Journal of Bio-Molecular Sciences (JBMS) (2013) 1(1-2): 11-16.

Antibacterial Activity of Leaf Extracts of *RicinusCommunis* Collected from Different Climatic Zones of Pakistan

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Received 05 November 2013; Accepted 15 December 2013; Available online 28 December 2013

Abstract: Methanolic leaf extracts of *Ricinuscommunis* collected from different climatic zones of Pakistan were screened for antimicrobial activity against different Gram-positive and Gram-negative human important pathogenic bacteria as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiellapnemoniae*, *Shegella*, *Vibreo cholera* and *Proteus*, by using agar cup diffusion method. It was found that all *Ricinuscommunis* species from different origin shows high zone of inhibition against the tested pathogens. These results demonstrated that *R. comminus* contain potential components that may be beneficial for evolution of pharmaceutical and can be use as a medicine against different types of bacterial phathogenic flora of human body.

Keywords: *Ricinus Communis*; Antimicrobial; Well diffusion method; Zone of Inhibition

Introduction

Importance of local resources is well known from centuries as a remedies, and play major role as a therapeutic agent in the basic health care facilities of rural and undeveloped areas (Banso and Bani, 2003). Plants produced different types of anti microbial substances (Shakasma et al., 1979; Banso and Bani, 2003; Nisar et al., 2012). Herbal medicine is used from centuries for treatments of different diseases (Kota and Manthri, 2011). Medicinal plants are rich in different type of important phyto chemicals which is used for Bacterial infections (Poonam and Pratap, 2012). According to Mothana and Linclequist (2005), 20 % of the plants all over the world are using for different biological and pharmaceutical activities.

Ricinuscommunis is poisonous, ornamental herb or tree both found in tropical and temperate region of the world having viable medical and industrial importance commonly used as disinfectant,

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remedy for eyes inflammation, in cosmetics and other pharmaceutical preparation (Mamma and prayaga, 2006; Raoof and yasmeen, 2006; Chanda, and Baravalia, 2010), as a Laxative (Capasso et al., 1994), against various pathogenic and dermatophytic bacteria. Eighty percent of the world population directly or in directly involved in using medicinal plants for therapeutic purpose alone or in combination with other pharmaceuticals (McGaw and Eloff, 2005). *Ricinus communis* methanolic extract was used in rats and show antimicrobial activity (Oyewole, 2010).

The Aim of the present study is to show out the antimicrobial activity of *Ricinus communis* and introduced it as a cheap therapeutic agent in coming future because of the major problems with antibiotics, because various men known important pathogenic micro flora produced resentence to these antibiotics causing many severe side effects.

Material and Methods

Sample Collection

Plants of *Ricinus communis* were collected from different climatic region of Pakistan as Swat, Peshawar, Kohat, Rawalpindi, and Lahore regions, and were characterized as *Ricinus communis* according to the standard procedure of I.C.B.N (International Committee for Botanical nomenclature). Leaves were separated from the plants, washed with Distilled water and make it Dry for 35 – 40^{o}C. Dry plant materials were grounded to fine powder with help Electric grinder.

Extract preparation

Plant extract was obtained according to Olorunclare et al. (1992) with slight modification. Ten grams of plant powder was extracted with 200 ml of methanol in conical flask covered with aluminum foil and plugged with sterile cotton, placed in shaker for 24 hours for complete homogenization at 35 ^{o}C. Homogenized extract was filter through Whatman #1 filter paper and stored in refrigerator.

Test Organisms

Bacterial strains as *Escherichia coli, Staphylococcus aurous, Salmonella typhi, Klebsiella pnemoniae, Shegella, Vibreo cholera, Proteus* were obtained from the Laboratory of Microbiology department, Kohat University of Science and Technology, Pakistan for antimicrobial activity. Sub Culture of these strains was obtained on nutrient agar for 24 hours, and dilutions of strains were made in distilled water with a loop full of inoculums.

Anti Bacterial Activity

Agar diffusion method (Boakye-yiadom, 1979) was used for anti bacterial activity. Clinical important microorganisms were uniformly spread in nutrient agar with the help of sterile swab inoculators. Uniform holes were make with the help of sterile tips 150 micro liter plant extract of different origins was poured using different concentration of amoxilline as a control with no plant extract. All the Petri plates were placed in sterile condition at room temperature for 1 hour that extract been diffused in the agar. After that place all the Petri plates in incubator for 24 hours at 37^{o}C and after 24 hours zone of inhibition was measured.

