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IN VITRO COMPARATIVE SCREENING OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF SOME COMMON WEEDS EXTRACTS

Sobia Mushatq[1], M. Saleem Haider, Amna Ali, Shabnam Javed, Ibatsam Khokhar, Irum Mukhtar

ABSTRACT

Herbal pesticides are gaining interest because of their cost effective and eco-friendly attributes. If this antifungal and antibacterial potency resides in weeds, it will be a great advantage. The present investigation is therefore undertaken to investigate the efficacy of three common weeds i.e., Amaranthus viridis, Lantana camara and Malvastrum coromandelianum against four bacterial species viz, Xanthomonas axonopodis, Pseudomonas syringae (gram negative bacteria), Corynebacterium minutissium, Clostridium difficile (gram positive bacteria) and major seed-borne fungi Aspergillus niger, Alternaria alternata, Drechslera biseptata, Fusarium solani were studied in vitro. The chloroform and aqueous leaf extract of these weeds were used in this study. The antimicrobial activity was tested by well diffusion method. Chloroform extract of M. coromandelianum were effective against all the four bacteria with maximum inhibition zone of 3.5cm in P. syringae while its aqueous extract showed the minimum inhibitory effect with 0.7cm inhibition zone of X. axonopodis; whereas the aqueous extract of L. camera was most active against the bacteria. Aqueous extracts of L. camera also showed good antifungal activity as compared to other weed extracts and chloroform extracts of all weeds were moderately active against seed-borne fungi tested. The antifungal components from these weeds can be used as an alternative to develop novel pesticides by replacing some chemical commercial antifungal and antibacterial for the plant diseases.

Key words: Antimicrobial activity, chloroform and aqueous extracts *Amaranthus viridis*, *Lantana camara, Malvastrum coromandelianum.*

INTRODUCTION

A major factor for the revival of weeds is their ability to resist pests and pathogens in their environment. Thus, they could be a

[1]First Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences University of the Punjab, Quaid-E-Azam campus, Lahore 54590 Pakistan.
Correspondence email: mushtaq.sobia@gmail.com

potential source of antimicrobial compounds and their identification is necessary to develop cheaper pesticides. The development of resistance in weeds to the common pesticides and the increasing restrictions on the use of toxic material in the environment have given an impetus to search for novel plant protectants that interfere with the pathogenicity factors. As the herbal medicines are gaining growing interest because of their eco-friendly attributes (Dwivedi and Singh, 1998; Karnwal and Singh, 2006). Similarly, the use of natural products for the control of diseases in plants is considered as an alternative source to synthetic pesticide due to their lower negative impacts on the environment. Besides being harmless and non-phytotoxic it has been proved that plant extracts exhibit inhibitory effect on pathogens. Several higher plants and their constituents have been successful in plant disease control and have proved to be harmless and non-phytotoxic, unlike chemical fungicides (Singh *et al*., 1986; Dubey, 1991; Alam *et al*., 2002). If the antimicrobial property resides in weed, that will be an added advantage. *Amaranthus viridis*, *Lantana camara* and *Malvastrum coromandelianum* are the common weeds in Pakistan. *L. camara* L. (Verbanaceae), commonly known as wild or red sage is the most widespread species of this genus and regarded as a notorious weed. *L. camara* contains lantadenes, the pentacyclic triterpenes, which is reported to possess a number of useful biological activities. Several previous reports have described antifungal (Tripathi and Shukla, 2002; Kumar *et al*. 2006), and antimicrobial activities of *L. camara* (Saxena *et al*. 1992; Juliani *et al*., 2002; Kasali *et al*., 2002; Rajakaruna *et al*., 2002) .

Similarly, *A. viridis* Linn.*,* an annual herb, serves as forage and as a wild leafy vegetable in Pakistan. Autecological studies including germination, growth behaviour and allelopathy have been done on many weeds, however no such information exists on *A. viridis* (Hussain *et al.*, 1994; 2003). Wheareas *M. coromandelianum* (L.) Garcke, an annual to perennial herb, commonly called False Mallow, belongs to family Malvaceae. Its antimicrobial activity have not been investigated for plant pathogens although its antimicrobial activity against human pathogens has been reported (Sittiwet *et al*., 2008).

