INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 9, No. 6, p. 19-27, 2016

RESEARCH PAPER OPEN ACCESS

Antibacterial potential of the crude extracts of *Trianthema portulacastrum* L.

Annam Zikrea*[1], Zaheer-ud-din Khan[2], Muhammad Waheed[2]

[1]*Department of Botany, Government College for Women University, Faisalabad, Pakistan*

[2]*Department of Botany, Government College University, Lahore, Pakistan*

Key words: Antibacterial, Agar well diffusion technique, Zone of inhibition, Minimum inhibitory concentration, Aizoaceae.

http://dx.doi.org/10.12692/ijb/9.6.19-27 Article published on December 11, 2016

Abstract

The weed indigenous to Punjab belonging to family Aizoaceae namely *Trianthema portulacastrum* was assessed for its antibacterial perspective. The steady-state maceration used for the extract preparation established that more phytochemical contents were macerated in fruit extract. The antibacterial potential was assessed employing agar well diffusion technique for measuring zone of inhibition and verified using agar dilution scheme by analyzing Minimum Inhibitory Concentration. Antibacterial activity ranged from 13.4±0.90 to 35.9±0.46mm, with maximum potential reported by ethanol extract of stem and minimum efficacy obtained by alcoholic macerate of root against *E. coli*. In addition, significant MIC (1.25mg/mL) was exhibited by ethanol extract of fruit against *B. subtilis*, ethanol extract of stem and *n*-hexane extract of fruit in opposition to *E. coli*, chloroform extract of fruit in contradiction to *P. aeruginosa* and ethanol extract of fruit against *S. aureus*. The correlation between zones of inhibition and MIC values concluded that negative association exist between the two categories i.e. increase in the zones of inhibition lead to the decrease in MIC and vice versa.The preliminary results presented in this study put forward some potential macerates derived from *T. portulacastrum*. However, further studies are needed to identify and isolate the active compounds responsible for the antibacterial action.

* **Corresponding Author:** Annam Zikrea ✉ azikrea@yahoo.com

Introduction

It is an alarming scenario that throughout the world, particularly in third world nations, primary cause of mortality in living creatures are the afflictions provoked by contagious microbial species (Livermore, 2000).The compound that exterminate or decelerate the multiplication frequency of microbes is characterized as antimicrobial (Abdullah *et al.*, 2012). If antimicrobial substance executes inhibition of microbial proliferation capacity then it is designated as micro-biostatic whereas, the prescription whose action bring about complete cessation of microbe is entitled as microbicidal (Jain *et al.*, 2012).

Mankind is enormously reliant upon the pharmacological organizations to combat with the resilient contagious strains. Nearly 70% of the incidents referred to the US hospitals had endowed bacterial contagions whom strains had acquired obstruction to certain magnitude. The utmost conspicuous in this concern isgram-positive, methicillin-resistant bacteria*Staphylococcus aureus* with its presence in more or less 50% of the samples macerated from the convalescent (Cushnie and Lamb, 2005). The microbes procure constraint at the pace in which its genome modifies in response to the certain antimicrobial chemosynthetic product. This time-passage can be restricted from a finite duration to the considerable span. Indeed, occasionally microbes did not obtain resistance but, the expedition for exploring contemporary antibacterial substitutes will be endured as a constant imperative concern (Berkowitz, 1995).

Therapeutic consultants of accustomed pharmaceuticals had already acknowledged the worth of voluminous aboriginal flora for combating numerous ailments. The plant-based medications having their origins in conventional prescriptions are presently assumed as advanced and compelling counterfeit for the synthetic antibiotics (Raja *et al.*, 2013). The extravagant cost, hostile aftermath reactions and unceasing procurement of defiance by the pathogens were constricting the encroachment of the chemotherapeutic business (Newman and Cragg, 2007).

The current study aims to evaluate the antibacterial efficacy of one of the local weed of Punjab, *Trianthema portulacastrum* L. to pave way towards the discovery of novel chemotherapeutic agents hence alleviating the health standard among the developing nations.

