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## Antibacterial Activity of the Broad Bean Extracts on Resistant Bacteria

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**Abstract:** The antimicrobial activities of four different extracts (ethanol, methanol, distilled water and 2-Methylbutan-1-01) obtained from different parts of broad bean (*Vicia faba* L.) such as flowers, leaves, seeds and seed hulls and were tested against *Bacillus subtilis*, *Serratia marcescens*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella* sp. and *Micrococcus pyogenes*. Better antimicrobial activity was observed with the leaves of broad bean sterile distilled water extract with a zone of inhibition in between 13-32 mm. Flower and seed hull showed by *Escherichia coli*, *Shigella* sp., *Bacillus subtilis*, *Serratia marcescens*, *Staphylococcus aureus* and *Micrococcus pyogenes* a zone of inhibition in between 15-29 and 14-19 mm, respectively. Leaves, seed hulls and flowers of broad bean with ethanol extract showed by *Escherichia coli* a zone of inhibition in between 25-34, 15-17 and 15 mm, respectively. Flower with ethanol extract had a zone of inhibition in between 15-16 mm by *Bacillus subtilis* and *Micrococcus pyogenes*, respectively. Different parts of Broad Bean with methanol and 2-Methylbutan-1-01 extract did not shown any antimicrobial affects.

**Key words:** Broad bean extracts activity, antimicrobial activity

### INTRODUCTION

Many of the plants used today were known to the people of ancient cultures throughout the world and they were valued their preservative and medicinal powers (Kivanc, 1997). The antimicrobial properties of plants have been investigated by a number of researchers world wide. The broad bean (*Vicia faba* L.), is a member of the Leguminosea family. It contains toxic substances such as saponins, lathrogens, cyanogenic and other glycosides, protease and amylase inhibitors and hemagglutinins (Rubatzky and Yamaguchi, 1997).

By a comparison of the compound Aglycon of Deoxy-Niazimicine (N-benzyl, S-ethyl thioformate) isolated from *Moringa oleifera* Lam. with crude chloroform extract showed a strong antibacterial activity against gram positive *Staphylococcus aureus* and gram negative *Shigella dysenteriae*, *Shigella boydii*, *Salmonella typhi* and *Pseudomonas aeruginosa* and produced zone of inhibition between 9 to 13 mm while the crud extract showed comparatively lower activity against gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Shigella dysenteriae*, *Shigella boydii* and produced zone of inhibition between 9 to 11 mm (Nikkon *et al.*, 2003). The effect of three different extracts (hexanic, ethyl acetate, methanol) obtained from various Brazilian Drosera species against different bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella*

*pneumoniae*, *Salmonella choleraesuis*, *Enterococcus faecium*, *Pseudomonas aeruginosa* and *Candida albicans* were evaluated. The best results were obtained with ethyl acetate extracts from *D. Montana* var. *montana* and *D. communis* (Ferreira *et al.*, 2004). Nascimento *et al.* (2000) also found antibacterial activity of *Achillea millifolium*, *Caryophyllus aromaticus*, *Melissa officinalis*, *Ocimum basilicum*, *Psidium guajava*, *Punica granatum*, *Rosmarinus officinalis*, *Salvia officinalis*, *Zyzygium joabolanum* and *Thymus vulgaris*. The phytochemicals benzoic acid, cinnamic acid, eugenol and farnesol were also utilized. The highest antibacterial potentials were observed for the extracts of *Caryophyllus aromaticus* and, *Zyzygium joabolanum*, which inhibited 64.2 and 57.1% of the tested microorganisms, respectively, with higher activity against antibiotic-resistant bacteria (83.3%). *Salvia officinalis*, *Achillea millifolium*, extracts did not present any antimicrobial activity.

As part of our investigation on the antibacterial activities of different parts of broad bean plant, a microbiological study of four different extracts was undertaken.

### MATERIALS AND METHODS

**Plant material:** Fresh plant parts (flowers, leaves, seeds and seed hulls) of broad bean were collected from the research field of Agricultural Faculty, The Guilan

University in Rasht, Iran in 2005 and dried in the sun for 5 days and finally in an oven below 50°C. The dried plant parts were ground in to fine powders.