The present investigation is therefore, undertaken to test the efficacy of these common weed extracts against the bacterial and fungal pathogens.

MATERIALS AND METHODS

Plant materials

Three common weeds were collected from different parts of the University of the Punjab Lahore Pakistan and are easily identified, as they are common weeds. Table-1 shows the list of common weeds

used in this study. The leaves of all weeds were air-dried, coarsely powdered and were then extracted.

Table-1. List of weeds selected for anti-microbial activity

S.No	Botanical Name	Common name	Part Used
1.	*Lantana camara*	Sleeper weed or Lantana	Leaves
2.	*Amaranthus viridis*	Green amaranth	Leaves
3.	*Malvastrum coromandelianum*	False Mallow	Leaves

Extract preparation

Thirty grams (30 g) of dried weed material were exhaustively extracted for 6 hours with 300 ml of solvent chloroform in soxhlet extractor. Extraction was allowed to proceed for 6 h. The resulting extract was concentrated over a rotary vacuum for complete solvent removal until a crude solid extract was obtained. The resulting solid masses were preserved in refrigerator at 4°C. Simultaneously, for aqueous extract preparation, 30 g each of the dried specific weed was soaked in 300 ml distilled water for 48 h at room temperature. The aqueous extract water was filtered with muslin cloth and filtered and resulting crude extract was freeze-dried. The dried powder extract was kept in refrigerator until use.

Preparation of the crude extracts of Weeds

The final concentration of weed extracts (aqueous and chloroform extract) were prepared in Sterile 100 % dimethylsulfoxide (DMSO) by dissolving 0.2g/ ml of each extract. This crude extract was stored at 4 ºC for further use. Sterile 100 % DMSO served as negative control.

Preparation of the tested organisms

The bacterial and fungal strains, *Xanthomonas axonopodis* (FCBP 007), *Pseudomonas syringae* (FCBP 009), *Corynebacterium minutissium* (FCBP 137), *Clostridium difficile* (FCBP 138), *Fusarium solani* (FCBP 438), *Aspergillus niger* (FCBP 628) and *Alternaria alternata* (FCBP 568) and *Drechslera biseptata* (FCBP 1042) were used for testing the antifungal activity. The microorganisms used in this investigation were obtained from the First Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences. The fungal strains were maintained at malt extract agar (MEA) and bacterial strains maintain at nutrient agar (NA) media at 4ºC. For the antimicrobial assay, seven days old fungal and one day old bacterial cultures were used.

Preliminary screening for anti microbial activity

Antifungal assay

To determine the antifungal activity, the *in vitro* tests were carried out to measure the effects of the leaf extracts on radial growth

of the seed-borne fungi by agar well diffusion method (Okeke *et al*., 2001). Pure isolate of each fungus was first sub-cultured on 2% MEA at 25ºC for 5 days. Eighty microlitres (80µL) of the standardized inoculum (10^5CFU/mL) of each test fungus was spread with the help of sterile spreader on to a sterile MEA plate so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 0.8 cm was used to bore wells in the agar plates. Subsequently, a 60µL volume of the each extract was introduced in wells into MEA plate. The plates were allowed to stand for at least 1 h for diffusion to take place and then incubated at 25ºC for 5 days. The zone of inhibition was recorded to the nearest size in cm. The experiment was carried out tin three replicates.

Antibacterial assay

The same method as for fungi was adopted for antibacterial assay of leaf extracts against tested bacterial species. Instead of malt extract agar, nutrient agar was used. The standard bacterial suspension (McFarland 0.5). The bacterial cultures were spread on the agar surface using sterile cotton swab. Then a well of 0.8cm was made in the medium using sterile cork borer, 60µl. of aqueous and chloroform weed extract were transferred into separate wells and plates were incubated at 37^{o}C for 24 hours. The zone of inhibition was recorded to the nearest size in cm. The experiment was carried out tin three replicates.

