

Preliminary Phytochemical Analysis and Antimicrobial Activity of Some Weeds collected from Marathwada Region

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ABSTRACT:

The aqueous and ethanolic extracts of selected weeds such as *Portulaca oleracea* L. (Portulacaceae), *Cardiospermum helicacabum* L. (Sapindaceae), *Euphorbia hirta* L. (Euphorbiaceae), *Crotalaria retusa* L. (Fabaceae) and *Euphorbia heterophylla* L. (Euphorbiaceae) were screened for phytochemical analysis and antimicrobial activity. The antimicrobial activities were tested against two gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), one gram-negative bacterium (*Pseudomonas aeruginosa*) and a mould *Aspergillus niger* by agar diffusion method. The phytochemicals like alkaloids, saponins, tannins, cardiac glycosides, steroids and flavonoids are investigated from plant samples. Tannins, steroids and flavonoids were absent in the *Cardiospermum helicacabum* L. Remarkable antibacterial activity was observed in the aqueous and ethanolic (root and leaves) extracts of *Portulaca oleracea* L. while *Cardiospermum helicacabum* L. showed no results in same experimental conditions as compared with standard antibiotics. The weed *Euphorbia hirta* L. possess significant antifungal activity as compared to nystatin used as a standard antifungal agent.

Keywords:

Weeds, Phytochemical analysis, Antimicrobial activity, Crude extract.

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INTRODUCTION:

The weeds are common, dominant and easily available in almost all the crop fields. These are produced in very huge quantity and competing to crops for water, nutrients and light. The total yield losses per year caused by weeds were more than 24 % (Newman, et al., 1973). But many phytochemicals from these plants were investigated for various preparations of novel natural remedies against many chronic diseases. The ethanopharmacological data provides an alternative approach for discovery of antimicrobial agents, particularly those medicinal plants that have a history of traditional use and have potential source with significant pharmacological and biological activities (Ambasta, 1992). The main objective of the present investigation was to search preliminary phytochemical analysis and evaluation of in-vitro antimicrobial activities using crude extracts of some common weeds. The detailed therapeutic usage of the selected the plants were summarized as below.

1. *Portulaca oleracea* L. (Family: Portulacaceae) *Portulaca oleracea* L contains many biological active compounds and also it is used as a good source of nutrients. This plant is used as antihelminthic, antidiarrhoeal and a bactericidal in bacillary dysentery. Fresh leaves were used externally for maturing of abscesses while seeds are effective as a refreshing agent and diuretic. Plant is very effective for wound healing and also used as anti-inflammatory treatment. (Leung and Foster, 1996).

2. *Cardiospermum helicacabum* L. (Family: Sapindaceae) The entire plant has medicinal properties and it is useful in the treatment rheumatism, fever, nervous disorder and in piles. The leaves are useful in the treatment of amenorrhoea (William, 2005).

3. *Euphorbia hirta* L. (Family: Euphorbiaceae) A prostrate ascending annual herb, the whole plant possesses medicinal property. A decoction of plant powder is very useful in the treatment of kidney

disorders, dysentery, asthma and curing many diseases of urenogenital tract. In many villages, leaves are chewed and spitted on wounds for prevention of infection, it acts as an antiseptic agent. (Ratnam, 2006).

4. *Crotalaria retusa* L. (Family: Fabaceae) In ayurveda, *Crotalaria retusa* L. is very useful in kapha and vata. It is bitter, astringent, expectorant, and specially the leaves are useful for curing pyalism, dyspepsia, fever and cardiac diseases. The seeds are powdered and boiled in milk and are said to be very useful for increasing body strength and life span (Prajapati, 2003).

5. *Euphorbia heterophylla* L. (Family: Euphorbiaceae). The leaves are used in traditional practices as antigonorrhoeal, laxative, migraine and have potential to eliminate wart (Rodriguez, 1976).

