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NOOR AFSHAN KHAN

SADAF QUERESHI

AKHILESH PANDEY

ASHUTOSH SRIVASTAVA

TÜBİTAK

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Antibacterial Activity of Seed Extracts of Commercial and Wild *Lathyrus* Species

Noor Afshan KHAN[1], Sadaf QUERESHI[2], Akhilesh PANDEY[2], Ashutosh SRIVASTAVA[1]

[1]Department of P.G. Studies and Research in Chemistry, R. D. University, Jabalpur, M.P. INDIA

[2]Department of P.G. Studies and Research in Bioscience, R. D. University, Jabalpur, M.P. INDIA

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Abstract: Butanolic extract of seeds from 2 different *Lathyrus* species (*Lathyrus ratan* [commercial variety] and *L. aphaca* [wild variety]), were investigated for their in vitro antibacterial activity. The antibacterial activity against plant and human pathogenic bacteria was evaluated on the basis of inhibition zones (IZs) measured by disc diffusion method. The maximum inhibition was shown by *L. ratan* against the gram-positive bacterium *Staphylococcus aureus*. *L. aphaca* seeds also showed inhibition but were inactive against *Klebsiella pneumoniae*. *L. ratan* extract was more active than *L. aphaca*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for both the seeds (in µg/ml) were also determined. The lowest value was obtained for *Staphylococcus aureus* [MIC-76.25 (L.a.) 78.5 (L.r.) MBC- 112.6 (L.a.) 98.35 (L.r.)]; thus this bacterium was most inhibited by the seed extract, whereas *Bacillus subtilis* was least inhibited, as indicated by MIC [(98.50 (L.a.) 96.2 (L.r.)] and MBC [169.50 (L.a.) 151.2 (L.r.)] values.

Key Words: Antibacterial, *Lathyrus ratan, Lathyrus aphaca*, MIC, MBC

Ticari ve Yabani Tip *Lathyrus* Türlerinin Tohum Ekstrelerinin Antibakteriyal Aktiviteleri

Özet: İki farklı *Lathyrus* türünün (*Lathyrus ratan*; ticari varyete ve *L. aphaca*; yabani varyete) tohumlarının butanolik ekstrelerinin in vitro antibakteriyal aktiviteleri çalışılmıştır. Bitki ve insan patojenlerine karşı antibakteriyal aktivite Disk Difizyon Yöntemi ile zon çapları ölçülerek yapılmıştır. Maksimum inhibisyon çapı *L. ratan* türünde gram pozitif bacteri olan-*Staphylococcus aureus* da gözlenmiştir. *L. aphaca* tohumları da inhibisyon göstermişlerdir, fakat *Klebsiella pneumoniae* bakterisinde aktiflik göstermemiştir. *L. ratan* ekstresinin *L. aphaca* dan daha aktif olduğu gözlenmiştir. Minimum inhibisyon konsantrasyonu (MIC) ve minimum bakteriyal konsantrasyon (MBC) değeri de hesaplanmıştır (µg/ml). En düşük değer *Staphylococcus aureus* [MIC-76,25 (L.a.) 78,5 (L.r.) MBC-112,6 (L.a.) 98,35 (L.r.)] şeklinde gözlenmiştir. Tohum ekstresi tarafından en çok etkilenen *S. aureus* bakterisi olmuştur. Buna karşılık *Bacillus subtilis* bakterisi en az etkilenen bakteri olmuştur MIC [(98,50 (L.a.) 96,2 (L.r.)] ve MBC [169,50 (L.a.) 151,2 (L.r.)].

Anahtar Sözcükler: Antibakteriyal, *Lathyrus ratan, Lathyrus aphaca*, MIC, MBC

Introduction

Plants are invaluable sources of pharmaceutical products that have attracted the attention of ethnopharmacologists from around the world (1,2). A number of scientific investigations have highlighted the importance and contribution of many plant families, namely Umbelliferae, Lauraceae, Leguminosae, Cupressacae, etc., used as medicinal plants. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (3,4). The search for new plants with potential antimicrobial properties has intensified owing to the side effects associated with traditional antibiotics (5,6).

The genus Lathyrus is large, with 190 species and subspecies being recognized, which are common plants of central India (7). *Lathyrus aphaca* (Jangli matar) is a

common weed of wheat fields and is commonly used as fodder. Its ripe seeds produce a narcotic effect and are considered one of the possible causes of lathyrism. *Lathyrus ratan* (grass pea) seeds are consumed as pulses. The seeds yield fatty oil, which is cathartic (8,9). *L. aphaca* flowers contain flavonol glycosides, mainly larycitrin and syringetin-3-O-rutinoside-7-O-β-D-glucopyranosides, accompanied by low levels of kaempferol, quercetin, isorhamnetin analogues, and the respective 3-O-rutinosides (10). The seeds of *L. ratan* contain homoarginine, which serves as a dietary source of lysine, the sterols cholesterol, 24-epicholesterol, and isoavenasterol, and triterpene alcohols (11). The proximate principles of *L. aphaca* and certain leguminous seeds have already been determined by the author (12,13).