Antifungal and antibacterial activity Index was calculated as:

$$\text{Activity Index (AI)} = Da/ Db - 1$$

Where: Da is the diameter (cm) of the growth zone in the experimental dish and Db is the diameter of the growth zone in the control dish.

Statistical evaluation

The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SE of three replicates.

RESULTS AND DISCUSSION

The aqueous and chloroform extracts of weeds gave varied results. Tables 2-4 show the results of antimicrobial activity of weeds extracts. Figures 1 and 2 show the inhibition percentage of weed leaf extracts against different bacterial and fungal strains.

Antibacterial assay

Chloroform extracts *of M. coromandalianum* and *A. viridis* showed evidence of high antibacterial activity against *X. axonopodis*; while *L. camara* showed low activity. *P. syringae* had least resistance against chloroform extract of *M. coromandalianum* (3.5cm) and aqueous extract of *L. camara* (3.3 cm) as compared to chloroform and

aqueous extracts of *A. viridis* with 1.5cm and 1.2 cm inhibition zone respectively.

The chloroform extracts of *A. viridis* and *M. coromandalianum* exhibited high activity against gram positive *C. minutissium* as compare to least activity of *L. camara*. In case of aqueous extracts of weeds, *L. camara* and *A. viridis* were highly active against *C. minutissium*. The growth of *C. difficile* was strongly inhibited by choloroform extracts of *M. coromandalianum* (3.5 cm) while its aqueous extract was inactive (0.9 cm).

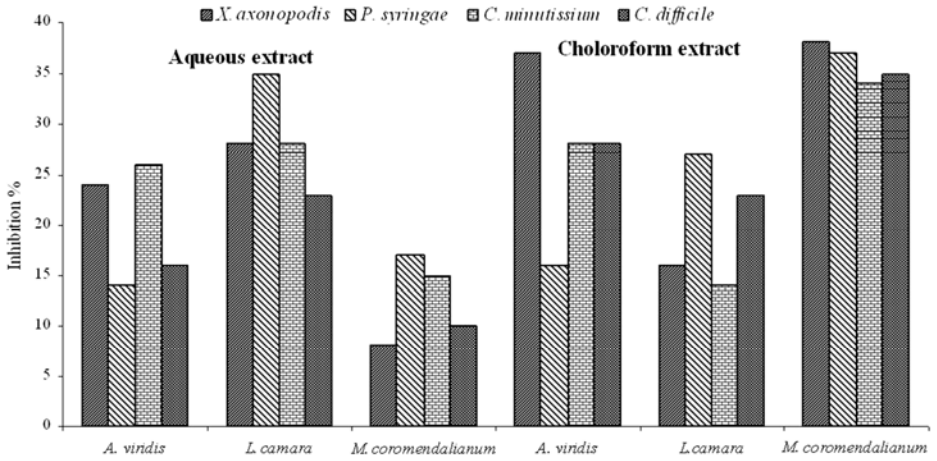

Figure 1. Percent inhibition of weed extracts on bacterial growth.

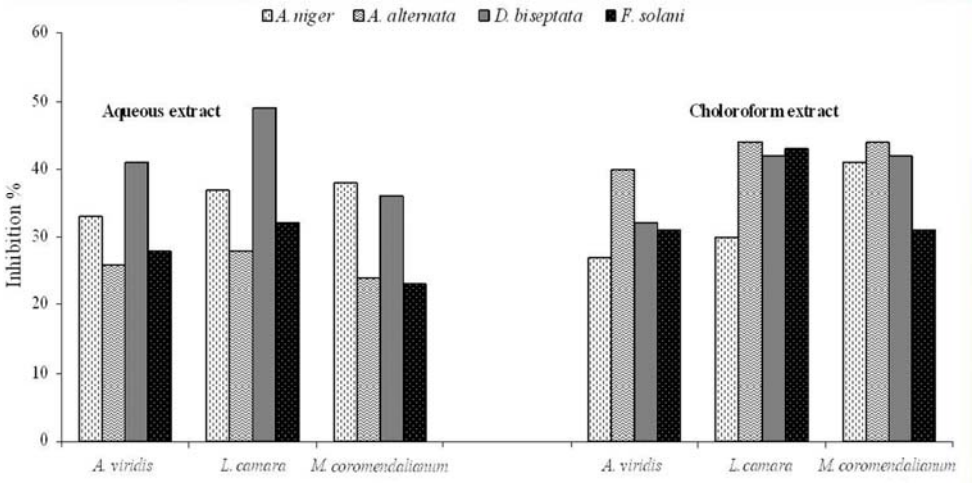

Figure 2. Percent inhibition of weed extracts on fungal growth.