Statistical Analysis

All the results were obtained from four times repeated experiments. The ANOVA and standard error were calculated by using statistic 9 Software.

Result and discussion

The determinations of the present study relieved that the tested five different locations of *Ricinus comminus* extracts possesses antibacterial activity against *E.coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pnemoniae*, *Shegella*, *Vibreo cholera* and *Proteus* at concentration of 1µg/µl, 0.1µg/µl, 0.01µg/µl and 0.001µg/µl. The results were compared with a standard antibiotic Amoxilline with concentration of 1µg/µl, 0.1µg/µl, 0.01µg/µl and 0.001µg/µl. All the extract exhibited significant activity.

Extract of swat showed highest activity against Klebsiella *pnemoniae* (23.8 mm), *Staphylococcus aureus* and *E.coli* (20.9 mm), while least activity was observed against *Salmonella typhi* (14.5 mm), *Shegella* (16.1 mm), *Proteus* (13.5 mm) *and Vibreo cholera* (12.5 mm) among other location of extract at concentration of 1 µg/µl. whereas antibiotic, amoxilline (1 µg/µl) showed activity against all bacterial strains ranged from 21.3-39.9 mm as illustrated in Table 1. The inhibition zone equal or above 14 mm is considered as a high antibacterial activity (Philip et al., 2009).

Peshawar and Kohat extracts showed activity against all tested organisms with zone of inhibition ranged from 1.1-9.7 mm, while Swat, Rawalpindi and Lahore extracts showed activity against all microorganism except *Shegella* and *Vibreo cholera* at 0.1 µg/µl concentration, Whereas the antibiotic showed activity against all tested organism ranged from 18.9-31.1 mm (Table 2).

Table 1. Antibacterial activity of leaf extracts of *Ricinus communis* at concentration of 1 µg/µl.

Bacterial strains	Extract concentration (1 µg/µl)					
	Diameter of zone of inhibition (mm)					Standard drug
	Swat	Peshawar	Kohat	Rawalpindi	Lahore	Amoxilline
Escherichia coli	22.9±.45	19.4±.808	19.1±.2	15.4±.321	15.8±.1	28.6±.360
Staphylococcus aureus	20.9±.208	22.8±.360	22.3±.057	19.6±.458	18.9±.378	39.9±.4
Salmonella typhi	14.5±.305	18.2±.4	17.5±.3	13.2±.3	12.8±.351	39.9±.208
Klebsiellapnemoniae	23.8±.305	24.2±.64	23.5±.4	18.6±.264	18.9±.115	26.4±.305
Shegella	16.1±.351	11.2±.346	11.6±.25	11.7±.650	11.2±.472	28.9±.5
Vibreo cholera	12.5±.351	9.9±.793	10.3±.152	9.8±.351	9.4±.435	22.5±.351
Proteus	13.5±1.26	14.7±.152	14.1±.43	16.5±.321	15.2±2.3	21.3±1.05

Table 2. Antibacterial activity of leaf extracts of *Ricinus communis* at concentration of 0.1 µg/µl.

Bacterial strains	Extract concentration (0.1 µg/µl)					
	Diameter of zone of inhibition (mm)					Standard drug
	Swat	Peshawar	Kohat	Rawalpindi	Lahore	Amoxilline
Escherichia coli	9.8±.305	7.9±.41	7.8±.3	5.7±.64	5.7±.152	25.7±.264
Staphylococcus aureus	8.4±.4	9.7±.057	9.6±.152	7.8±1.44	7.4±.288	31.1±.351
Salmonella typhi	5.4±.556	6.3±.556	5.4±.321	4.9±1.09	4.8±1.05	30.3±.1
Klebsiellapnemoniae	10.1±.709	8.4±.305	8.3±.1	5.8±.602	6.1±.251	15.1±.264
Shegella	-	1.5±.351	1.1±.208	-	-	22.8±.4
Vibreo cholera	-	2.5±.32	2.7±.208	-	-	26.3±.503
Proteus	2.5±.351	5.8±.360	5.9±.435	4.2±.057	3.9±.1	18.9±.2

