e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(1); 704-709

Original Research Article

Evaluation of Antimicrobial Potential of Crude Plant Extracts of Begonia *Semperflorens, Morus Alba*, and *Myrisitica Fragrans* against Mentioned Bacterial and Fungal Species

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Received: 02-11-2022 / Revised: 30-11-2022 / Accepted: 30-12-2022
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Conflict of interest: Nil

Abstract

In the present study crude extracts of leaves of two plants, Begonia semperflorens, *Morus alba* and seeds *Myristica fragrans* in distilled water, ethanol and methanol, respectively were subjected to preliminary phytochemical and antimicrobial studies on certain microorganisms. The phytochemical analysis done using the chemical methods showed the presence of various phytochemicals in all the plants. The results of antimicrobial studies showed that only ethanolic and methanolic extracts of *Morus alba* have good antimicrobial activity against the test organisms: *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Aspergillus niger*, *A. flavus*. No extract had any activity against *E. coli*, *Candida albicans* and *Cryptococcus neoformans*. Complete inhibition of sporulation was seen in *A. fumigatus* by methanolic extract of *Myristica fragrans* and ethanolic extract of *Morus alba*. This study supports the use of plants in traditional medicine and the potential of using them as food supplements. This opens future prospects of these plant constituents to be used as potential drugs for the treatment of infectious diseases, especially those caused by antibiotic resistant organisms.

Keywords: Antimicrobial Resistance, Phytotherapy, Alternative Medicine.

Introduction

Bacteria and fungi cause a wide variety of diseases in humans and animals. With the advent of antibiotics, the control of these diseases became possible and easier. But, over the years, several cases of antibiotic resistance have been reported. In the latest report by WHO, it has warned the world against many type of pathogens which cannot be treated with the currently available antibiotics. Antibiotic resistance is a phenomenon where the microorganisms evolve so that the antibiotics have no effect on the microbes. E.g. include Methicillin Resistant *Staphylococcus Aureus*, Vancomycin Resistant Enterococcus, Multi Drug Resistant Tuberculosis etc.

Causes of antimicrobial resistance can be Natural like absence of target of antibiotic, horizontal gene transfer by conjugation, transduction or transformation. But, the main cause is the misuse and overuse of the antibiotics.

Availability of antibiotics over the counter, missing the antibiotic doses, use of antibiotics in livestock and agriculture increase the incidence of antimicrobial resistance. Pets also act as reservoir of

resistant microorganisms. It has been reported that microorganisms and antibiotics have coevolved. Microorganisms resistant to penicillin were discovered within 2 years of discovery of penicillin (D'Costa *et al.*, 2011).

Medicinal plants are a part of most traditional medicines and a large number of civilizations were dependent on plant products for fighting against pathogens and other diseases. Plants produce a number of secondary metabolites, called as phytochemicals, many of which have antimicrobial activity.

A combination of these phytochemicals such as phenols, flavanoids, tannins, saponins etc, results in the beneficial medical effects of these plant extracts (Cowan, 1999). Ayurveda is an example of Indians using and depending upon plants for their medicinal properties.

The rationale for the current study is based on the phenomenon of combating antimicrobial resistance. Due to growing resistance scientists are now looking for alternative therapies like Probiotics, Phage Therapy, Medicinal Plants, Developing new antibiotics etc. Plants are exposed to a number of microorganisms constantly. To check the growth of these pathogens, plants also possess innate mechanisms that employ a number of defense responses. Phytochemicals, both preformed and pathogen induced can be thus used as potential antimicrobials (Lamothe *et al.*, 2010).

The current study involves preparation of crude extracts of different plants and the objective are to test their antimicrobial potential. The chosen plants are *Begonia semperflorens*, *Morus alba* (Mulberry) and *Myristica fragrans* (Nutmeg). The leaves (Begonia and Mulberry) and seed (Nutmeg) have been chosen for preparation of the crude extracts in different organic solvents such as Ethanol, Methanol, Distilled Water etc.