Even though weeds were considered as unwanted for a number of reasons, the most important one is that they interfere with food and fiber production in agriculture, but there are many weeds having ethno-medicinal and pharmacological value. The plant under investigation belong to carpet-weed family, known as Aizoaceae (Ficoidaceae). It is an exotic weed contemplated to be the indigene of tropical America encroaching 39 crops beyond 40 countries, frequently prevailing in maize, mustard, potato, onion, cotton, rice and sugarcane, eminently amid rainy periods (Holm *et al.*, 1997).

Horse purslane, Black pigweed, Narma, Bishkapra and Itsit are a few trivial nomenclatural terminologies utilized by the local people (Gledhill, 2008). This plant is still employed in Ayurvedic medication as anodyne, purgative, stomachic, cure of blood ailments, anemia, night blindness and tenderness; consequently, replenishing motivation for appropriate appraisal of the plant in medical prescriptions (Khare, 2006).

Materials and methods

Test organisms

The bacterial test organismsemployed for the respective appraisal included *Bacillus subtilis(*ATCC 15029*), Escherichia coli(*ATCC 14962*), Pseudomonas aeruginosa(*ATCC 14971*)* and *Staphylococcus aureus(*ATCC 14923*).*

Plant specimen

The healthy specimens of *Trianthema portulacastrum*L. were collected in October, identified, assigned authenticated voucher number and deposited in Dr. Sultan Ahmed herbarium, Department of Botany, GC University, Lahore.

Methodology

The steady-state maceration was employed after the plant material was rinsed with water, mildly brushed to eliminate soil and remaining detritus, separated into components, subjected to shade desiccation and pulverized; to prepare crude extracts with *n*-hexane, chloroform, ethanol and distilled water as per polarity gradient. Dissimilar quantity of plant components were engaged as per accessibility and quantity of the fluent was adjusted correspondingly (Table 1). Subsequently, the % extraction yield was calculated as:

% Extraction yield = (Wt. of plant extract / Wt. of initial plant sample) ×100.

Estimation of zone of inhibition

The zone of inhibition was determined by employing Agar well diffusion technique following Jorgensen *et al.* (2007); having antibiotic discs as positive and respective solvents (*n*-hexane, chloroform, ethanol and distilled water) as negative control.

To set up the analysis plates, inoculum with 1.5×10^8 CFU/mL turbidity adjusted using 0.5 McFarland Barium chloride grade (Hindler *et al.*, 2007) was spread homogeneously on the Petri plates containing 20mL sterilized nutrient agar medium, prepared in accordance to Cruick-Shank *et al.* (1975) protocol. Subsequently, with cork borer no.4, a well was created in the middle and 1mL of 20mg/mL plant extract was poured in it. The prepared plates were lodged in incubator at 37±2°C for 24hrs. After incubation, the diameter of zone due to the inhibition of microbial growth around the plant extract was documented in mm by means of ruler.

The Activity index (AI) was estimated by comparing the zone of inhibition of the extracts with standard antimicrobial agents employing the following formula:

Activity index = Zone of inhibition by extract / Zone of inhibition by standard antimicrobial agent.

Estimation of minimum inhibitory concentration (MIC)

The agar dilution method used by Jorgensen *et al.* (2007) was applied for the inquisition of MIC.The sterilized same-sized Petri-plates comprising 18mL nutrient agar medium and 2mL of either 10, 5, 2.5, 1.25 or 0.625mg/mL plant macerate were prepared followed by homogenous spreading of inoculum with the assistance of disinfected cotton mop, the lid of the plate was positioned on it, secured with cling film and incubated at 37±2°C for 24hrs. After incubation, Petri-plates were analyzed for the presence (+) or absence (-) of microbial proliferation. The least concentration that had successfully obviated microbial growth was treated as MIC.

Statistical analysis

All parameters were carried out in triplicates and results obtained were analyzed statistically applying STATISTX version 8.1.

Results

The percentage extraction yield of the plant was considered as a measure of the efficiency of the solvents employed during maceration to extract specific components from the original material.

Table 1. Quantity of plant material (g) of *T. portulacastrum* dissolved in test solvents (mL) during maceration.