Highest activity was observed in Swat extract that showed activity against *E.coli*, *Staphylococcus aureus* and *Klebsiella pnemoniae* with a zone of inhibition of 2.6, 2.7 and 5.9 mm while other showed no activity at 0.01 µg/µl concentrations (Table 3). Extract of Peshawar and Kohat showed activity against all except *Shegella* and *Vibreo cholera*, whereas Rawalpindi and Lahore extracts at 0.01 µg/µl concentrations recorded activity against *E.coli*, *Staphylococcus aureus* and *Klebsiella pnemoniae* with zone of inhibition ranged from 1.7-2.7 mm. While the antibiotic showed activity against all tested organisms with zone of inhibition ranged from 9-23.6 mm (Table 3).

The highest zone of inhibition at 0.001 µg/µl concentration showed only in Swat extract against *E.coli*, *Staphylococcus aureus* and *Klebsiella pnemoniae* with a values 0.8, 0.5 and 0.6 mm, while Peshawar, Kohat, Rawalpindi and Lahore extract do not show any activity against the tested organisms (Table 4).. However, anitibiotic at 0.001 µg/µl concentration, showed activity against *Staphylococcus aureus* (19.1 mm), *Klebsiella pnemoniae* (2.5 mm), *Shegella* (9.4 mm)and *Proteus* (14.2 mm) while no activity were observed against *E.coli, Salmonella typhi and Vibreo cholera* (Table 4).Uddin *et al.* (2005)showed that methanol extract of *R. communis* has antimicrobial activity against *S. aureus.* They also observed that antimicrobial activity against *E. coli, Klebsiell apnemoniae, Salmonella typhi, Vibreo cholera and Proteus* in *R. communis.* It has been well documented that that methanol is highly potent solvent for extracting the phytochemicals from the plant material (Parekh and Chanda, 2007). The substantial activity of methanol extract, which is slightly lesser than the standard antibiotics, tends to show that the active compounds of the plants have the capability to use as a medicine against different types of bacterial phathogenic flora of human body.

Table 3. Antibacterial activity of leaf extracts of *Ricinus communis* at concentration of 0.01 µg/µl.

Bacterial strains	Extract concentration (0.01 µg/µl)					
	Diameter of zone of inhibition (mm)					Standard drug
	Swat	Peshawar	Kohat	Rawalpindi	Lahore	Amoxilline
Escherichia coli	2.6±.305	2.6±.3	2.7±.305	1.7±.152	1.8±.057	15.2±305
Staphylococcus aureus	2.7±.288	2.8±.602	2.5±.351	2.0±.378	2.1±.351	22.7±.458
Salmonella typhi	-	1.63±.513	1.0±.150	-	-	23.6±.37
Klebsiellapnemoniae	5.9±.321	4.4±.503	4.6±.230	2.5±.461	2.7±.2	9.0±.152
Shegella	-	-	-	-	-	14.5±.264
Vibreo cholera	-	-	-	-	-	17.2±.115
Proteus	-	1.2±.208	1.4±.435	-	-	15.5±1.35

Table 4. Antibacterial activity of leaf extracts of *Ricinus communis* at concentration of 0.001 µg/µl.

Bacterial strains	Extract concentration (0.001 µg/µl)					
	Diameter of zone of inhibition (mm)					Standard drug
	Swat	Peshawar	Kohat	Rawalpindi	Lahore	Amoxilline
Escherichia coli	.8±.07	-	-	-	-	-
Staphylococcus aureus	.5±.064	-	-	-	-	19.1±.650
Salmonella typhi	-	-	-	-	-	-
Klebsiellapnemoniae	.6±.015	-	-	-	-	2.5±.832
Shegella	-	-	-	-	-	9.4±1.07
Vibreo cholera	-	-	-	-	-	-
Proteus	-	-	-	-	-	14.2±.680

Conclusion

The present study concluded that methanolic leaf extracts of *R. comminus* possess significant inhibitory activity at different concentration against the tested organism. *R. comminus* contain potential components that may be beneficial for evolution of pharmaceutical and for the therapy of ailments. Further studies on these plant was going on in order to isolate, identify, characterized and elucidate the structure of the bioactive principles to develop new antibacterial medications.

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