Antimicrobial Activity of *Vigna unguiculata* L. Walp Seed Oil

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Abstract: The antimicrobial activity of three varieties of *Vigna unguiculata* L. Walp seed oil (LBS-1, LBS-2 and LBS-3) were investigated against five Gram positive bacteria (*Bacillus megaterium, Bacillus subtilis, Sarcina lutea, Salmonella typhi* and *Staphylococcus aureus*) and four Gram negative (*Escherichia coli, Shigella dysenteriae, Shigella sonnei, Shigella shiga*) and four fungi (*Penicilium spp., Mucor spp., Candida albicans and Aspergillus fumigatius*). The LBS-1 oil at the concentration of 400 µg/ disc showed the highest activity against *Sarcina lutea* (19±0.1 mm) than that of LBS-2 (14±0.3 mm) and LBS-3 (12±0.3 mm) oil whereas LBS-3 oil showed highest activity against *Staphylococcus aureus* (16±0.1 mm) than that of LBS-1 (10±0.6 mm) and LBS-2 (13±0.4 mm) oil. All the three oils are active against the three tested fungi namely *Penicilium spp., Mucor spp.* and *Candida albicans* but showed no sensitivity against *Aspergillus fumigatius*.

Keywords: Plant oil, seed oil, *Vigna unguiculata*, antibacteria, antifungi.

INTRODUCTION

In the treatment of infectious diseases, microorganisms have developed resistance to many antibiotics [1]. The increasing resistance to microorganisms forced scientists to the search for new infection-fighting substances from various sources [2]. By a number of studies worldwide, the antimicrobial properties of plants have been investigated and because of their antimicrobial properties, many of them have been used as therapeutic alternatives [3]. The plants such as *Orthosiphon stamineus* Benth [4], *Coccinia grandis* L. [5], *Moringa oleifera* L. [6], *Calotropis gigantean* L. [7], *Callistemon viminalis* [8], *Diospyros peregrine, Coccinia grandis,* and *Swietenia macrophylla* [9] exhibits antimicrobial activity.

The plant *Vigna unguiculata* L. Walp (Bengali name: Barbati, English name: Cowpea), grows widely in Asia, Africa and South America [10]. By mixing seed powder with oil, it is used as medicine of stubborn boils [11]. The seed is diuretic and also used to strengthen the stomach. Boiled seeds are eaten to destroy worms in the stomach [12]. Powdered roots are eaten with porridge to treat chest pain, epilepsy and painful menstruation, [13]. Physico-chemical Properties and Enzyme contents of *Vigna unguiculata* L. Walp seeds [14], Extraction and Characterization of *Vigna unguiculata* L. Walp seed oil [15] and Antidiabetic effects of *Vigna unguiculata* L. Walp seed oil have been investigated [16]. This seed is rich in oil and contain some essential fatty acids [15]. Many seed oils exhibit antimicrobial activity have been reported [17-19]. Antimicrobial activity of *Vigna unguiculata* leaf extracts of acetone and ethanol against several fungal and bacterial strains have also been reported [20]. However, as far as the literature is concerned, no report on the antimicrobial activity of *Vigna unguiculata* L. Walp seed oil has found thus far. Hence the present study was undertaken to investigate the role of *V. unguiculata* seed oil as a potential antibacterial and antifungal agent.

MATERIALS AND METHODS

Collection of Seeds

Ripe pods of barbati (*Vigna unguiculata* L. Walp) used in this work were collected from the experimental at Rajshahi city, Rajshahi, Bangladesh in the year 2007. The authenticity of the barbati was identified by Professor A.T.M. Naderuzzaman, Department of Botany and University of Rajshahi, Bangladesh. The varieties reported herein, which were all cultivated in homogeneous conditions and different morphologically from each other, were: LBS-1 (pods are short, straight and red; seeds are somewhat round, small, smooth and red), LBS-2 (pods are long, sylindrical and deep green; seeds are somewhat elongated, kidney shaped, and deep red), and LBS-3 (pods are short, somewhat curved and light green; seeds are small, kidney shaped and red), To remove the foreign materials, the seeds

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were separated manually from the flesh of the fruits and washed several times with water. Afterward, the seeds were dried in the sunlight for four consecutive days and again in an electric oven at 40°C until a constant weight were reached. The seeds were ground to a fine powder, packed and stored in a refrigerator at 4°C prior to analysis.