MATERIAL AND METHODS**Plant Materials and Preparation of Plant Extracts**

The weeds were collected from various crop fields from Nanded district of Marathwada region (MS) India. The plants were identified and authenticated by a taxonomist Dr. S. S. Bodke, Head, Dept. of Botany, Yeshwant Mahavidyalaya, Nanded using "Flora of Marathwada" (Naik, V.N., 1998). The voucher plant specimens were deposited in the herbarium section of Department of Botany, Yeshwant Mahavidyalaya, Nanded (MS). The herbarium voucher specimens no. are *Portulaca oleracea* L. (412A), *Cardiospermum helicacabum* L. (413A), *Euphorbia hirta* L. (414A), *Crotalaria retusa* L. (415A) and *Euphorbia heterophylla* L. (416A). The collected plants were shade dried and made fine powder. Sequential extraction was carried out using Soxhlet extraction apparatus in (water, ethanol) for six hours. Obtained extract were filtered, evaporated and stored in refrigerator for further experiments.

Preliminary Phytochemical Analysis

The selected plants were analyzed for

Table 1: Preliminary phytochemical analysis of selected weeds

Sr. No	Name of the Weeds	Preliminary Phytochemical Analysis					
		Alkaloids	Saponins	Tannins	Cardiac glycosides	Steroids	Flavonoids
1	<i>Portulaca oleracea</i> L.	+	+	+	+	+	+
2	<i>Cardiospermum helicacabum</i> L.	+	+	-	+	-	-
3	<i>Euphorbia hirta</i> L.	+	+	+	+	+	+
4	<i>Crotalaria retusa</i> L.	+	+	+	+	+	+
5	<i>Euphorbia heterophylla</i> L.	+	+	+	+	+	+

(+ Present, - absent)

preliminary phytochemical analysis such as alkaloids, saponins, tannins, steroids and flavonoids according to standard protocols (Jigna et al., 2007).

Microorganisms used

In present investigation two gram-positive bacteria [*Bacillus subtilis* (MTCC-2415), *Staphylococcus aureus* (MTCC-96)], one gram-negative organism *Pseudomonas aeruginosa* (MTCC-2488) and one mould *Aspergillus niger* (MTCC-1781)] were included. All the microbial cultures were purchased from Institute of Microbial Technology, Chandigarh, India.

Antimicrobial Activity

The screening of antimicrobial activity of crude extracts was carried out by measuring the zone of inhibition using agar diffusion method (Sahoo et al., 2006). The sterile disc were loaded aseptically with the various concentration of crude

extracts (water and ethanolic) 100, 250, 500, 750µg/ml. Streptomycin (30µg), Tetracycline (30 µg) were used as standard antibiotics (a positive control) and appropriate controls were prepared for corrections using respective solvents.

For antifungal activity potato dextrose agars plates were used. Nystatin (30µg) was used as a standard antifungal agent.

RESULTS

The phytochemicals like alkaloids, saponins, tannins, cardiac glycosides, steroids and flavonoids are present in all selected plants except *Cardiospermum helicacabum* L. which showed positive results towards alkaloids, saponins and cardiac glycoside (Table 1).

The antimicrobial activities of five weed species (root and leaves) extracts were assayed in

