Comparison of Fresh with Dry Extracts for Antibacterial Activity of *Vigna radiate* L. on Pathogenic Bacteria

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ABSTRACT

Introduction: *Vigna radiat*a L. is common vegetable plant cultivated all of the world. It's belonging to the family Leguminosae (Fabaceae**). Methods:** To investigate for antibacterial activity of *Vigna radiata*, fresh and dried extracts of plant parts were tested on five strains of bacteria using standard well agar diffusion method. **Results:** Dried extracts showed more effective action on tested bacteria than of fresh extracts. Extracts prepared from dried stem and root exhibited better antibacterial activities than those prepared from fresh plant. Furthermore, there was no difference in the activity of ethanolic and aqueous extracts on isolated bacteria. Both of gram positive and negative bacteria showed approximately the same ratio of susceptibility to each part of plant.**Conclusions:** *V. radiata* has a potential antibacterial activity on clinically isolated bacteria. Dried extracts showed more effective action on tested bacteria than fresh extracts.

Key words: aqueous extract, ethanolic extract, Vigna radiata**,** bacteria

INTRODUCTION

For testing antimicrobial activity of any suggesting plant, preparation of extract from fresh parts is preferred due to retain the components of plant in active state.[1] This is not always available because selected plants needed to collect from so far distance from the place of actually extraction work. Thus, plant must be dried until extraction time.

Many studies are designed to compare between antimicrobial activities of fresh and dry plant. Results are variable to determine which state of plant is effective against organisms. Pepeljnjak et al[2] found that extracts prepared from fresh leaves of *Pelargonium radula* have significant higher antimicrobial activity than those prepared from dried leaves. Fresh fruit shell of Pomegranate is also has antibacterial effect on the bacterium *Ralstonia solanacearum* than dry fruit shell.[3] Whereas, Goyal et al[4] demonstrated that dry powder extracts of all *Catharanthus roseus* parts showed more antibacterial activity than extracts prepared from fresh parts.

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Vigna radiata L. or also called mungbean is belonging to the family Leguminosae (Fabaceae). It is very important economic plant through its contents of valuable nutrients. Furthermore, *V. radiata* facilitates the nitrogen fixation in soil by producing nodules on its root in combines with *Rhizobium*.[5] However, *V. radiata* contains within its species many genotypes resistance to bacterial infection.[6]

To determine the variation between antibacterial activity of fresh and dry parts of *Vigna radiate*, extracts of these plant parts was tested on many bacteria. Furthermore, this study tried to detect the differences between the activity of fresh and dry plant against bacteria.

MATERIALS AND METHODS

Plant preparation

Seeds of *Vigna radiate* L. (Fabaceae) were obtained from institute of agriculture of Karbala province (Iraq). Cultivation was performed in prepared field with suitable soil during July to August 2009. Mature plants were harvested and washed under running tap water. Root nodulation and damaged parts were removed. Plant materials (leaves, stem, and root) were separated and washed once again with distilled water.

Plant extracts

Extraction was performed by two different modes: (1) Extraction of fresh plant materials without drying and (2) Extraction after each plant part was air-dried

under room temperature. The 20 g of grounded plant part was extracted in electrical blender with 100 ml of ethyl alcohol (70%) for obtaining ethanolic extract and with sterilize distilled water for obtaining aqueous extract for 5 min and left for 1 h. Extracts were filtered through sterilized gauze and concentrated to dryness at room temperature.

Test organisms

Tested pathogenic bacteria were clinically isolated from AL-Hussein general hospital in August 2009. Five strains of bacteria were isolated. Strains were diagnosed using API 20 system (Biomérieux, Netherlands-France). The isolated bacteria were: *E. coli, Staphylococcus aureus, Klebisella pneumoniae, Proteus vulgaris,* and *Bacillus subtilis,*.

Antibacterial assay

Standard culture of bacteria for antibacterial assay was prepared by culturing of isolated bacteria in Mueller-Hinton broth (HiMedia, Mumbai-India) to become equivalent to 0.5 MacFarland standard (reading to 1×10^8 cfu/ml) and diluted 1:10.

All extracts were sterilized by sterile membrane syringe filter (pore size 0.45 µm, manufactured by Pall Life Science). Well agar diffusion recommended by NCCLS[7] was used. A well of 6 mm was performed in plate with Mueller-Hinton agar (HiMedia, Mumbai-India) inoculated with isolated bacterial strains. Various concentrations (3.125, 6.25, 12.5, 25, 50 mg/ml) of fresh and dried extracts were prepared in sterilize distilled water. Each well filled with 50 µl of specific concentration of extract. Cefotaxime sodium (30 µg) supplied by KonTam pharmaceuticals co. Zhongshan-China and distilled water were used as controls.