Extraction of Oil from Seed

For solvent extraction (Soxhlet method), 500g of ground barbati seeds were placed into a cellulose paper cone and extracted using n-Hexane in a 5-l Soxhlet extractor for 8 h [21]. By using rotary evaporator the oil was recovered and the residual solvent was removed by drying in an oven at 60 °C for 1 h.

Test Microorganisms

Nine pathogenic bacteria were selected for the antibacterial activity test, five of which were gram positive (*Bacillus megaterium, Bacillus subtilis, Sarcina lutea, Salmonella typhi* and *Staphylococcus aureus*) and the remaining were gram negative (*Escherichia coli, Shigella dysenteriae, Shigella sonnei, Shigella shiga*) and four fungi (*Penicilium ssp., Mucor spp. Candida albicans and Aspergillus fumigatius*). The pure cultures were collected from the microbiological research laboratory, Institute of Biological Sciences (IBSc), Rajshahi University.

Antimicrobial Study

The bacterial and fungal strains were cultured in nutrient Agar medium and Dextrose Agar medium respectively for 12 and 24hrs. The antibacterial activity of leaf extracts were tested by disc diffusion assay method [22]. The Nutrient Agar plates used for antibacterial tests were incubated at 37°C for 24 hrs whereas the Dextrose Agar plates for antifungal tests were incubated at 30°C for 72 hrs. In nutrient agar plates, an inoculums size, 10^6cfu/ml for bacteria was used and on dextrose agar plates 2×10^5 spores were used for fungi. As positive control, Doxycycline is used for antibacterial tests whereas Nystatin is used as positive control for antifungal tests. Antifungal and antibacterial activity was determined by measuring the diameter of the zone of inhibition (Mean±SD) surrounding fungal and bacterial growth.

RESULTS AND DISCUSSION

Results

The antibacterial activity of LBS-1 oil, LBS-2 oil and LBS-3 oil were tested against nine bacteria at concentrations of 200 μg/disc and 400 μg/disc. Standard antibiotic disc Doxycycline (DXT-30 μg/disc) was used for comparison. The results obtained are shown in Tables **1**, **2**, **3**.

The produced zone of inhibition for LBS-1 oil against Bacillus megatorium, Bacillus subtilis, Sarcina lutia, Staphylococcus aureus, Escherichia colli, Shigella dysenteriae, Shigella sonnei and Shigella shiga were 12±0.2, 12±0.3, 19±0.1, 10 ±0.6, 11±0.1, 12±0.3, 11±0.4 and 8±0.1 mm at 400 μg/disc dose respectively. At 200 μg/disc dose, the produced zone of inhibition against the bacteria, Bacillus megatorium, Bacillus subtilis, Sarcina lutia, Escherichia colli and Shigella dysenteriae were 6±0.3, 8±0.1, 10±0.5, 7±0.2 and

Table 1: ***In Vitro*** **Antibacterial Activity of LBS-1 Oil and Doxycycline**

Test bacteria	Zone of inhibition (diameter in mm.)		
	Oil 200 μg/disc(le 200)	Oil 400 μg/disc (le 400)	Doxycycline (μg/disc)
Gram positive			
Bacillus megatorium	6±0.3	12±0.2	29±0.1
Bacillus subtilis	8±0.1	12±0.3	25±0.4
Sarcina lutia	10±0.5	19±0.1	27±0.2
Salmonella typhi	-	-	26±0.1
Staphylococcus aureus	-	10±0.6	26±0.2
Gram negative			
Escherichia colli	7±0.4	11±0.1	28±0.3
Shigella dysenteriae	9±0.2	12±0.3	25±0.1
Shigella sonnei	-	11±0.4	28±0.1
Shigella shiga	-	8±0.1	30±0.5

LBS =Local Barbati Seed. Results are presented as average-means ± standard mean deviations (n = 4).