Table-2. Antimicrobial activity of *Amaranthus viridis* leaf extracts.

Microorganisms	Chloroform extract				Aqueous Extract			
	Control (cm)	Inhibition zone (cm)	Experimental growth zone	Index	Control (cm)	Inhibition zone (cm)	Experimental growth zone	Index
Xanthomonas axonopodis	9.0±0.0	3.3±0.05	5.6±0.03	0.37	9.0±0.0	2.2±0.10	6.8±0.03	0.24
Pseudomonas syringae	9.0±0.0	1.5±0.01	7.5±0.03	0.16	9.0±0.0	1.2±0.03	7.7±0.03	0.13
Corynebacterium minutissium	9.0±0.0	2.5±0.06	6.4±0.03	0.28	9.0±0.0	2.4±0.10	6.6±0.06	0.25
Clostridium difficile	9.0±0.0	2.6±0.05	6.4±0.03	0.28	9.0±0.0	1.5±0.05	7.5±0.03	0.16
Aspergillus niger	7.0±0.0	1.9±0.18	5.5±0.18	0.26	7.0±0.0	2.5±0.11	5.0± 0.11	0.33
Alternaria alternate	5.0±0.0	2.0±0.08	3.0±0.08	0.40	5.0±0.0	1.3±0.08	3.7±0.08	0.26
Drechslera biseptata	5.5±0.0	1.8±0.20	3.7±0.20	0.32	5.5±0.0	2.3±0.15	3.2±0.31	0.41
Fusarium solani	6.5±0.0	2.0±0.11	4.5±0.12	0.30	6.5±0.0	1.8±0.16	4.7±0.15	0.27

Table-3. Antimicrobial activity of *Lantana camara* leaf extracts.

Microorganisms	Chloroform extract				Aqueous Extract			
	Control (cm)	Inhibition zone (cm)	Experimental growth zone	Index	Control (cm)	Inhibition zone (cm)	Experimental growth zone	Index
Xanthomonas axonopodis	9.0±0.0	1.5±0.02	7.6±0.10	0.15	9.0±0.0	2.6±0.10	6.4±0.03	0.28
Pseudomonas syringae	9.0±0.0	2.5±0.01	6.5±0.03	0.27	9.0±0.0	3.2±0.10	5.8±0.03	0.35
Corynebacterium minutissium	9.0±0.0	1.2±0.03	7.7±0.02	0.15	9.0±0.0	2.6±0.05	6.4±0.03	0.28
Clostridium difficile	9.0±0.0	2.1±0.05	6.9±0.03	0.23	9.0±0.0	2.1±0.10	6.9±0.03	0.23
Aspergillus niger	7.0±0.0	2.1±0.11	4.9± 0.11	0.30	7.0±0.0	2.6±0.10	4.4±0.10	0.37
Alternaria alternate	5.0±0.0	2.2±0.08	2.8±0.20	0.44	5.0±0.0	1.3±0.32	3.6±0.03	0.28
Drechslera biseptata	5.5±0.0	2.3±0.14	3.2±0.14	0.42	5.5±0.0	2.8±0.08	2.7±0.08	0.50
Fusarium solani	6.5±0.0	2.7±0.06	3.7±0.03	0.43	6.5±0.0	2.1±0.16	4.4±0.12	0.32

Table-4. Antimicrobial activity of *Malvestrum coromendalianum* leaf extracts.