Determination of Minimum Inhibitory Concentration (MIC)

MICs were determined as described by NCCLS.[7] Crude extracts were twofold diluted in Mueller-Hinton broth for bacteria. A 100 µl of each dilution was dispensed in well of microdilution plates (96-wells). Well was inoculated with 50 µl of previously prepared standard culture of bacteria. The inoculated plates were incubated at 35ºC for 24 h and examined for visible growth in order to determine MIC. The previous controls were also included.

Table 1: Antibacterial activity of fresh parts of *V. radiata* on isolated bacteria by well agar diffusion method

Plant parts	Extract type	Concen. (mg/ml)	Zone of inhibition (mm)				
			S. aureus	*E. coli*	*K. pneumoniae*	*B. subtilis*	*P. vulgaris*
Leaves	Ethanolic	50	–	14*	–	19*	16*
		25	–	12*	–	18*	16*
		12.5	–	–	–	15*	14*
		6.25	–	–	–	13	–
		3.125	–	–	–	–	–
	aqueous	50	–	15*	–	17*	18*
		25	–	12*	–	16*	15*
		12.5	–	11	–	16*	11
		6.25	–	–	–	10	–
		3.125	–	–	–	–	–
Stem	Ethanolic	50	–	–	–	–	–
		25	–	–	–	–	–
		12.5	–	–	–	–	–
		6.25	–	–	–	–	–
		3.125	–	–	–	–	–
	Aqueous	50	15*	17*	–	20*	22*
		25	–	16*	–	20*	19*
		12.5	–	9	–	18*	15*
		6.25	–	–	–	13	11
		3.125	–	–	–	10	–
Root	Ethanolic	50	–	13*	–	–	–
		25	–	11	–	–	–
		12.5	–	–	–	–	–
		6.25	–	–	–	–	–
		3.125	–	–	–	–	–
	Aqueous	50	–	–	–	–	–
		25	–	–	–	–	–
		12.5	–	–	–	–	–
		6.25	–	–	–	–	–
		3.125	–	–	–	–	–
Cefotaxime		30 µg/ml	27	22	–	34	21

*** Significant differences ($P < 0.05$) between parts of plant and cefotaxime**

Statistical analysis

Results were statistically analyzed by using two-way variance of analysis (ANOVA) with less significant difference (L.S.D.) at $P < 0.05$.

RESULTS

Antibacterial activity of fresh and dried parts of *Vigna radiata* was investigated using standard well agar diffusion method. Data of this study revealed variable results of antibacterial effect of different plant parts. Extracts prepared from dried stem and root exhibited better antibacterial activities with significant differences ($P < 0.05$) from standard antibacterial agent (cefotaxime) than those prepared from fresh plant, while leaves extract showed reflective results. The extract of fresh leaves revealed more effective than of dried leaves. Otherwise, there is no significant differences ($P < 0.05$) was noted between the activity of ethanolic and aqueous extracts on all tested bacteria (Table 1 and 2).

Fresh aqueous extracts of root and aqueous and ethanolic extract of stem exhibited no activity on all isolated strains, while dried extracts of the same plant parts showed much more antibacterial effects.

Based on bacteria strain, *K. pneumoniae* showed resistance to all types of extracts of all plant parts, followed by *S. aureus* in most concentrations. On the other hand, *B. subtilis* as one of gram positive bacteria showed more susceptibility to most extracts, especially to dried extracts of stem and root with least MIC value (Table 3). Meanwhile, gram negative bacteria represented by *P. vulgaris* were also susceptible to most concentrations of plant parts, especially to dried extracts. Thus, both of gram positive and negative could be considered have the same susceptibility to *V. radiata* extracts.