Table 2: *In Vitro* Antibacterial Activity of LBS-2 Oil and Doxycycline

Test bacteria	Zone of inhibition (diameter in mm.)		
	Oil 200 µg/disc(le 200)	Oil 400 µg/disc (le 400)	Doxycycline (µg/disc)
Gram positive			
Bacillus megatorium	5±0.5	11±0.4	29±0.3
Bacillus subtilis	6±0.1	11±0.1	25±0.1
Sarcina lutia	8±0.4	14±0.3	27±0.4
Salmonella typhi	-	-	26±0.2
Staphylococcus aureus	-	13±0.4	26±0.1
Gram negative			
Escherichia colli	6±0.1	10±0.1	28±0.5
Shigella dysenteriae	8±0.3	10±0.3	25±0.2
Shigella sonnei	-	11±0.1	29±0.3
Shigella shiga	-	6±0.5	27±0.1

LBS =Local Barbati Seed. Results are presented as average-means ± standard mean deviations (n = 4).

Table 3: *In Vitro* Antibacterial Activity of LBS-3 Oil and Doxycycline

Test bacteria	Zone of inhibition (diameter in mm.)		
	Oil 200 µg/disc(le 200)	Oil 400 µg/disc (le 400)	Doxycycline (µg/disc)
Gram positive			
Bacillus megatorium	8±0.1	12±0.4	29±0.4
Bacillus subtilis	9±0.2	11±0.1	25±0.1
Sarcina lutia	10±0.3	12±0.3	27±0.3
Salmonella typhi	-	-	26±0.1
Staphylococcus aureus	-	16±0.1	26±0.5
Gram negative			
Escherichia colli	5±0.4	10±0.6	28±0.4
Shigella dysenteriae	8±0.1	11±0.5	25±0.1
Shigella sonnei	-	11±0.4	28±0.3
Shigella shiga	-	8±0.1	26±0.2

LBS =Local Barbati Seed. Results are presented as average-means ± standard mean deviations (n = 4).

9±0.2 mm respectively but this amount of oil has no activity against the bacteria such as Staphylococcus aureus, Shigella sonnei and Shigella shiga while it showed no activity against Salmonella typhi in both the concentration of oil. From the observation it was found that the 400 µg/disc dose is more potent on Sarcina lutia, (19±0.1).

The produced zone of inhibition for LBS-2 oil against Bacillus megatorium, Bacillus subtilis, Sarcina lutia, Staphylococcus aureus, Escherichia colli, Shigella dysenteriae, Shigella sonnei and Shigella shiga were 11±0.4, 11±0.1, 14±0.3, 13±0.4, 10±0.1, 10±0.3, 11±0.1 and 6±0.5 mm at 400 µg/disc dose respectively. At 200 µg/disc dose, the produced zone of inhibition against the bacteria, Bacillus megatorium, Bacillus subtilis, Sarcina lutia, Escherichia colli and Shigella dysenteriae were 5±0.5, 6±0.1, 8±0.4, 6±0.1 and 8±0.3 mm respectively but this amount of oil has no activity against the bacteria such as Staphylococcus aureus, Shigella sonnei and Shigella shiga. Like LBS-1, LBS-2 oil has no activity against Salmonella typhi.

The produced zone of inhibition for LBS-3 oil against Bacillus megatorium, Bacillus subtilis, Sarcina lutia, Staphylococcus aureus, Escherichia colli, Shigella dysenteriae, Shigella sonnei and Shigella shiga were 12±0.4, 11±0.1, 12±0.3, 16±0.1, 10±0.6, 11±0.5,

Table 4: ***In vitro*** **Antifungal Activities of LBS-1, LBS-2 and LBS-3 Oil and Nystatin**

Test fungi	Zone of inhibition (diameter in mm.)						Nystatin (100µg/disc)
	Oil of LBS-1		Oil of LBS-2		Oil of LBS-3		
	(100µg/ disc)	(200µg/ disc)	(100µg/ disc)	(200µg/ disc)	(100µg/ disc)	(200µg/ disc)	
Penicilium spp.	11±0.3	13±0.5	12±0.1	14±0.1	12±0.4	15±0.1	25±0.1
Mucor spp.	8±0.1	9±0.3	9±0.3	11±0.2	9±0.4	10±0.2	25±0.3
Candida albicans	9±0.2	11±0.1	9±0.4	10±0.1	10±0.3	12±0.4	23±0.5
Aspergillus Fumigatius	-	-	-	-	-	-	24±0.1

LBS =Local Barbati Seed. Results are presented as average-means ± standard mean deviations (n = 4). **'-'** no sensitivity.