**Preparation of the extracts:**

Each parts of plant material were extracted with the flowing solvents:

14 gram dried seed powder + 100 cc ethanol, methanol and sterile distilled water,

14 gram flower powder + 100 cc ethanol, sterile distilled water and 2-Methylbutan-1-01,

14 gram seed hull powder + 100 cc ethanol, sterile distilled water and 2-Methylbutan-1-01,

14 gram leaves powder + 100 cc ethanol, sterile distilled water and 2-Methylbutan-1-01.

The extracts were concentrated to a dark syrupy residue. This syrupy was dissolved once again in 10 cc in the above solvents. This liquid was used for testing inhibitory activity.

**Antimicrobial screening:** *In vitro* antimicrobial screening is generally performed by disc diffusion method (Vander and Vlietnck, 1999). Disc diffusion method is highly effective for rapidly growing microorganisms and the activities of the test drugs are expressed by measuring the diameter of the zone of inhibition (Nikkon *et al.*, 2003).

The microorganisms in this research were collected from the Faculty of Science, Microbiological Department, the Guilan University in Rasht, Iran. For antibacterial activity seven pathogenic bacteria (four gram negative and three gram positive) were selected. The plant extracts were dissolved separately in the solvents to get a concentration of 100, 200 and 300 µL. Discs were dried in an oven below 37°C for 8 days and then applied against seven pure cultured microorganisms.

**RESULTS**

The antimicrobial activity of the plant parts of broad bean (flowers, leaves, seeds and seed hulls) are shown in Table 1 and 2.

While seed extract did not showed any antibacterial activity against gram positive and gram negative bacteria, leaves extract in sterile distilled water showed higher antibacterial activity against gram positive *Bacillus subtilis* (Fig. 1), *Staphylococcus aureus* (Fig. 2), *Micrococcus pyogenes* (Fig. 3) and gram negative *Escherichia coli* (Fig. 4), *Shigella* sp. (Fig. 5), *Serratia marcescens* (Fig. 6). The leaves extract on *Klebsiella pneumonia* did not showed any antibacterial activity. The different parts of broad bean extracts in sterile distilled water produced against all tested organisms zone of inhibition between 13 to 32 mm by a concentration of 100 to 300 µL.

Table 1: *In vitro* antibacterial activity of sterile distilled water extract Zone of inhibition (Diameter in mm)

Name of bacterial strains	Flower			Seed			Leaves			Seed hull		
	100	200	300	100	200	300	100	200	300	100	200	300
	(µL)			(µL)			(µL)			(µL)		
<b>Gram positive</b>												
<i>Bacillus subtilis</i>	00	00	00	00	00	00	14	16	18	00	00	00
<i>Staphylococcus aureus</i>	13	20	22	00	00	00	13	17	22	00	00	00
<i>Micrococcus pyogenes</i>	00	00	00	00	00	00	23	31	32	00	00	00
<b>Gram negative</b>												
<i>Escherichia coli</i>	25	27	29	00	00	00	20	21	23	14	17	19
<i>Shigella</i> sp.	-	25	27	00	00	00	17	20	23	00	00	00
<i>Serratia marcescens</i>	00	15	17	00	00	00	00	13	16	00	00	00
<i>Klebsiella pneumonia</i>	00	00	00	00	00	00	00	00	00	00	00	00

Table 2: *In vitro* antibacterial activity of ethanol extract Zone of inhibition (Diameter in mm)

Name of bacterial strains	Flower			Seed			Leaves			Seed hull		
	100	200	300	100	200	300	100	200	300	100	200	300
	(µL)			(µL)			(µL)			(µL)		
<b>Gram positive</b>												
<i>Bacillus subtilis</i>	00	15	16	00	00	00	00	00	00	00	00	00
<i>Staphylococcus aureus</i>	00	00	00	00	00	00	00	00	00	00	00	00
<i>Micrococcus pyogenes</i>	00	00	15	00	00	00	00	00	00	00	00	00
<b>Gram negative</b>												
<i>Escherichia coli</i>	00	15	15	00	00	00	25	30	34	00	15	17
<i>Shigella</i> sp.	00	00	00	00	00	00	00	00	00	00	00	00
<i>Serratia marcescens</i>	00	00	00	00	00	00	00	00	00	00	00	00
<i>Klebsiella pneumonia</i>	00	00	00	00	00	00	00	00	00	00	00	00