Agar well diffusion and Minimal Inhibitory Concentration (MIC) have been used to test the susceptibility of the microorganism to the extract. Phytochemical analysis of the extracts was also done.

Materials and Methods

Preparation of crude extract

1. The extracts were prepared using classic Solvent Extraction method.
2. The powdered material was extracted with following solvents: Distilled water, Ethanol and Methanol.
3. The powder was dissolved in the solvents in a ratio of 1:10 (10 grams in 100ml solvent) and kept in an incubator shaker at 37°C for 48 hours.
4. Further, the extracts were filtered using Whatman Filter Paper no.1 and poured in petriplates.
5. Blank weight of petriplates was taken to calculate the yield.
6. Petriplates were then kept in a hot air oven set at 45°C for evaporation of the solvent and concentration of the extract.
7. The petriplates were again weighed after complete evaporation of the solvent.
8. The remaining solid was scrapped off using blade and stored in an eppendorf at 4°C till further use.

Microbial Strains

Bacterial Strains	***Fungal Strains:***
• *Escherichia coli*	• *Aspergillus niger*
• *Pseudomonas aeruginosa*	• *Aspergillus fumigatus*
• *Proteus mirabilis*	• *Aspergillus flavus*
• *Staphylococcus* aureus	• *Cryptococcus neoformans*
	• *Candida albicans*

Agar Well Diffusion Assay- Bacteria (Rao *et al*., 2012)

1. The organisms were inoculated in Nutrient Broth tubes and incubated at 37°C for 18 hours.
2. The tubes were matched with 0.5 McFarland Standards visually.
3. Nutrient Agar (NA) was used for screening the antibacterial activities of the extracts.
4. The NA plates were prepared by pouring 20mL autoclaved NA into sterile petriplates and allowed to solidify for 5 minutes under aseptic conditions.
5. A sterile swab was dipped in the NB tube containing the test organism and the inoculum was uniformly swabbed across the surface of the agar.
6. Using 1mL pipette tip, wells were punched (0.5mm) in the agar.
7. Serial dilutions of extracts were prepared in mother solvents (100mg/ml, 50mg/ml, 25mg/ml and 12.25mg/ml) and 0.0025ml of each was added in 4 different wells.
8. Gentamycin and mother solvents were taken as positive and negative controls, respectively.
9. The plates were incubated at 37°C for 18 hours and later checked for Zone of Inhibition. All the experiments were performed in duplicates.

Agar well diffusion- Fungal cultures Similar methodology was used for fungal strains as mentioned above, except:

1. The organisms were inoculated in Czapek Dox Broth tubes and incubated at 25°C for 48 hours.
2. Terbinafine and mother solvents were taken as positive and negative controls, respectively.
3. The plates were incubated at 25°C for 48 hours and later checked for Zone of Inhibition. All the experiments were performed in duplicates.

Results and Discussion

Agar Well Diffusion

a) Agar well diffusion for Nutmeg methanolic extract (N_M) and Begonia aqueous extract (B_A) against bacterial and fungal cultures didn't show any activity against the chosen strains.

But, only complete inhibition of sporulation was observed in *A. fumigatus,* incubated for 12 days.

b) Agar well diffusion for Mulberry Ethanolic extract (M_E) against bacterial and fungal cultures.

The tables below show the activity of M_E against the tested organisms. Maximum zones (27.5 mm) were observed against *Pseudomonas aeruginosa*, in a dose dependent manner and No activity was observed against *Escherichia coli.*

Table 1: Zone Diameters M_E (mm)

S. No.	Organism ↓ / Conc. → (mg/ml)	100	50	25	12.5	P	N
a)	*Escherichia coli*	0	0	0	0	27	0
b)	*Pseudomonas aeruginosa*	27.5	15	15	0	27	0
c)	*Proteus mirabilis*	21	17.5	13	10	21	0
d)	*Staphylococcus aureus*	15	14	10	0	25	0

P: Positive control, Gentamycin; N: Negative control, Ethanol.

