 respectively.

Characterization and identification of isolated endophytic fungi [5,9,12].

Plate 15: Endophytic fungi1 Slide culture.

Plate 16: Endophytic fungi 1 Subculture on SDA.

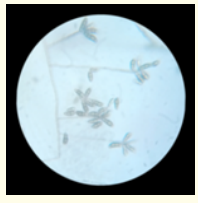

Plate 17: Endophytic fungi 1 Subculture on SDA.

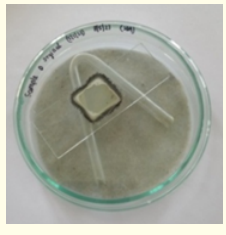

Plate 18: Endophytic fungi 1 Subculture on SDA.

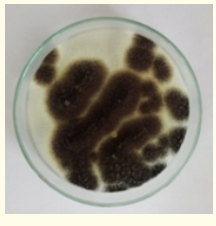

Plate 19: Endophytic fungi 2 Subculture on SDA.

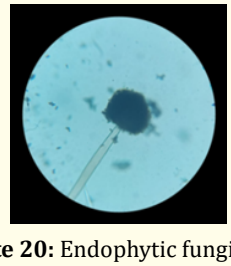

Plate 20: Endophytic fungi 2 LPCB.

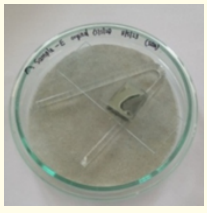

Plate 21: Endophytic fungi 3 Slide culture.

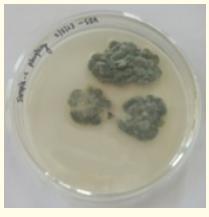

Plate 22: Endophytic fungi 3 Subcultulture on SDA.

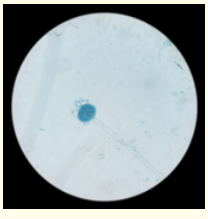

Plate 23: Endophytic fungi 3 LPCB.

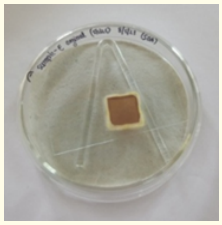

Plate 24: Endophytic fungi 4 Slide culture.

Plate 25: Endophytic fungi 4 Subculture on SDA.

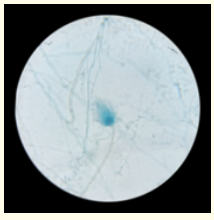

Plate 26: Endophytic fungi 4 LPCB.

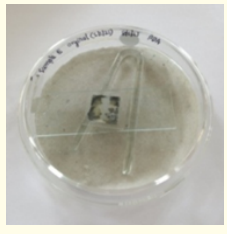

Plate 27: Endophytic fungi 5 Slide culture.

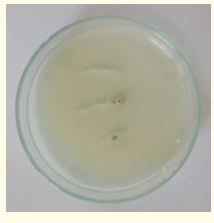

Plate 28: Endophytic fungi 5 Subculture on SDA.

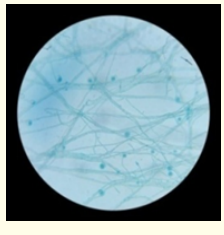

Plate 29: Endophytic fungi 5 LPCB.

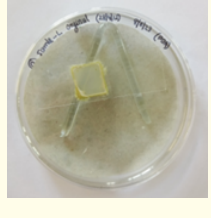

Plate 30: Endophytic fungi 6 Slide culture.

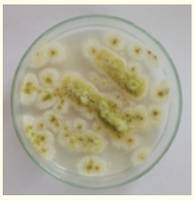

Plate 31: Endophytic fungi Subculture on SDA.

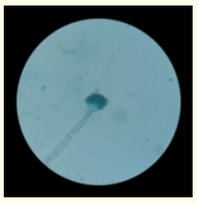

Plate 32: Endophytic fungi 6 LPCB.

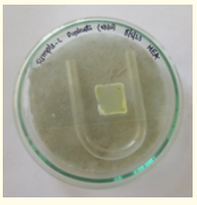

Plate 33: Endophytic fungi 7 Slide culture.

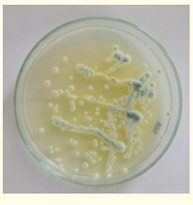

Plate 34: Endophytic fungi 7 Subculture on SDA.