Plant part	Plant material used (g)	Solvent used (mL)
Root	17.71	100
Stem	56.60	250
Leaf	62.35	250

The % extraction yield of *T. portulacastrum* L. range from 0.92-27.28% with maximum maceration capability reported in aqueous extract of fruit and minimum extract recovery obtained from chloroform macerate of root (Fig. 1).

Antibacterial activity was estimated by measuring zone of inhibition followed by measuring MIC for further affirmation. The antibiotic standard discs used as positive control (Table 2) determine the susceptibility of bacterial specimens in accordance to

which one gram-positive (*S. aureus*) and one gram-negative bacterial specimen (*E. coli*) had offered intermediate susceptibility while, the resistance was offered by *B. subtilis* (gram-positive) and *P. aeruginosa* (gram-negative) bacteria. Moreover, all bacterial samples had demonstrated negligible response against the solvents employed during the extraction of plant components (Table 3).

Table 2. Zone of inhibition (mm) produced by the test bacterial strains against antibiotics standard discs.

Antibiotic standard disc	Conc. (µg)	Zone of Inhibition (mm)			
		B. subtilis	*E. coli*	*P. aeruginosa*	*S. aureus*
Amikacin	30	18±1.3	13±0.3	14±0.8	17±0.5
Ampicillin	10	11±1.5	12±2.5	11±0.5	22±3.9
Erythromycin	15	13±0.6	18±0.7	-	19±1.0
Gentamicin	10	12±2.5	14±2.2	12±0.5	13±3.5
Streptomycin	10	-	14±1.5	14±0.9	18±0.8
Tetracycline	10	14±2.4	18±0.7	13±1.4	15±0.4
Final response		Resistant	Intermediate	Resistant	Intermediate

*Source: delrio.dcccd.edu/jreynolds/microbiology/2421/lab.../KB_antibiotic.pdf

*The results reported were run in triplicates and stated as Mean ± Standard error.

Table 3. Zone of inhibition (mm) produced by test bacterial strains against solvents used in maceration.

Solvents	Quantity (mL)	Zone of Inhibition (mm)			
		B. subtilis	*E. coli*	*P. aeruginosa*	*S. aureus*
n-hexane	1.5	0±0	0±0	0±0	0±0
Chloroform	1.5	0±0	0±0	0±0	0±0
Ethanol	1.5	0±0	0±0	0±0	0±0
Aqueous	1.5	0±0	0±0	0±0	0±0
Final response		Negligible	Negligible	Negligible	Negligible

*The results reported were run in triplicates and stated as Mean ± Standard error.

The overall antibacterial activity of *Trianthema portulacastrum* L. ranges from 13.4±0.90 to 35.9±0.46mm, with maximum potential reported by ethanol extract of stem and minimum efficacy obtained by alcoholic macerate of root against *E. coli*. The zones of inhibition obtained in opposition to *Bacillus subtilis* (Table 4) varied from 14.5±0.42 to 29.1±0.30mm, against *Escherichia coli* (Table 5) ranged from 13.4±0.90 to 35.9±0.46mm, in contradiction to *Pseudomonas aeruginosa* (Table 6) range within 14.8±0.83 to 29.9±0.98mm and against *Staphylococcus aureus* varied from 14.0±0.26 to 28.5±0.68mm (Table 7).

Table 4. Zone of inhibition (mm) produced by *T. portulacastrum* L. against *Bacillus subtilis*.

Plant Part	Zone of Inhibition (mm)			
	n-hexane	Chloroform	Ethanol	Aqueous
Root	17.6 ± 0.93^{b}	20.5 ± 0.35^{c}	17.6 ± 0.31^{c}	14.5 ± 0.42^{c}
Stem	15.5 ± 2.25^{b}	16.6 ± 0.23^{d}	16.1 ± 0.58^{c}	15.7 ± 0.38^{c}
Leaf	15.6 ± 0.22^{b}	22.0 ± 0.52^{b}	22.7 ± 1.09^{b}	23.3 ± 0.47^{a}
Fruit	22.6 ± 0.56^{a}	27.8 ± 0.31^{a}	29.1 ± 0.30^{a}	17.2 ± 0.47^{b}
LSD	4.53	1.19	2.13	1.43

*The results reported were run in triplicates and stated as Mean ± Standard error.