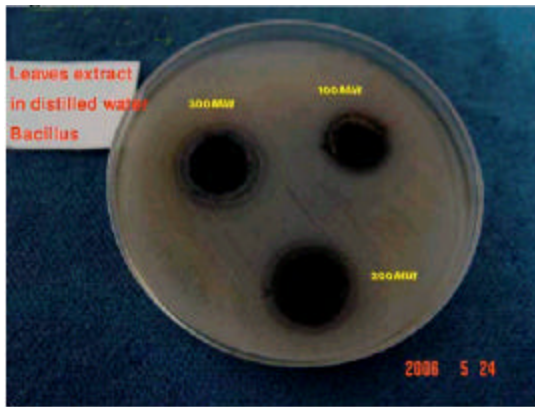


Fig. 1: Leaves extract in sterile distilled water by *Bacillus subtilis*

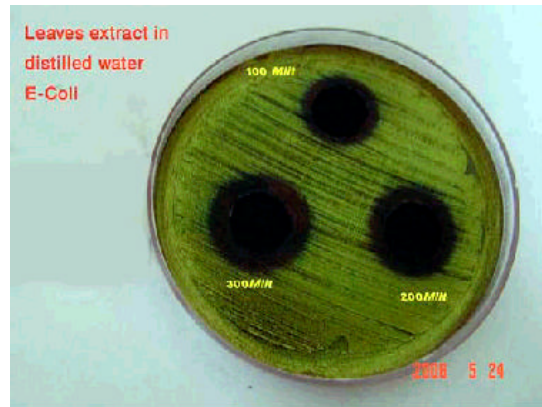


Fig. 4: Leaves extract in sterile distilled water by *Escherichia coli*

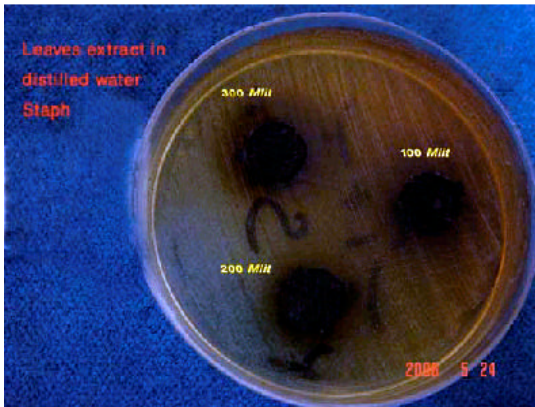


Fig. 2: Leaves extract in sterile distilled water by *Staphylococcus aureus*

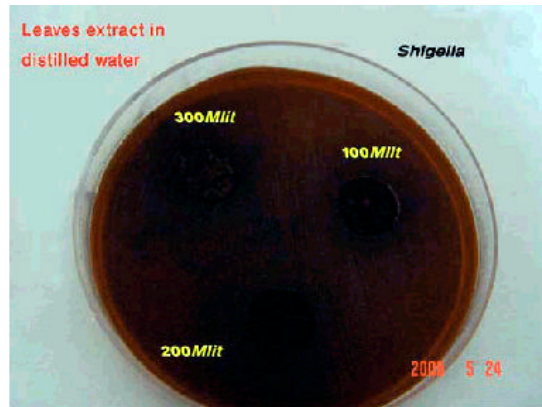


Fig. 5: Leaves extract in sterile distilled water by *Shigella* sp.