According to the available literature, no scientific evaluation of the antibacterial activity of these plants has been performed. The present study was therefore designed to investigate the antibacterial properties of the butanolic extract from the seeds against human and plant pathogenic bacteria.

Materials and Methods

Plant materials and extraction procedure

The seeds of *L. ratan* (commercial variety) and *L. aphaca* (wild variety) were collected from the Plant Breeding Department and wheat fields of Jawaharlal Nehru Krishi Vishwavidalaya, Jabalpur, in April 2005. The plant materials were further identified by Prof. K.S. Verma, Head, Dept. of Biological Sciences, R. D. University, Jabalpur. A voucher specimen was deposited in the Herbarium of National Research Centre for Weed Sciences, Jabalpur. Dried and milled plant materials were extracted sequentially with petroleum ether and methanol. Methanol extracts produced using 500 g of seeds were vacuum dried and further partitioned with n-hexane, $CHCl_3$, and BuOH to give a total of 4 fractions. The butanol extract was evaporated to dryness in a vacuum resulting in 150 g of brownish-yellow mass, which was stored at low temperature for further study.

Test Micro-organism

Five human and plant pathogenic bacteria—*Escherichia coli, E. coli* (MTCC-443), *Staphylococcus aureus* (MTCC-737), *Klebsiella pneumoniae* (MTCC-2405), and *Bacillus subtilis* (MTCC-1789)—were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, whereas the *Streptococcus* species was obtained from Chandraker pathology laboratory, Jabalpur, and were used in this study. The disc diffusion method outlined by the National Committee for Clinical Laboratory standards (NCCLS) (14) was used with 40 µg/ml Gentamicin sulphate disc as reference antibiotic. Zone size was measured after incubation of 37 °C for 24 h.

Antibacterial activity

The antibacterial activity was determined by disc diffusion method (15-17), in which 3 to 5 bacterial colonies from each agar plate were lifted with a sterile loop and transferred into a tube containing 5 ml of nutrient broth. The turbidity of each bacterial suspension was adjusted to reach an optical comparison with a 0.5 McFarland Standard, resulting in a suspension containing approximately 1×10^8 cfu/ml. The test solution was prepared by dissolving butanol extract in 20% MeOH. The final concentration of solution so obtained was 100 µg/ml. Nutrient agar plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking 2 more times, rotating the plates approximately 60° each time to ensure even distribution of the inoculum. After allowing the inoculum to dry at room temperature, the disc loaded with the extract of desired concentrations was placed on the agar plates. The positive control was maintained using gentamicin sulphate solution of concentration 40 µg/ml. The plates were then incubated at 37 °C for 18-24 h. Subsequently, the plates were examined for bacterial growth inhibition and the inhibition zone (IZ) diameter was measured.

The MIC and MBC was determined by well assay method (18). The solutions for MIC determination were prepared by 2-fold serial decrease dilution up to 65.25 µg/ml and that for MBC from 285.25 to 105.25 µg/ml. Wells of 6 mm diameter were bored in the agar plates. Each well was loaded with extract of different concentration. Microbial growth was determined macroscopically and recorded after 18-24 h of incubation at 37 °C. The MIC was determined as the lowest dilution corresponding to absence of IZs, whereas the lowest dilution yielding no growth was considered the MBC. Each assay in this experiment was performed 3 times.

Results and Discussion

In the present investigation butanolic extracts of plant seeds of *L. aphaca* and *L. ratan* were assessed for their antibacterial activity against some bacteria by disc diffusion method. As given in Table 1, among the 5 selected bacteria, the maximum zone of inhibition was formed by butanolic seeds extract of *L. ratan* against *Staphylococcus aureus* followed by the *Streptococcus* species, while very low inhibition was shown by *E. coli* and *K. pneumoniae* at a concentration of 100 µg/ml (Table 1). The butanolic seeds extract of *L. aphaca* exhibited lower antibacterial potential than *L. ratan*. *Staphylococcus aureus* was inhibited the most, followed by *Streptococcus species* and *E. coli*, while no zone of inhibition was observed for *K. pneumoniae*. The least antibacterial activity was recorded against the gram-positive bacteria *Bacillus subtilis* by extracts of both seeds.