*LSD stands for Least Significant Difference.

*Different superscripted alphabets in the same column indicate significant ($p < 0.05$) differences between means.

The comprehensive antibacterial analysis of *T. portulacastrum* L. had affirmed that fruit macerates were most promising with efficacy decreasing in leaf, stem and root extracts. The antibacterial components extraction pattern followed by solvents employed for maceration was chloroform > ethanol >*n*-hexane > aqueous (Fig. 2). *P. aeruginosa* had revealed much resistance while, other gram-negative clinical isolate *E. coli* was reported to be most susceptible test organism.

Table 5. Zone of inhibition (mm) produced by *T. portulacastrum*against *Escherichia coli.*

Plant Part	Zone of Inhibition (mm)			
	n-hexane	Chloroform	Ethanol	Aqueous
Root	18.0±0.55^{c}	18.2±1.46^{b}	13.4±0.90^{d}	14.1±0.88^{b}
Stem	20.8±0.62^{b}	16.5±0.57^{b}	35.9±0.46^{a}	15.7±0.42^{ab}
Leaf	17.3±0.40^{c}	26.2±0.46^{a}	16.4±0.83^{c}	17.4±1.19^{a}
Fruit	29.3±0.22^{a}	26.9±0.35^{a}	20.9±0.74^{b}	18.1±0.09^{a}
LSD	1.40	2.73	2.45	2.52

*The results reported were run in triplicates and stated as Mean ± Standard error.

*LSD stands for Least Significant Difference.

*Different superscripted alphabets in the same column indicate significant ($p < 0.05$) differences between means.

Table 6. Zone of inhibition (mm) produced by *T. portulacastrum* against *Pseudomonas aeruginosa.*

Plant Part	Zone of Inhibition (mm)			
	n-hexane	Chloroform	Ethanol	Aqueous
Root	16.1±0.67^{b}	18.2±0.54^{b}	15.7±0.62^{b}	15.1±0.18^{b}
Stem	19.8±1.02^{a}	16.4±0.26^{bc}	15.9±0.75^{b}	14.8±0.83^{b}
Leaf	15.0±0.29^{b}	16.1±0.12^{c}	21.2±0.12^{a}	18.2±0.15^{a}
Fruit	20.1±0.58^{a}	29.9±0.98^{a}	21.2±0.85^{a}	15.1±0.69^{b}
LSD	2.40	1.89	2.12	1.81

*The results reported were run in triplicates and stated as Mean ± Standard error.

*LSD stands for Least Significant Difference.

*Different superscripted alphabets in the same column indicate significant ($p < 0.05$) differences between means.

The activity index (Table 8) concluded that highest AI was reported by alcoholic macerate of stem against *E. coli*. Though significant values were reported by chloroform extract of leaf as well as *n*-hexane and chloroform macerate of fruit against *E. coli*, in addition to chloroform extract of fruit against *P. aeruginosa*;none of the extract had displayed insignificant potential.

Table 7. Zone of inhibition (mm) produced by *T. portulacastrum* against *Staphylococcus aureusl.*

Plant Part	Zone of Inhibition (mm)			
	n-hexane	Chloroform	Ethanol	Aqueous
Root	15.5±0.52^{c}	20.8±0.76^{b}	20.3±0.43^{ab}	14.0±0.26^{b}
Stem	18.9±0.61^{b}	15.4±0.46^{d}	24.8±3.33^{a}	17.7±0.36^{a}
Leaf	24.8±0.78^{a}	18.7±0.25^{c}	18.5±0.52^{b}	14.2±0.56^{b}
Fruit	15.9±1.02^{c}	28.5±0.68^{a}	21.5±0.48^{ab}	15.5±0.82^{b}
LSD	1.76	1.86	5.59	1.77

*The results reported were run in triplicates and stated as Mean ± Standard error.