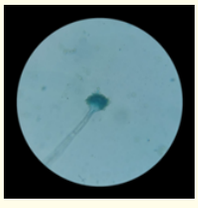

Plate 35: Endophytic fungi 7 LPCB.

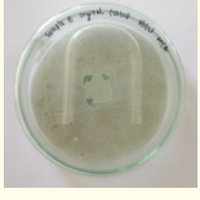

Plate 36: Endophytic fungi 8 Slide culture.

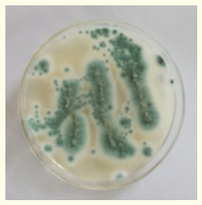

Plate 37: Endophytic fungi 8 Subculture on SDA.

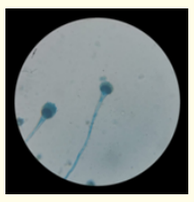

Plate 38: Endophytic fungi 8 LPCB.

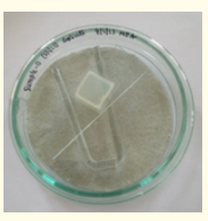

Plate 39: Endophytic fungi 9 Slide culture.

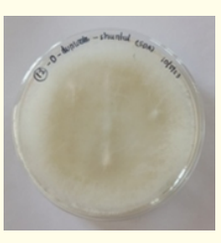

Plate 40: Endophytic fungi 9 Subculture on SDA.

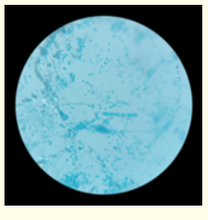

Plate 41: Endophytic fungi 9 LPCB.

Characterization and genus level identification of endophytic fungal isolates were done by observing macroscopic cultural characters on sabouraud dextrose agar (SDA) and microscopic characters using lactophenol cotton blue technique.

Fungal Isolates	Characteristic features		Genus level identification
	Macroscopic	Microscopic	
ENF1	Black to grey thick wooly like appearance. Fast growing colonies.	Ovoid, pale brown, smooth-walled conidia present single or in acropetal chains.	*Alternaria sps*
ENF2	Cultural growth is initially white but they change to black after a few days producing conidial spore.	Conidial heads are large. Uniseriate or biseriate conidial heads, spherical to pyriform vesicles and black coloured conidia.	*Aspergillus sps*
ENF3	Green shaded, flat slow growing colonies without margin.	Conidial heads are globose or subglobose, smooth, thin-walled conidia.	*Aspergillus sps*
ENF4	White coloured velvet like colonies with an entire margin.	Conidial heads are typically columnar and uniseriate. Conidiophore stipes are short, smooth-walled and have conical-shaped terminal vesicles. Conidia are globose to subglobose, green and finely roughened.	*Aspergillus sps*
ENF5	Thread like colonies white in color.	Smooth walled macro and microconidia. Conidia either rounded or tear shaped. Macroconidia mostly borne directly on the hyphae.	*Trichophyton sps*
ENF6	Colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age.	Septate hyphae and swollen vesicle giving raise to phialides from which chains of conidia araise.	*Aspergillus sps*
ENF7	Fast growing colonies, in shades of green surrounded by white area.	Chains of single-celled conidia. Conidiophores are hyaline, smooth, green coloured. Phialides are flask-shaped, branched metulae, giving a brush-like appearance.	*Penicillium sps*
ENF8	Typically blue-green with a slight yellow reverse. Mature colonies turn slate gray. Texture is woolly to cottony to somewhat granular.	Conidial heads are typically columnar. Conidiophore stipes are short. Conidia are globose to subglobose	*Aspergillus sps*
ENF9	Initially white, then turned to pink coloured thread like, fast growing colonies.	Smooth walled macro and microconidia. Conidia rounded to tear shaped. Macroconidia mostly borne directly on the hyphae , thin or thick walled.	*Trichophyton sps*

Table 1: Macroscopic and Microscopic characterization and identification.

By observing both microscopic and macroscopic characters ENF1 identified as *Alternaria sps*. ENF2, ENF3, ENF4, ENF6 and ENF8 *Aspergillus sps* while ENF5 and ENF9 were identified as *Trichophyton sps and* ENF7 as *Penicillium sps.* Similarly, genus level identification of fungi on the basis of microscopic and macroscopic characters was reported in literature [9,12].