Microorganisms	Chloroform extract				Aqueous Extract			
	Control (cm)	Inhibition zone (cm)	Experimental growth zone	Index	Control (cm)	Inhibition zone (cm)	Experimental growth zone	Index
Xanthomonas axonopodis	9.0±0.0	3.3±0.10	5.6±0.03	0.37	9.0±0.0	0.7±0.05	8.2±0.03	0.08
Pseudomonas syringae	9.0±0.0	3.5±0.06	5.5±0.03	0.37	9.0±0.0	1.6±0.06	7.4±0.03	0.17
Corynebacterium minutissium	9.0±0.0	3.1±0.05	5.9±0.03	0.34	9.0±0.0	1.4±0.05	7.6±0.03	0.14
Clostridium difficile	9.0±0.0	3.2±0.11	5.8±0.03	0.35	9.0±0.0	0.9±0.08	8.1±0.03	0.09
Aspergillus niger	7.0±0.0	2.9±0.08	4.1±0.08	0.41	7.0±0.0	2.7±0.11	4.3±0.11	0.38
Alternaria alternate	5.0±0.0	2.2±0.20	2.8±0.17	0.44	5.0±0.0	1.2±0.23	3.8±0.05	0.24
Drechslera biseptate	5.5±0.0	2.4±0.15	3.2±0.03	0.42	5.5±0.0	2.0±0.23	3.5±0.23	0.36
Fusarium solani	6.5±0.0	2.0±0.02	4.5±0.05	0.30	6.5±0.0	1.5±0.26	5.0±0.26	0.23

Antifungal assay

The chloroform extracts of all weeds of were found to be moderately active against all organisms tested. The chloroform extract of weed of *L. camara* showed high activity (2.7 cm) against *F. solani* in comparison of moderate activity of *A. viridis* and *M. coromendalianum* with inhibition zone of 2.0cm each. The aqueous and chloroform weed extracts of *M. coromendalianum* was more active against *A. niger* (2.7 cm, 2.9 cm) whereas the least activity was showed by *A. viridis* (1.9 cm). The growth of *A. alternata* was moderately inhibited by the chloroform extracts of all weeds while aqueous extracts were least active against this pathogen. *D. biseptata* was strongly inhibited by aqueous extract of *L. camara* (2.8 cm) whereas chloroform extract of *A. viridis* were least effective (1.8 cm).

Testing the Antimicrobial activity of plants remains an area of intense interest. Many reports are available on the antiviral, antifungal, antibacterial, antihelmintic, antimolluscal, and anti-inflammatory properties of plant (Samy and Ignacimuthu, 2000; Palombo and Semple, 2001; Kumaraswamy *et al.*, 2002; Stepanovic *et al.,* 2003; Bylka *et al.*, 2004; Behra and Misra*,* 2005; Govindarajan *et al.,* 2006; Malik and Singh*,* 2010).

However, reports on the exploitation of antimicrobial property of weeds are scanty. Despite serious environmental implications associated with the excessive use of chemicals, it still remains the first line of defense against plant pathogen. When fungicides consumed by human beings and animals through food and water cause various ailments in the body; whereas search of natural herbicides from the plant sources would definitely be a better alternative to these hazardous chemicals.

L.camara possess many important biological activities viz., antipyretic, antimicrobial, antimutagenic, antimicrobial, fungicidal, insecticidal, nematicidal, and others (Siddiqui *et al*., 1995; Deena and Thoppil, 2000; Mello *et al.,* 2005; Verma and Verma, 2006) and these studies are in agreement with our results. The antifungal activity of *L. camera* against seed borne fungi has also been reported by Patel *et al*. (2007).

Flavanoids (Mendoza *et al*., 1997), terpenoids (Aurelli *et al*., 1992; Cowan, 1999), tannins and phlobatanins (Stern *et al*., 1996) are phytochemicals that have been demonstrated to have antimicrobial activity. The current results corroborate the findings of Harish *et al.* (2008), who demonstrated the presence of antimicrobial activity in *A. viridis* the antimicrobial activity of *M. coromandelianum* extracts has been studied by Jain *et al*. (2010) who also reported the presence of above mentioned antimicrobial compounds.

CONCLUSION

It can be concluded in light of the present experimental results that the leaf extracts of these weeds exhibit antimicrobial effects which offer a scientific basis for using these weeds as a good source of antimicrobial compounds. Further work is however required to be done for its formulation.

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