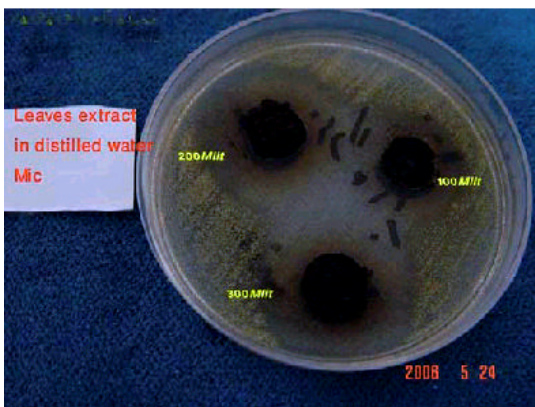


Fig. 3: Leaves extract in sterile distilled water by *Micrococcus pyogenes*

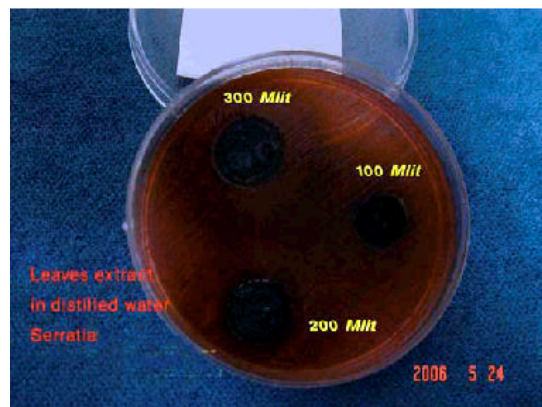


Fig. 6: Leaves extract in sterile distilled water by *Serratia marcescens*

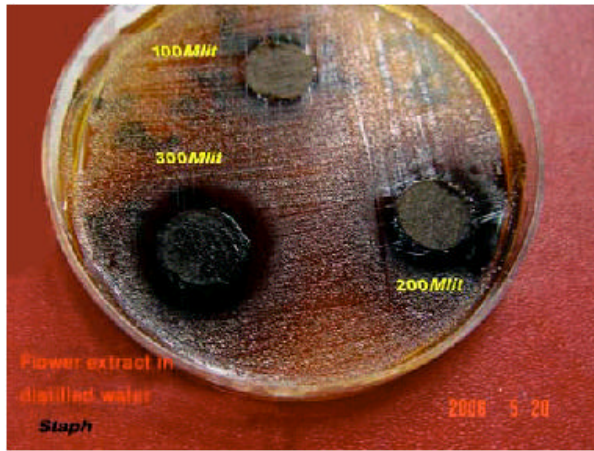


Fig. 7: Flowers extract in sterile distilled water by *Staphylococcus aureus*

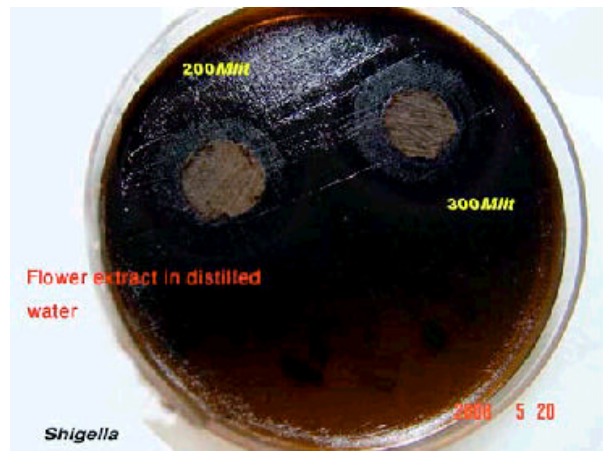


Fig. 10: Flowers extract in sterile distilled water by *Shigella* sp.