Preparation of fungal extract for doing antimicrobial assay [1]

The fungal extracts prepared were stored at 4°C for further assays.

Antimicrobial activity of isolated endophytic fungal extracts [8,10].

Antimicrobial activity of endophytic fungal extract against *E. coli.*

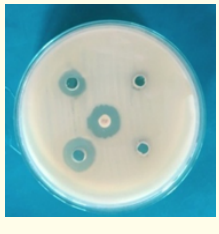

Plate 42: Antimicrobial activity of ENF 3 against *E. coli.*

Antimicrobial activity of endophytic fungal extract against *Klebsiella pneumoniae*.

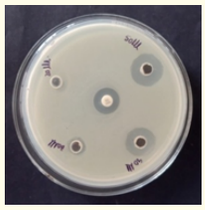

Plate 43: Antimicrobial activity of ENF1 against *Klebsiella pneumoniae.*

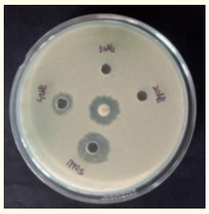

Plate 44: Antimicrobial activity of ENF2 against *Klebsiella pneumoniae.*

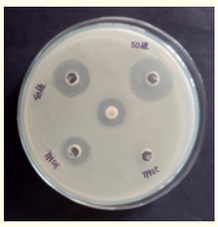

Plate 45: Antimicrobial activity of ENF4 against *Klebsiella pneumoniae.*

Antimicrobial activity of endophytic fungal extract against *Staphylococcus aureus*.

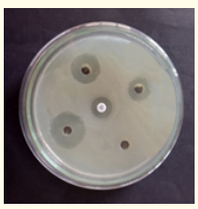

Plate 46: Antimicrobial activity of ENF1 against *Staphylococcus aureus.*

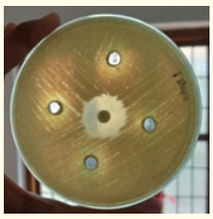

Plate 47: Antimicrobial activity of ENF2 against *Staphylococcus aureus.*

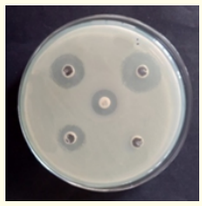

Plate 48: Antimicrobial activity of ENF3 against *Staphylococcus aureus*.

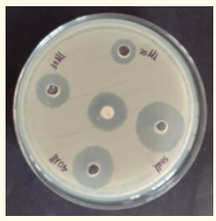

Plate 49: Antimicrobial activity of ENF4 against *Staphylococcus aureus*.

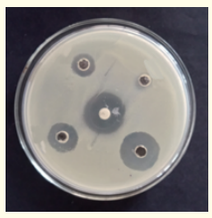

Plate 50: Antimicrobial activity of ENF7 against *Staphylococcus aureus*.

Antimicrobial activity of endophytic fungal extract against *Pseudomonas aeruginosa*.

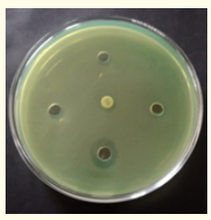

Plate 51: Antimicrobial activity of ENF3 against *Pseudomonas aeruginosa*.

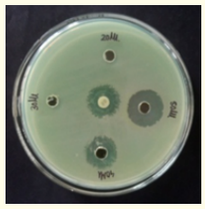

Plate 52: Antimicrobial activity of ENF4 against *Pseudomonas aeruginosa*.

Antimicrobial activity of endophytic fungal extract against *Candida albicans.*

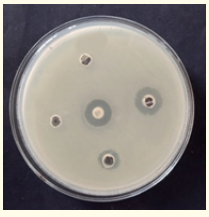

Plate 53: Antimicrobial activity of ENF4 against *Candida albicans.*

The results indicated that the inhibitory activity of endophytes was dose dependent. When the concentration of the sample increased, an increase in the diameter of zone of inhibition was observed. ENF4 showed best antimicrobial activity against *Klebsiella pneumoniae* followed by ENF2,ENF1 respectively. ENF5 showed best antimicrobial activity against *Staphylococcus aureus* followed by ENF3, ENF1, ENF2 and ENF4.ENF3 and ENF4 showed antimicrobial activity against *Pseudomonas aeruginosa.* ENF3 alone showed antimicrobial activity against *E.coli* and ENF4 alone showed antimicrobial activity against *Candida albicans.* None of