*LSD stands for Least Significant Difference.

*Different superscripted alphabets in the same column indicate significant ($p < 0.05$) differences between means.

Table 8. Activity index (AI) of *Trianthema portulacastrum* against bacterial test strains.

Plant part	Solvent	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. aureus*
Root	*n*-hexane	0.97	1.38	1.15	0.91
	Chloroform	1.14	1.40	1.30	1.22
	Ethanol	0.97	1.02	1.12	1.19
	Aqueous	0.80	1.08	1.08	0.82
Stem	*n*-hexane	0.86	1.60	1.41	1.11
	Chloroform	0.92	1.27	1.17	0.91
	Ethanol	0.89	2.76	1.13	1.46
	Aqueous	0.87	1.21	1.06	1.04
Leaf	*n*-hexane	0.86	1.33	1.07	1.46
	Chloroform	1.22	2.01	1.15	1.10
	Ethanol	1.26	1.26	1.51	1.09
	Aqueous	1.29	1.34	1.30	0.83
Fruit	*n*-hexane	1.26	2.25	1.44	0.93
	Chloroform	1.54	2.07	2.13	1.68
	Ethanol	1.62	1.61	1.51	1.26
	Aqueous	0.96	1.39	1.07	0.91

*The zones of inhibition produced by Amikacin against bacterial isolates were treated as standard.

MIC verified the antimicrobial potential of *T. portulacastrum* L. (Table 9) with maximum efficacy reported byethanol extract of fruit against *B. subtilis*, ethanol extract of stem and *n*-hexane extract of fruit in opposition to *E. coli*, chloroform extract of fruit in contradiction to *P. aeruginosa* and ethanol extract of fruit against *S. aureus*.

Table 9. Minimum inhibitory concentration of different extracts of *Trianthema portulacastrum* against bacterial pathogens.

Bacterial isolates	Plant macerate (mg/mL)	Plant parts															
		Root				Stem				Leaf				Fruit			
		Hex	Chl	Eth	Aq.	Hex	Chl	Eth	Aq.	Hex	Chl	Eth	Aq.	Hex	Chl	Eth	Aq.
Bacillus subtilis	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2.5	+	-	+	+	-	+	+	+	+	-	-	-	-	-	-	+
	1.25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	0.625	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2.5	+	-	+	+	-	+	-	+	+	-	+	+	-	-	-	+
	1.25	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+
	0.625	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pseudomonas aeruginosa	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2.5	+	-	+	+	-	+	+	+	+	+	-	+	-	-	-	+
	1.25	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
	0.625	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Staphylococcus aureus	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2.5	+	-	-	+	-	+	-	+	-	+	+	+	+	-	-	+
	1.25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	0.625	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

*Key: - = Absence of bacterial growth, + = Presence of bacterial growth.

*Macerates: Hex = *n*-hexane, Chl = Chloroform, Eth = Ethanol, Aq. = Aqueous.

Zones of inhibition were ultimately correlated with MIC(Fig. 3) concluding negative association between the two categories such thatincrease in zones of inhibition lead to the decrease in MIC and vice versa. The gram-negative bacteria including *E. coli* (R^2 = 0.7398) and *P. aeruginosa* (R^2 = 0.7562) had revealed significant correspondence between the two parameters followed by *B. subtilis* (R^2 = 0.6658) and *S. aureus* (R^2 = 0.5958).