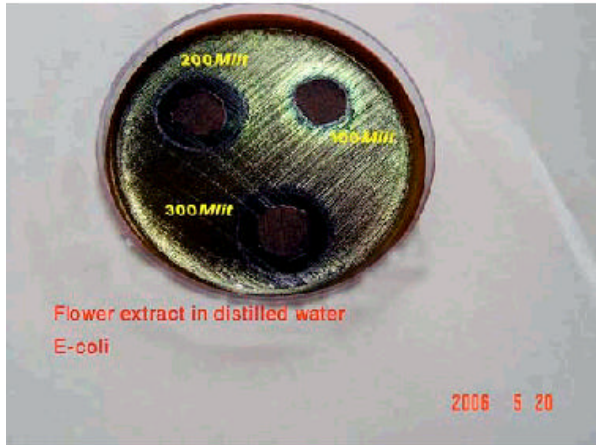


Fig. 8: Flowers extract in sterile distilled water by *Escherichia coli*

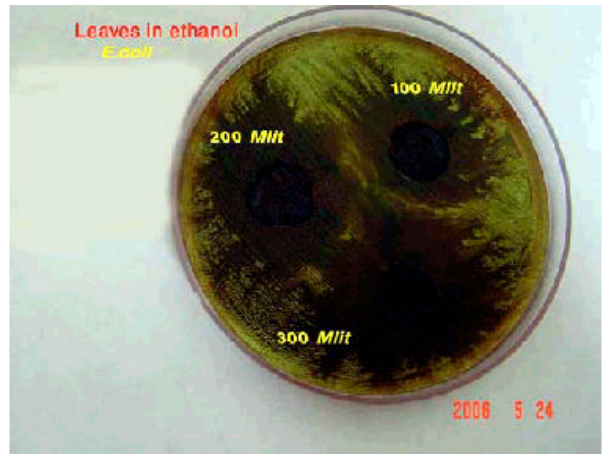


Fig. 11: Leaves extract in sterile distilled water by *Escherichia coli*

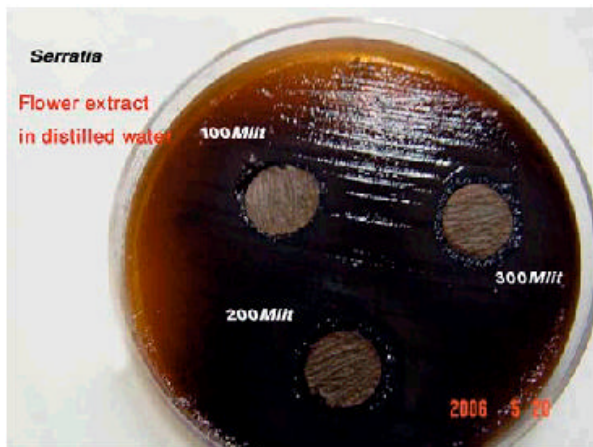


Fig. 9: Flowers extract in sterile distilled water by *Serratia marcescens*

Flower extract in sterile distilled water affected also *Staphylococcus aureus* between 13 to 22 mm (Fig. 7), *Escherichia coli* between 25 to 29 mm (Fig. 8) and *Serratia marcescens* (Fig. 9), between 0 to 17 mm by a concentration of 100 to 300 μL, respectively. *Shigella* sp. (Fig. 10) produced by 200 and 300 mm zone of inhibition 25 and 27 mm, respectively.

Leaves extract in ethanol produced against *Escherichia coli* zone of inhibition between 25 to 34 mm by a concentration of 100 to 300 μL (Fig. 11). Flower and Seed hull extracts in ethanol showed in the tested concentration lower zone of inhibition.

Different parts of broad bean extracts in Methanol and 2-Methylbutan-1-01 couldn't affect against all organisms tested in our research.

## DISCUSSION

Our investigation showed that different parts of broad bean extracts could produce relative highly antimicrobial activity against various bacteria. Best results were obtained with sterile distilled water and ethanol extracts from leaves and flowers as shown in Table 1 and 2. Seed and seed hull of broad bean did not produced valuable zone of inhibition. With the exception of *Klebsiella pneumoniae* (resistant), the other gram-negative and gram-positive bacteria were sensitive to all extracts (*Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus pyogenes*, *Escherichia coli*, *Shigella* sp., *Serratia marcescens*). We consider these as preliminary results and a larger number of bacterial isolated must be tested, with different concentrations of broad bean extracts in order to establish their real antimicrobial activity. Our investigation determined the relation between extract concentration and bactericidal effect. The isolation of essential oil of mature leaves of *Cestrum diurnum* (Bhattacharjee *et al.*, 2005), the root of *Moringa pterygosperma* (Kurup and Rao, 1952) and different species of *Drosera* (Ferreira *et al.*, 2004), are agreement with the tests accomplished in this study.

## CONCLUSIONS

Data from our results reveal the great potential of leaves and flower of broad bean for therapeutic treatment, in spite of the fact that they have not been completely investigated. Therefore, more studies need to be conducted to search for new compounds. In conclusion, this investigation reports that flower and leaves of broad bean extracts in sterile distilled water possesses antibacterial activity and could be used in medicine after toxicity test.

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