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Original Research Article

Bioefficacy Of *Luffa Acutangula* Var. Amara For Antimicrobial Activities

Hara Prasad Sahoo[1], Prakash Kumar Sarangi[2,] Mahasweta Das*[3] and Bhabjit Pattnaik[4]
[1]Department of Botany, BJB College, Bhubaneswar, Odisha, India-751014
[2]CAEPHT, Central Agricultural University, Gangtok, Sikkim, India-737135
[3]Orissa University Agriculture and Technology, Bhubaneswar-751003
[4]Department of Botany, Christ College, Cuttack, Odisha, India-753008

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Abstract: Work has been conducted for evaluating the efficacy of *Luffa acutangula var. amara* seed oil and meal for antimicrobial activities by modified cup-plate method. The antibacterial activity of extracts was evaluated against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus fecalis*. The antifungal activity of extracted oil and powder was evaluated against *Candida albicans, Trichophyton rubrum, and Microsporum canis*. The minimal inhibitory concentration (MIC) and zone of inhibition were calculated for both extracts. The oil extract with highest MIC resulted to play better antimicrobial activity than the meal.

Keywords: Minimal Inhibitory Concentration, Efficacy, Antibacterial Activity, Zone Of Inhibition

Introduction

Infectious diseases now account for high percentage of health problems around the globe. It has been detected that development of antibiotic resistance against various microorganism causing greater hindrances for treatment of above symptoms[1]. The major cause serving the resistance is the use of huge amount of commercial antimicrobial medicines that so forces the academicians and researchers to seek for new antimicrobial substances from different herbal formulation[2]. Keeping in view of the excessive use of synthetic antibiotics, replacement of natural herbal by-products having more specificity for the disease treatment. Many of the antimicrobial compounds produced by plant products and their formulation are active against plant and human pathogenic microorganisms[3].

This is an ancient practice for the treatment of infectious diseases by using plants and their preparations and this was the only past probably method available at that time. However, the methodical study of higher plants for exploring antimicrobial activity is of recent practices[4]. Synthesis of active secondary metabolites like major phenolic compounds has showing different potent insecticidal and antimicrobial[5], thereby enhancing their applications in many pharmaceuticals as alternative medicines and natural therapies[6]. In this paper, we have focussed the application of *Luffa acutangula Roxb var. amara* oil for its antimicrobial activities against different bacterial and fungal diseases.

Materials and Methods

Collection of samples

The indigenous plant *Luffa acutangula var. amara* belongs to family Cucurbitaceae was collected from Nuagaon village, Nayagarh district, Odisha (In eastern India) and identified by Prof. Haraprasad Sahoo, Department of Botany, BJB College, Bhubaneswar, and Odisha. The fruits of *Luffa acutangula var. amara* were shown in figures 1-3. The local people of that area used the raw plant seeds for curing infectious skin diseases. The seeds were removed from the fruits by manual process. Seeds were ovoid, 0.6-0.8 cm long, 0.5-0.6 cm wide; much compressed, slightly corrugated on the edges, black colour and bitter in taste. Following harvest, seeds were stored at 4°C for oil extraction. The steps are involved in extraction of oil i.e. storage, cleaning, decortications shelling, milling, grinding, oil expelling, crude oil, filtering, setting tanks.

***Corresponding Author:**
Dr. Prakash kumar Sarangi,
CAEPHT, Central Agricultural university,
Gantok, Sikkim,India, 737135.
Email: sarangi77@yahoo.co.in

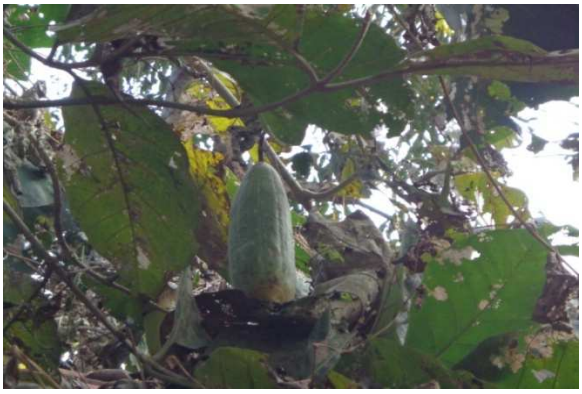

Fig.1: Raw fruit of Luffa acutangula

Fig.2: Raw fruit of Luffa acutangula

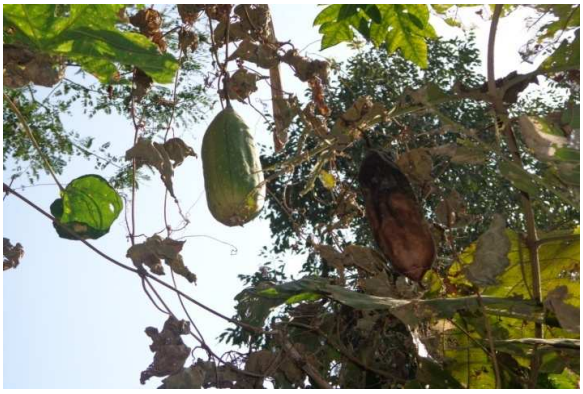

Fig.3: Raw fruit of Luffa acutangula

Microorganisms:

The antibacterial activity of extracted oil and powder was evaluated against two Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and two Gram positive bacteria *Staphylococcus aureus, Enterococcus fecalis*. The antifungal activity of extracted oil and powder was evaluated against *Candida albicans, Trichophyton rubrum,* and *Microsporum canis*. The strains were collected from Department of Microbiology, College of Basic sciences and Humanities, OUAT, Bhubaneswar, Odisha, India. Cultures of these bacteria and fungi were maintained on Nutrient agar and Sabouraud dextrose agar media and incubated at 37ºC for 24h and 28ºC for 48h respectively. Gentamycin and Fluconazol were used as positive control for bacteria and fungus study respectively. Three replicates were carried for each microorganism. Freshly prepared cultures were used for testing antimicrobial activity using cup plate method with some modifications[7,8,9,10]. Minimum inhibitory concentration (MIC) values were also calculated for the microorganisms.

Antimicrobial activities:

Antimicrobial activity was calculated by Cup-plate method with some modifications[11]. 2ml of bacterial suspension were poured in the Petri-plates containing 15 ml of Nutrient agar media at 50ºC temperature and plates were rotated to mix the suspension with medium. After the agar got solidified bores were made in the plate with sterile borer of 8 mm diameter. In each plate six and five bores were made for antibacterial and antifungal activities respectively. In each plate, one was treated for positive control and another was for blank without any extracts. Study was conducted in triplicates for both the extracts. 100µl of sample was added in each bore. The plates were allowed for diffusion at room temperature for two hours and then incubated in the upright position in incubator at 37ºC and 27ºC for about 24h for bacteria and 48 h for fungus growth. The diameter of inhibition zone (mm) was accurately measured by zone reader in each plate.

Determination of MIC:

The minimum inhibitory concentration (MIC) was defined as the lowest concentration which inhibits the visible growth of bacteria and fungus during the defined incubation period for each species. The plant extracts like oil and powder were tested for MIC using serial broth dilution technique [12] with modifications. 10 ml of respective broth for bacteria and fungus was taken in a test tube and quantities of 75, 150, 300, 450 and 600ul of extracted oil were added. For antimicrobial activity of powder, 10, 25, 50, 100 and 150mg of extracted samples were taken. 1 ml of inoculums of each test organism was added in each sample. The tubes were incubated at 37°C and for 27°C for 48h for bacteria and fungi respectively. The growth of organisms was detected by a double beam spectrophotometer (**Jasco V-530**) at 620 nm.

Results and Discussion

Antibacterial and antifungal activities were evaluated as diameter of zone of inhibition. Both the extracts were showing inhibition power against different microorganisms (Table 1). The highest and lowest inhibition was detected by extracted oil against *Trichophyton rubrum* and *Pseudomonas aeruginosa* respectively. Although the inhibition by positive controls was higher than the extracts, the latter also showing greater importance against different microorganisms. As compared to oil, extracted powder showing lesser effect on microorganisms. The highest and lowest inhibition was detected against *Staphylococcus aureus* and *Enterococcus fecalis respectively*.

Table 1: Antimicrobial activity of *Luffa acutangula var. amara* seed oil and powder (n=3)

Extracts/ Positive control	Zone of Inhibition (mm)						
	E.coli	***P. aeruginosa***	***S. aureus***	***E.fecalis***	***C.albicans***	***T. rubrum***	***M. canis***
Powder	10±0.3	10±0.8	11±0.1	8±0.4	9±0.6	8±0.7	10±0.3
Oil	11±0.8	10±0.5	12±0.8	10±0.6	12±08	12±0.9	11±0.4
Gentamycin	14±0.6	15±0.8	16±0.8	18±0.5	-	-	-
Fluconazol	-	-	-	-	16±0.5	13±0.9	15±0.7

The effect of control by plant extracts was also detected by calculating the minimum inhibitory concentration (MIC) helping the inhibition of visible growth of bacteria and fungus during the defined incubation period (Table 2). The growth of inhibition was drastic lowered in case of extracted oil as sample against *Trichophyton rubrum* taking MIC very less amount. On the other hand, the extracted powder has the lower MIC against *Pseudomonas aeruginosa.* Both the extracts were showing the higher MIC against the positive controls. It has been also found out that the efficacy of oil is more against fungus than the bacteria. But the extracted powder has the much inhibition power against the bacteria than the fungus. This may be due to the presence of different active phytocompounds inside the extracts. The full spectrum of efficacy can be evaluated after the phyotochemical analysis of active compounds inside the extracts. The local people were using broken seeds for curing of different skin diseases. Hence this may concluded that the ethnic traditional knowledge of local people about various botanicals cannot be avoided at any circumstances.

Table 2: MIC for *Luffa acutangula var. amara* seed oil and powder (n=3)

Microorganisms	Extracts		Positive control	
	Oil	Powder	Gentamycin	Fluconazol
Escherichia coli	30	5	2	-
Pseudomonas aeruginosa	60	2.5	1	-
Staphylococcus aureus	45	5	0.75	-
Enterococcus fecalis	60	15	2	-
Candida albicans	15	5	-	1
Trichophyton rubrum	7.5	10	-	0.75
Microsporum canis	15	5	-	0.75

MIC: minimum inhibitory concentration (mg / ml) for others and (ul/ml) for oil

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