DISCUSSION

Although large numbers of plants are constantly being screened for their antimicrobial effects, plant kingdom still holds many species containing substances of medicinal value that have yet to be discovered. *V. radiata* considers important plant enrichment with valuable contents including

Table 2: Antibacterial activity of dried parts of *V. radiata* on isolated bacteria by well agar diffusion method

Plant parts	Extract type	Concen. (mg/ml)	Zone of inhibition (mm)				
			S. aureus	*E. coli*	*K. pneumoniae*	*B. subtilis*	*P. vulgaris*
Leaves	Ethanolic	50	–	14*	–	20*	17*
		25	–	–	–	–	–
		12.5	–	–	–	–	–
		6.25	–	–	–	–	–
		3.125	–	–	–	–	–
	Aqueous	50	–	18*	–	18*	18
		25	–	11*	–	11	–
		12.5	–	–	–	–	–
		6.25	–	–	–	–	–
		3.125	–	–	–	–	–
Stem	Ethanolic	50	17*	16*	–	24*	19*
		25	–	14*	–	20*	17*
		12.5	–	13*	–	16*	14*
		6.25	–	11*	–	13	13*
		3.125	–	–	–	11	11
	Aqueous	50	15*	17*	–	20*	22*
		25	–	16*	–	20*	19*
		12.5	–	9	–	18*	15*
		6.25	–	–	–	13	11*
		3.125	–	–	–	10	–
Root	Ethanolic	50	18*	15*	–	23*	19*
		25	–	13*	–	21*	18*
		12.5	–	11*	–	19*	15*
		6.25	–	–	–	13	12*
		3.125	–	–	–	12	10
	Aqueous	50	–	11*	–	22*	20*
		25	–	10	–	20*	15*
		12.5	–	–	–	17*	10
		6.25	–	–	–	11	9
		3.125	–	–	–	–	–
Cefotaxime		30 µg/ml	27	22	–	34	21

*** Significant differences ($P < 0.05$) between parts of plant and cefotaxime**

Table 3: MICs (mg/ml) of *V. radiata* extracts in pathogenic bacteria

Plant parts	Extract type	*S. aureus*	*E. coli*	*B. subtilis*	*P. vulgaris*
Fresh leaves	Ethanolic	>50	16	4	10
	Aqueous	>50	8	4	10
Dried leaves	Ethanolic	>50	30	40	32
	Aqueous	>50	16	16	36
Fresh stem	Ethanolic	>50	>50	>50	>50
	Aqueous	>50	>50	>50	>50
Dried stem	Ethanolic	32	4	1	1
	Aqueous	38	10	0.8	4
Fresh root	Ethanolic	>50	16	>50	>50
	Aqueous	>50	>50	>50	>50
Dried root	Ethanolic	48	8	1.2	0.8
	Aqueous	>50	10	0.8	>50

proteins, arachidic acid, arginine, ascorbic acid, genstein and shikimic acid.[8] Other components that protect *V. radiata* from microbial infection may also present. Thus, exposure of *V. radiata* to pathogenic microorganisms stimulated production of antioxidant activity and phenolic compounds.[9]

Based on present study, it is well demonstrated that extracts prepared from dried plant parts revealed better antibacterial activity than those prepared from fresh parts. Extracts of dried stem and root showed greater diameter of zone of inhibition on cultured of most isolated bacteria. While, fresh extract of *Pelargonium radula*, in previous study, showed more effects on both gram positive and negative bacteria than dried extract.[2]

Data also indicated that both strains of gram positive and negative were affected by plant materials at the equal level with no difference in the susceptibility of these groups to each of plant extracts. *K. pneumoniae* as one strain of gram negative and *S. aureus* as one strain of gram positive showed more resistant to plant extracts. Meanwhile, *B. subtilis* (gram positive) and *P. vulgaris* (gram negative) revealed susceptibility to these extracts. The similarity in the response of two main groups of bacteria to plant extracts may related to the presence of broad spectrum compounds in *V. radiata* that active against gram positive and gram negative bacteria.

There is frequently much variable suggestion about which type of solvent is preferred to use in extraction method that can exhibit the potential activity of plant on bacteria. In some cases, ethanolic extract showed more efficiency to inhibit microorganism's growth as noted with the activity of ethanolic extracts of *Catharanthus rose* on six strains of bacteria.[4] Otherwise, two strains of *S. aureus* inhibited by aqueous extracts of V. *radiata*, while ethanolic extract effected on other strain (*S. subflava*).[8] Furthermore, dried alcoholic extract of *V. radiata* seeds was failed to inhibit the bacterium *Burkholderia pseudomallei*.[10] However, parts of *V. radiata* in the form of ethanolic and aqueous extracts revealed approximately the same manner of activity on isolated bacteria.

CONCLUSIONS

V. radiata has a potential antibacterial activity on clinically isolated bacteria. Dried extracts showed more effective action on tested bacteria than fresh extracts. Furthermore, no differences were noted between ethanolic and aqueous extracts on both of gram positive and negative bacteria with no differences in the susceptibility of each group of bacteria to any part of plant.

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