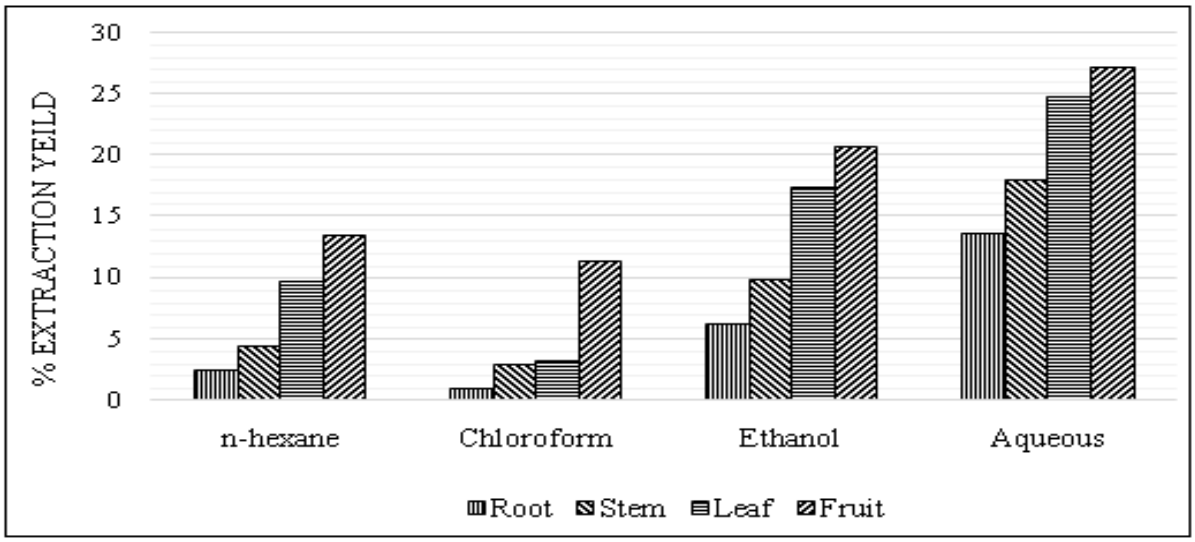


Fig. 1. Percentage extraction yield of the different extracts of *Trianthema portulacastrum*.

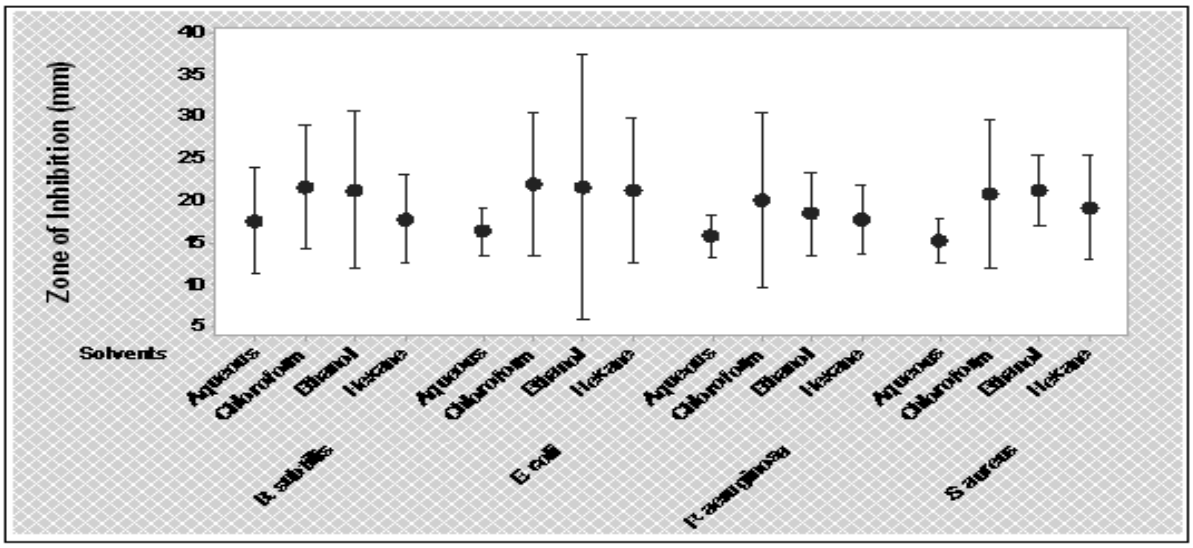
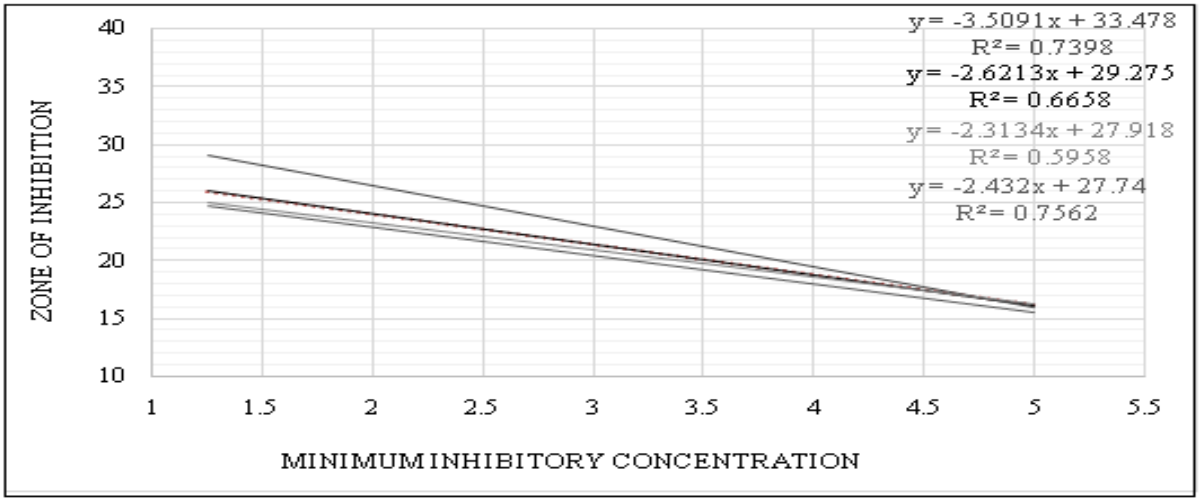