Table 2: Profile of antimicrobial activity (root extracts) of selected weeds

Sr. No	Name of the weeds	Test organisms	Zone of Inhibition (mm)							
			Aqueous extract (µg/ml)				Ethanolic extract (µg/ml)			
			100	250	500	750	100	250	500	750
1	<i>Portulaca oleracea</i> L	<i>B. subtilis</i>	29	32	37	39	26	29	30	31
		<i>S. aureus</i>	23	31	35	47	31	34	37	40
		<i>P. aeruginosa</i>	23	31	33	35	29	32	44	46
		<i>A. niger</i>	09	12	13	14	05	10	11	13
2	<i>Cardiospermum helicacabum</i> L.	<i>B. subtilis</i>	NR	NR	NR	NR	NR	NR	NR	NR
		<i>S. aureus</i>	NR	NR	NR	NR	NR	NR	NR	NR
		<i>P. aeruginosa</i>	NR	NR	NR	NR	NR	NR	NR	NR
		<i>A. niger</i>	NR	NR	NR	NR	NR	NR	NR	NR
3	<i>Euphorbia hirta</i> L.	<i>B. subtilis</i>	06	08	11	13	12	16	21	24
		<i>S. aureus</i>	05	07	09	12	11	13	19	21
		<i>P. aeruginosa</i>	08	10	13	14	21	25	26	28
		<i>A. niger</i>	20	23	24	27	21	24	27	32
4	<i>Crotalaria retusa</i> L	<i>B. subtilis</i>	15	17	21	29	15	18	21	23
		<i>S. aureus</i>	04	07	09	14	07	11	19	21
		<i>P. aeruginosa</i>	31	33	43	45	37	39	42	43
		<i>A. niger</i>	NR	NR	NR	NR	03	05	06	09
5	<i>Euphorbia heterophylla</i> L	<i>B. subtilis</i>	10	12	13	14	03	08	11	13
		<i>S. aureus</i>	06	07	10	13	NR	NR	NR	NR
		<i>P. aeruginosa</i>	14	18	26	31	14	17	23	28
		<i>A. niger</i>	08	12	14	16	10	11	13	15
6	Standard drug		A	B	C	A	B	C		
		<i>B. subtilis</i>	31	ND	ND	30	ND	ND		
		<i>S. aureus</i>	ND	34	ND	ND	32	ND		
		<i>P. aeruginosa</i>	ND	19	ND	ND	20	ND		
		<i>A. niger</i>	ND	ND	18	ND	ND	19		

[*B. subtilis* – *Bacillus subtilis*, *S. aureus* - *Staphylococcus aureus*,
P. aeruginosa - *Pseudomonas aeruginosa*, *A. niger* - *Aspergillus niger*]
 A-Streptomycin (30µg/ml), B-Tetracycline (30µg/ml), C- Nystatin (30µg)
 NR-No results under experimental conditions, ND- Not determined
 The results presented are mean values of n=2.

vitro by agar diffusion method against three bacterial species (two gram-positive and one gram-negative bacterium) and one fungal species. The antimicrobial activity of root extracts of different weed species are summarized in (Table 2). The highest antibacterial activity was observed in *Portulaca oleracea* L. at the concentration of 750 µg/ml of aqueous extract against *Staphylococcus aureus* having zone of inhibition (47 mm) as compared to standard antibiotics Tetracycline. While in case of ethanolic extract of *Portulaca oleracea* L. showed maximum antimicrobial activity against *Pseudomonas aeruginosa* showing zone of inhibition (46 mm). The aqueous and ethanolic extracts of *Cardiospermum helicacabum* L. showed no results under the similar experimental conditions. The aqueous extract of *Crotalaria retusa* L. showed no results against *Aspergillus niger* while in ethanolic extracts of *Euphorbia hirta*

L. showed maximum antifungal activity against *Aspergillus niger* at 750 µg/ml having zone of inhibition (32 mm) as compared to standard reference compound of Nystatin. Remaining selected plants exhibited considerable antimicrobial activity.

The summary of antimicrobial activity (leaves extracts) of selected weeds are shown in Table 3. Among the selected weeds aqueous extract of *Portulaca oleracea* L. showed maximum antimicrobial activity against *Bacillus subtilis* at 750 µg/ml showing zone of inhibition (35mm). The ethanolic extract of *Euphorbia heterophylla* L. showed no results against *Staphylococcus aureus* in similar conditions. All other aqueous and ethanolic (leaves) extracts exhibited considerable antimicrobial activity.