11±0.4 and 8±0.1 mm at 400 µg/disc dose respectively. At 200 µg/disc dose, the produced zone of inhibition against the bacteria, Bacillus megatorium, Bacillus subtilis, Sarcina lutia, Escherichia colli and Shigella dysenteriae were 8±0.1, 9±0.2, 10±0.3, 5±0.4 and 8±0.1 mm respectively but this amount of oil has no activity against the bacteria such as Staphylococcus aureus, Shigella sonnei and Shigella shiga. LBS-3 oil also showed no activity against Salmonella typhi.

The LBS-1 oil at the concentration of 400 µg/ disc showed the highest activity against *Sarcina lutea* (19±0.1) than that of LBS-2 (14±0.3) and LBS-3 (12±0.3) oil whereas LBS-3 oil showed highest activity against *Staphylococcus aureus* (16±0.1) than that of LBS-1 (10±0.6) and LBS-2 (13±0.4) oil.

The antifungal activities of oil of LBS-1, LBS-2 and LBS-3 against five pathogenic fungi were investigated by using the doses of 100µg/ disc and 200µg/ disc. The standard antibiotic disc of nystatin (100µg/disc) was used for comparison. The results of antifungal activity (zone of inhibition) of test materials against respective fungi were given in the Table **4**.

It was found that all the three oils are active against the three tested fungi namely *Penicilium spp., Mucor spp.* and *Candida albicans* but showed no sensitivity against *Aspergillus fumigatius*. All the three varieties of LBS-1, LBS-2 and LBS-3 oil are more active against *Penicilium spp.,* but LBS-3 oil showed the more activity than that of LBS-1 and LBS-2 oil.

DISCUSSION

By many researchers the antimicrobial activities of various plants and herbs have been reported [23, 24, 25] Various phytochemicals present in plants namely alkaloids, flavonoids, terpinoids and tannins are producing very exciting opportunity for the expansion of modern chemotherapies against a very wide range of microorganisms [26]. The antimicrobial efficacy of plant phenolic compounds against Salmonella and E. coli have been reported [27] .The flavonoid aglycones, namely quercetin be present in *V. unguiculata* L. is known to inhibit the growth of various fungi and bacteria [28, 29]. A pathway for the development of new antimicrobial agents may be provided by the natural alternative treatments for bacterial and fungal infections.

In the present study a variety of gram positive and gram negative and fungal strain for the screening of antimicrobial effect of three selected seed varieties were selected to perceive the antimicrobial spectrum as well to authenticate ethnomedicinal claims. The results of this study showed that the LBS-1, LBS-2 and LBS-3 oil have varied antimicrobial activities against the tested microorganisms. Among these three varieties, LBS-1 oil at the concentration of 400 µg/disc was found more effective against *Sarcina lutia* (19±0.1) followed by LBS-1 and LBS-2 oil. LBS-3 oil at the concentration of 400 µg/disc was found more effective against *Staphylococcus aureus* (16±0.1) and LBS-3 oil at the concentration of 200 µg/disc was found more effective against *Penicilium spp.,* in order of effectiveness. The seed oil extracted from *Cannabis sativa* L. posses the antimicrobial activity has also reported [30].

Thus in the search of novel antimicrobial agents, this study has not only scientific basis for some of the therapeutic uses of traditional plant oils, but it also confirmed the ethnomedicinal claims for the selected plants.

CONCLUSION

From the above experiment it has been found that *V. unguiculata* seed oil might have some

phytochemicals, which are responsible for the antimicrobial activity. Finally it can conclude that *V. unguiculata* seed oil may be considered as the medicine for diarrhea and dysentery and can be useful as probiotics or as the sources of antifungal agents.

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