Fig. 2. Comparative antibacterial inhibition potential of *T. portulacastrum*.

Discussion

The extraction scheme adopted was considered because of its comparative cost effectiveness and good solute yield.The extraction yield of*T. portulacastrum*macerates were found to be in agreement with Iqbal *et al.* (2012), Bari *et al.* (2012) and Anwar *et al.* (2009). The variation in % maceration efficacy might be due to the use of different solvents, different plant parts, time, temperature, mode of extraction as and on chemical nature of the sample (Priya *et al.*, 2012).

The range of zones of inhibition presented by the respective plant macerates against the test bacterial strains were similar to the findings reportedby Shibu *et al.* (2013), Baloch *et al.* (2013), Dastagir *et al.* (2012), Iqbal *et al.* (2012) and Woldeyes *et al.* (2012).



*Key: *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus.*

Fig. 3. Correlation between Zone of inhibition and MIC displayed by the extracts of *T. portulacastrum* against bacterial isolates.

Pseudomonas aeruginosa had demonstrated more resistance as compared to the other gram-negative test organism i.e., *Escherichia coli* which might be attributed to the poor permeability of the outer membrane of *P. aeruginosa* (Brown, 1975).

References

Abdullah N, Syida WS, KamarudinW, Samicho Z, Aziman N, Zulkifli KS. 2012. Evaluation of *in vitro* antioxidant and antimicrobial activities of the various parts of *Benincasa hispida*. International Journal of Pharm Tech Research **4(4),** 1367-1376.

Anwar F, Ali M, Hussain AI, Shahid M. 2009. Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Pakistan. Flavour and Fragrance Journal **24,** 170-176.
http://dx.doi.org/10.1002/ffj.1929

Baloch N, Nabi S, Al-Kahraman YMSA. 2013. *In vitro* antimicrobial, insecticidal, antitumor activities and their phytochemical estimation of methanolic extract and its fractions of *Medicago lupulina* leaves. World Applied Sciences Journal **23(4),** 500-506.
http://dx.doi.org/10.5829/idosi.wasj.2013.23.04.368

Bari MN, Zubair M, Rizwan K, Rasool N, Bukhari IH, Akram S, Bokhari TH, Shahid M, Hameed M, Ahmad V. 2012. Biological activities of *Opuntia monacantha* cladodes. Journal of the Chemical Society of Pakistan **34(4),** 990-995.

Berkowitz FE. 1995. Antibiotic resistance in bacteria. Southern Medical Journal **88(8),** 797-780.