The above obtained results are in contrast to the reported antibacterial activity of alcoholic extract of *Lathyrus odoratus* flowers (19). The isolated antibacterial compound was effective against *Bacillus subtilis* and *E. coli*. Reports are also available on the screening of hexane and methanol extracts of 16 plants of the family Caesalpiniaceae and the isolated phytochemicals were tested for their antibacterial activity (20). The methanol extracts of all the examined plants exhibit stronger growth inhibition against tested bacteria than hexane extracts. Reports revealed that the gram-positive bacterium *Staphylococcus aureus* was more sensitive only to n-hexane extract of the aerial parts of *Astragalus verrucosus* in comparison with other solvent extracts (21).

The antibacterial activity of the extracts and their potency were quantitatively assessed by determining the MIC and MBC values respectively (Table 2). It was

Table 1. Antibacterial activity of the butanolic extract (100 µg/ml) of seeds of *L. aphaca* and *L. ratan*.

Bacteria	Zone of inhibition (in mm)		
	L.a.	L.r.	S[b]
Gram positive			
Bacillus subtilis (MTCC-1789)	6.66 ± 0.08	6.76 ± 0.26	33 ± 0.04
Staphylococcus aureus (MTCC-737)	19.83 ± 0.44	31.0 ± 0.57	28 ± 0.02
Streptococcus species	18 ± 0.57	27.50 ± 0.50	40 ± 0.11
Gram negative			
Escherichia coli (MTCC-443)	10.07 ± 0.52	13.66 ± 0.33	38 ± 0.50
Klebsiella pneumoniae (MTCC-2405)	- -	9.33 ± 0.44	38 ± 0.02

Values are means ± SEM of 3 replicate analysis; - = not active against tested micro-organism; S = Standard: Gentamicin sulphate (40 µg/ml); L.a. = *L. aphaca;* L.r. = *L. ratan*.

Table 2: MIC and MBC Results (in µg/ml) against bacteria.

Bacteria	MIC µg/ml		MBC µg/ml	
	L.a	L.r.	L.a.	L.r
Escherichia coli	90.5	89.6	142.5	135.3
Klebsiella pneumoniae	N.T.	95.3	N.T.	142.50
Staphylococcus aureus	76.25	78.5	112.6	98.35
Streptococcus species	85.5	82.0	122.4	118.5
Bacillus subtilis	98.50	96.2	169.50	151.2

MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration, L.r. = *Lathyrus ratan*, L.a. = *Lathyrus aphaca*
NT = Not tested

considered that if the extract displayed MIC less than 100 µg/ml, the antimicrobial activity was good; from 100 to 500 µg/ml the antimicrobial activity was moderate; from 500 to 1000 µg/ml the antimicrobial activity was weak; over 1000 µg/ml the extract was considered to be inactive. The *Lathyrus* species, i.e. *L. aphaca* and *L. ratan*, show MIC values below 100 µg/ml, and therefore present good activity against the selected bacteria. However, the MIC value is maximum for *B. subtilis* for both species, and therefore this bacterium is the least effective. The lowest value is for *Staphylococcus aureus*, which shows that the seed extracts are more effective against *Staphylococcus aureus*. The results of MIC were in accordance with the MBC values, where the maximum value was obtained for *B. subtilis* and minimum for *Staphylococcus aureus*.

The antibacterial activities of various organic and aqueous extracts of leaves of *Indigofera suffruticosa* (Fabaceae) were determined by Leite et al. and it was found that only the aqueous extract showed strong inhibitory activity against the gram-positive bacterium *Staphylococcus aureus* with a MIC of 5000 µg/ml (22). A study on *Tamarindus indica* was conducted to evaluate antimicrobial activities of extracts of the stem, bark, and leaves. MIC and MBC of aqueous and organic extracts were found in gram-negative and gram-positive bacteria. The lowest MIC and MBC were demonstrated against *Salmonella paratyphi*, *Bacillus subtilis*, and *Salmonella typhi*, and the highest MIC and MBC were exhibited against *Staphylococcus aureus* (23).

Conclusions

From the above studies it can be concluded that the antimicrobial activity of the selected seeds extract would be helpful in treating various kinds of diseases. Crude extracts and their mechanism of interaction with different active fractions of the plants need to be explored. The bioactive compounds from *L. aphaca* and *L. ratan* seeds can be used as antibacterials after further studies.

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Corresponding Author:

Ashutosh SRIVASTAVA

Dept. P.G. Studies and Research in Chemistry,

R. D. University, Jabalpur, M.P. 482001. INDIA

E-mail: noor_afshan25@yahoo.com

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