In the fungal assay, maximum zone was observed in *A. niger and A. flavus* of 16 mm at a concentration of 50 mg/ml and 12.5 mg/ml, respectively. Complete inhibition of sporulation was observed in *A. fumigatus,* incubated for 12 days.

Table 2: Zone Diameters M_E (mm)

S. No.	Organism ↓	Conc. → (mg/ml)	100	50	25	12.5	P	N
a)	*A. niger*		13	16	0	13	25	0
b)	*A. flavus*		12	11	9	16	21	0
c)	*A. fumigatus*		0	0	0	0	23	0
d)	*Cryptococcus neoformans*		0	0	0	0	0	0
e)	*Candida albicans*		0	0	0	0	0	0

P: Positive control, Terbinafine; N: Negative control, Ethanol.

d) Agar well diffusion for Mulberry Methanolic extract (M_M) against bacterial and fungal cultures.

The tables below show the activity of M_E against the tested organisms. Maximum zones (19 mm) were observed against *Staphylococcus aureus*, in a dose dependent manner and No activity was observed against *Escherichia coli.*

Table 3: Zone Diameters M_M (mm)

S. No.	Organism ↓	Conc. → (mg/ml)	100	50	25	12.5	P	N
a)	*Escherichia coli*		0	0	0	0	17	0
b)	*Pseudomonas aeruginosa*		18	15	14	9	29	0
c)	*Proteus mirabilis*		17	11	0	0	19	0
d)	*Staphylococcus aureus*		19	15	14	12	14	0

P: Positive control, Gentamycin; N: Negative control, Ethanol.

Discussion

The study was conducted on three crude plant extracts, namely Begonia, Mulberry and Nutmeg. The whole project comprised of two phases: 1.) Preparation of crude plant extracts, and 2.) Antibacterial and antifungal assay of the crude plant extracts.

In the present study on the aqueous extracts of *Begonia semperflorens*, no antimicrobial activity was observed against the tested organisms (*E. coli, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, A. niger, A. flavus, A. fumigatus, Candida albicans* and *Cryptococcus neoformans.* However, Suresh and Nagarjan used methanolic extracts of *Begonia malabracia* and reported antimicrobial activity against *E. coli, A. niger, A. fumigatus* and *A. flavus*. This shows that choice of solvent and the species of the plant have an effect on the antimicrobial activity of the extract. This can be linked to different phytochemical profiles of the plant species and polarities of the different solvents used for extraction.

Gupta *et al* have reported antimicrobial activity of Methanolic extracts of Nutmeg seeds against *A. flavus*, *A. fumigatus, Pseudomonas aeruginosa* and *S. aureus.* In the present study, no antimicrobial activity was observed in against any test organism. However, in this study, inhibition of sporulation was observed in *A. fumigatus.* To our best knowledge, no paper has reported

the inhibition of sporulation in *A. fumigatus* by methanolic extracts of Nutmeg seeds.

Rao *et al* have reported antimicrobial activity of *Morus alba* methanolic extracts against Gram negative bacteria; in our study also it was observed that the extracts could inhibit the growth of *Pseudomonas* and *Proteus.*

This opens future prospects of these plant constituents to be used as potential drugs for the treatment of infectious diseases, especially those caused by antibiotic resistant organisms. These phytochemicals can also be screened for treatment of other diseases such as Cancer.

Conclusion

This pilot study demonstrated that Begonia, Mulberry and Nutmeg contain several phytochemicals. Mulberry has potent antibacterial activity against *Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcus aureus* and antifungal activity against *A. niger* and *A. flavus.*

This opens future prospects of these plant constituents to be used as potential drugs for the treatment of infectious diseases, especially those caused by antibiotic resistant organisms.

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