Brown MRW. 1975. Resistance of *Pseudomonas aeruginosa*. London, UK: John Wiley and Sons, 71.

Cruick-Shank R, Dugid JP, Marininon BP, Swain RHA. 1975. Screening of some Greek aromatic plants for antioxidant activity. Phytotherapy Research **17(2),** 194-195.

Cushnie TPT, Lamb AJ. 2005. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents **26,** 343-356.
http://dx.doi.org/10.1016/j.ijantimicag.2005.09.002

Dastagir G, Hussain F, Khan AA. 2012. Antibacterial activity of some selected plants of family Zygophyllaceae and Euphorbiaceae. Journal of Medicinal Plants Research **6(40),** 5360-5368.
http://dx.doi.org/10.5897/JMPR12.539

Gledhill D. 2008. The Names of Plants, 4th Ed. New York, USA: Cambridge University Press, 50, 312, 386.

Hindler FJ, Hochstein L, Howell A. 2007. Preparation of routine media and reagents used in antimicrobial susceptibility testing. In:Isenberg HD, Ed. Clinical Microbiology Procedures Handbook. Washington DC, USA: American Society for Microbiology (ASM) press **2(2),** 5.14.1.1-5.14.1.4.

Holm L, Doll J, Holm E, Pancho J, Herberger J. 1997. World Weeds: Natural Histories and Distribution. New York, USA: John Wiley and Sons, Inc., 854-861.

Iqbal MJ, Hanif S, Mahmood Z, Anwar F, Jamil A. 2012. Antioxidant and antimicrobial activities of Chowlai (*Amaranthus viridis* L.) leaf and seed extracts. Journal of Medicinal Plants Research **6(27),** 4450-4455.
http://dx.doi.org/10.5897/JMPR12.822

Jain RA, Agarwal RC, Dubey D, Verma R, Jain R. 2012. Evaluation of antibacterial and antioxidant activity of fruits extract of *Argemone mexicana* Linn. International Journal of Pharmaceutical Innovations **2(1),** 45-51.

Jorgensen JH, Turnidge JD. 2007. Susceptibility test methods: Dilution and disk diffusion methods. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, Eds. Manual of Clinical Microbiology, 9th Ed. Washington DC, USA: ASM Press, 1152-1172.
http://dx.doi.org/10.1128/9781555817381.ch71

Khare CP. 2006. Indian Medicinal Plants, an Illustrated Dictionary. New Delhi, India: Springer-Verlag, 668.

Livermore DM. 2000. Antibiotic resistance in *Staphylococci*. International Journal of Antimicrobial Agents **16,** 3-10.
http://dx.doi.org/10.1016/S0924-8579(00)00299-5

Newman DJ, Cragg GM. 2007. Natural products as sources of new drugs over the last 25 years. Journal of Natural Product **70(3),** 461-477.
http://dx.doi.org/10.1021/np068054v

Priya, GS, Radhika R, Siddhuraju P. 2012. Antioxidant and antimicrobial activity of traditional Indian leafy vegetables: *Mukia maderaspatana* and *Solanum trilobatum*. International Journal of Pharmacy and Pharmaceutical Sciences **4(2),** 513-521.

Raja W, Ovais M, Dubey A. 2013. Phytochemical screening and antibacterial activity of *Lawsonia inermis* leaf extract. International Journal of Microbiology Research **4(1),** 33-36.
http://dx.doi.org/10.5829/idosi.ijmr.2013.4.1.6679

Shibu BS, Devi BC, Moin S, Wesley S. 2013. Evaluation of bioactive potential of *Coelogyne nervosa* A. Rich. An endemic medicinal orchid of western Ghats, India. Asian Journal of Pharmaceutical and Clinical Research **6(1),** 114-118.

Woldeyes S, Adane L, Tariku Y, Muleta D, Begashaw T. 2012. Evaluation of antibacterial activities of compounds isolated from *Sida rhombifolia* Linn. (Malvaceae). Natural Products Chemistry and Research **1,** 101.
http://dx.doi.org/10.4172/2329-6836.1000101