Fungal Isolate	Concentration of the fungal extract	Test organisms				
		E. coli	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Candida albicans*
ENF1	50µl	0 ± 0	18 ± 1.00	23 ± 1.52	0 ± 0	0 ± 0
	40µl	0 ± 0	17 ± 2.00	19 ± 1.52	0 ± 0	0 ± 0
	30µl	0 ± 0	0 ± 0	17 ± 1.15	0 ± 0	0 ± 0
	20µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
ENF2	50µl	0 ± 0	21 ± 1.52	22 ± 1.00	0 ± 0	0 ± 0
	40µl	0 ± 0	16 ± 1.52	18 ± 1.58	0 ± 0	0 ± 0
	30µl	0 ± 0	0 ± 0	14 ± 2.51	0 ± 0	0 ± 0
	20µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
ENF3	50µl	17 ±1.52	0 ± 0	25 ± 2.00	15 ± 1.52	0 ± 0
	40µl	16 ±2.00	0 ± 0	20 ± 1.00	0 ± 0	0 ± 0
	30µl	0 ± 0	0 ± 0	15 ± 1.00	0 ± 0	0 ± 0
	20µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
ENF4	50µl	0 ± 0	26 ± 2.51	25± 1.52	19 ± 2.00	14 ± 3.21
	40µl	0 ± 0	20 ± 1.52	16 ± 2.51	13 ± 1.52	10 ± 1.52
	30µl	0 ± 0	13 ± 1.00	13 ± 0.57	0 ± 0	0 ± 0
	20µl	0 ± 0	0 ± 0	9 ± 1.00	0 ± 0	0 ± 0
ENF5	50µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	40µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	30µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	20µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
ENF6	50µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	40µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	30µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	20µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
ENF7	50µl	0 ± 0	0 ± 0	26± 1.52	0 ± 0	0 ± 0
	40µl	0 ± 0	0 ± 0	22 ± 1.52	0 ± 0	0 ± 0
	30µl	0 ± 0	0 ± 0	19 ± 1.00	0 ± 0	0 ± 0
	20µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
ENF8	50µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	40µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	30µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	20µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
ENF9	50µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	40µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	30µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	20µl	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 2: Antimicrobial activity of endophytic fungal isolates.

the endophytes showed antimicrobial activity against all the five organisms tested, still ENF1, ENF2, ENF3 and ENF4 showed antimicrobial activity against any of the two test organisms. According to previous research work done by Rivai., *et al.* 2016 endophytic fungi showed activity against *E.coli and Staphylococcus aureus.* This indicates the possibility of exploiting these fungi in developing natural therapeutic products which can benefit society.

Conclusion

This study demonstrates the isolation of endophytic fungi from *Garcinia mangostana* plant. Mangosteen is an ideal source for isolation of endophytes with various applications. Sabouraud dextrose agar, potato dextrose agar and malt extract agar are the culture media used for the isolation of endophytic fungi. Nine types of endophytic fungi were isolated namely, ENF1, ENF2, ENF3, ENF4, ENF5, ENF6, ENF7, ENF8 and ENF9 respectively. By observing both microscopic and macroscopic characters ENF1 identified as *Alternaria sps*. ENF2, ENF3, ENF4, ENF6 and ENF8 *Aspergillus sps* while ENF5 and ENF9 were identified as *Trichophyton sps.* ENF7 was identified as *Penicillium sps.* Antimicrobial activity of the fungal isolates were also studied using agar well diffusion method. ENF4 showed best antimicrobial activity against *Klebsiella pneumoniae* followed by ENF2,ENF1 respectively. ENF5 showed best antimicrobial activity against *Staphylococcus aureus* followed by ENF3, ENF1, ENF2 and ENF4.ENF3 and ENF4 showed antimicrobial activity against *Pseudomonas aeruginosa.* ENF3 alone showed antimicrobial activity against *E.coli* and ENF4 alone showed antimicrobial activity against *Candida albicans.*

From this study it's clear that *Garcinia mangostana* is a potential source of fungal endophytes with various beneficial properties. Out of nine, more than five endophytes showed antimicrobial activity. This indicates the possibility of exploiting various endophytic fungi isolated from *Garcinia mangostana* in the production of industrially important natural therapeutic products, which can be beneficial for the society.

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