Table 3: Summary of antimicrobial activity (leaves extracts) of selected weeds

Sr. No.	Name of the weeds	Test organisms	Zone of Inhibition (mm)							
			Aqueous extract (µg/ml)				Ethanolic extract (µg/ml)			
			100	250	500	750	100	250	500	750
1	<i>Portulaca oleracea</i> L.	<i>B. subtilis</i>	20	24	31	35	21	22	24	25
		<i>S. aureus</i>	14	17	18	20	22	26	27	29
		<i>P. aeruginosa</i>	14	15	20	25	07	14	21	24
		<i>A. niger</i>	04	07	12	14	03	07	10	12
2	<i>Cardiospermum helicacabum</i> L.	<i>B. subtilis</i>	NR	NR	NR	NR	NR	NR	NR	NR
		<i>S. aureus</i>	NR	NR	NR	NR	NR	NR	NR	NR
		<i>P. aeruginosa</i>	NR	NR	NR	NR	NR	NR	NR	NR
		<i>A. niger</i>	NR	NR	NR	NR	NR	NR	NR	NR
3	<i>Euphorbia hirta</i> L.	<i>B. subtilis</i>	05	09	12	13	12	15	16	18
		<i>S. aureus</i>	06	09	12	13	08	13	16	24
		<i>P. aeruginosa</i>	03	07	11	13	03	05	09	11
		<i>A. niger</i>	10	13	15	16	12	15	25	27
4	<i>Crotalaria retusa</i> L.	<i>B. subtilis</i>	20	24	25	27	22	23	26	29
		<i>S. aureus</i>	22	26	30	31	15	17	21	24
		<i>P. aeruginosa</i>	16	19	21	23	12	14	17	20
		<i>A. niger</i>	14	23	24	27	05	07	11	15
5	<i>Euphorbia heterophylla</i> L.	<i>B. subtilis</i>	20	24	25	27	13	14	17	18
		<i>S. aureus</i>	22	26	30	31	NR	NR	NR	NR
		<i>P. aeruginosa</i>	16	19	21	23	13	15	17	20
		<i>A. niger</i>	14	23	24	27	05	09	12	13
6	Standard drugs		A	B	C	A	B	C		
		<i>B. subtilis</i>	29	ND	ND	31	ND	ND		
		<i>S. aureus</i>	ND	33	ND	ND	32	ND		
		<i>P. aeruginosa</i>	ND	18	ND	ND	20	ND		
		<i>A. niger</i>	ND	ND	19	ND	ND	20		

A-Streptomycin (30µg/ml), B-Tetracycline (30µg/ml), C- Nystatin (30µg)

NR-No results under experimental conditions, ND- Not determined

[*B. subtilis* – *Bacillus subtilis*, *S. aureus* - *Staphylococcus aureus*,

P. aeruginosa - *Pseudomonas aeruginosa*, *A. niger* - *Aspergillus niger*]

The results presented are mean values of n=2.

DISCUSSION

The potential source of antimicrobial agents developing from plants species is an alternative strategy for the production of safe and standardization of a phytomedicines against harmful microbes. The plant based antimicrobial agents have enormous therapeutic potential while they don't have any major side affects to the human beings (Lwu et al .,1999). The plant *Portulaca oleracea* possesses several phytochemicals and has significant antifungal properties against *Aspergillus* species (Bongoh et al., 2000).

The phytochemicals like alkaloids, saponins, flavonoids and phenolic compounds present in plants are responsible for many biological activities (Rabe, 2000). The ethanopharmacological exploration of plant species derived antimicrobial agents is needed for the production of safe and standardization of therapeutic drugs against harmful microbes. *Portulaca oleracea* L., *Crotalaria retusa* L. *Crotalaria speciosa* L. and *Euphorbia hirta* L. extract possess a broad spectrum of antimicrobial activity against a panel of bacteria responsible for the most common bacterial diseases.

CONCLUSIONS

Further research is necessary to determine the active phytochemicals ingredients contributes for antimicrobial activity from weed species and also to determine their minimum inhibitory concentration (MIC) values required for demonstrating the antimicrobial activity.

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