

## Comparative Account of Antifungal Activity of two weeds

Tutakne Neeraja\* and Golatkar Vikas

Assistant Professor, Department of Botany, SIES College, Sion (W) Mumbai 400022, India.

\*Corresponding author Email: neerajaba@gmail.com

Manuscript details:	ABSTRACT
<p>Available online on <a href="http://www.ijlsci.in">http://www.ijlsci.in</a></p> <p>ISSN: 2320-964X (Online) ISSN: 2320-7817 (Print)</p> <p><b>Editor: Dr. Arvind Chavhan</b></p> <p><b>Cite this article as:</b> Tutakne Neeraja and Golatkar Vikas (2016) Comparative Account of Antifungal Activity of two weeds, Int. J.of. Life Sciences, Special Issue, A7:25-32.</p>	<p>The weeds, <i>Lantana camara</i> Linn. and <i>Parthenium hysterophorus</i> Linn. were subjected to different solvent extractions to prepare crude extracts. The antifungal activity of these crude extracts was tested against two plant pathogenic fungi, viz., <i>Alternaria alternata</i> and <i>Fusarium oxysporum</i> and also against three animal pathogenic (dermatophytic) fungi, viz., <i>Candida albicans</i>, <i>Microsporum gypseum</i> and <i>Trichophyton mentagrophytus</i>. All the extracts efficiently inhibited the growth of all the fungi under study.</p> <p><b>Keywords:</b> Antifungal activity, inhibition zone, plant pathogenic fungi, animal pathogenic fungi, dermatophytes</p>
<p><b>Acknowledgements:</b> Authors are grateful to Principal, SIES College Sion (W), Mumbai and Principal, D. G. Ruparel College, Matunga, Mumbai, for providing support and all the facilities. Thanks are also due to Librarians, D. G. Ruparel College, Matunga, Mumbai; Haffkin Institute, Mumbai, and UICT, Mumbai for providing library facilities. The authors would also like to acknowledge the faculty members, Department of Microbiology, Bhavans College, Andheri, Mumbai, for their help in providing the authentic bacterial cultures.</p> <p><b>Copyright:</b> © Author, This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derives License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.</p>	<p><b>INTRODUCTION</b></p> <p>The term 'Weed' refers to any wild harmful unwanted plant, that grows on cultivated land and not only compete with the crop plants for light, space, moisture and soil nutrients but also harbours harmful insect pests and diseases. These weeds interfere with agricultural operations, increase labour, add to the cost of crop cultivation, impair the quality of crops and finally reduce the crop yields. The main problem of the environmentalists and agriculturists is, how to control the rapid expansion of the weeds across the region. The weeds, <i>Lantana camara</i> Linn. and <i>Parthenium hysterophorus</i> Linn. are notorious shrubs with strong odour. They have received the status of world's worst weeds because of their invasiveness, potential for spread and economic and environmental impacts.</p> <p>As per ancient Indian literature, however, every plant on this earth possesses some useful medicinal properties for human beings, animals and also for other plants (Oudhia, 1999 a, b and c). It is sheer the ignorance of human beings that they have classified some plants as useful and others as weeds.</p> <p>The use of higher plants and their extracts to treat infections is an age-old practice. Ethnopharmacologists, botanists, microbiologists and natural-product chemists are combing the Earth for phytochemicals which could be developed for the treatment of infectious diseases (Marjorie, 1999).</p>

Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Patel *et al.*, 2007). Herbal medicines are gaining growing interest because of their cost effective and eco-friendly attribute. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has also been increased. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized (Patel *et al.*, 2007). If this medicinal (antifungal) property resides in a weed then that will be of great advantage. Therefore, in the present investigations, the weeds, *Lantana camara* Linn. and *Parthenium hysterophorus* Linn. were screened to explore their antifungal potentials.

The extracts of *Lantana camara* Linn. and *Parthenium hysterophorus* Linn. prepared in petroleum ether, chloroform, methanol and water were screened against two plant pathogenic fungi, viz., *Alternaria alternata* and *Fusarium oxysporum* and also against three animal pathogenic (dermatophytic) fungi, viz., *Candida albicans*, *Microsporum gypseum* and *Trichophyton mentagrophytus*.

## MATERIALS AND METHODS

### Collection of Plant material:

Fresh wild samples of the weeds, *Lantana camara* Linn. (Yellow-Orange variety) and *Parthenium hysterophorus* Linn. were collected in monsoon by conducting the field excursions to different parts of Mumbai and Thane districts in the months of June to September.

### Extraction of weeds:

The cleaned, washed aerial parts (stem, leaves, flowers and fruits) of the weeds were dried at 45°C in hot air oven. The dried aerial parts of both these weeds were homogenized separately in the mixer to get fine powder of 30 mesh (500µm) particle size. The extractions of both the weed powders in petroleum ether, chloroform and methanol were carried out separately by Soxhlet's extraction method while aqueous extraction was carried out by hot decoction method.

### Soxhlet's Extraction:

50g of weed powder was wrapped in the thimble of filter paper and placed in Soxhlet extractor along with the respective solvent for extraction. The powder : solvent proportion was 1:10. The extraction was continued till the solvent became colourless. The extracts so obtained were concentrated; collected in glass vials and dried completely at 40°C in hot air oven. The glass vials containing extracts were given identification codes and stored in a refrigerator at 4°C.

### Aqueous Extraction:

10g of weed powder was mixed with distilled water in the proportion of 1:10. The mixture was boiled for two hours. It was then filtered through Whatman's filter paper no. 1 and the filtrate was concentrated to achieve the consistency of thick paste. The extract was collected in glass vial; given identification code and stored in refrigerator at 4°C. The identification codes given to the extracts, before storing, were as follows:

Solvent used for extraction	Identification code given to the extracts	
	<i>Lantana camara</i>	<i>Parthenium hysterophorus</i>
Petroleum ether	LP	PP
Chloroform	LC	PC
Methanol	LM	PM
Water	LW	PW

All the extracts except water extract, were insoluble in water hence there was a need to dissolve them in a suitable vehicle to carry out further experiments. Tween 80 (Polysorbate 80) was used as a vehicle (not more than 1%) and six different concentrations viz., 5%, 10%, 20%, 40%, 80% and 100% of each extract were prepared. Different concentrations of water extract were made in similar manner but without using Tween 80.

### Screening of weed extracts for antifungal activity:

#### a. Standardization of Fungal inoculum:

Stock cultures of fungi under study were maintained at 4°C on PDA medium. Each fungal culture was standardized separately.

#### For animal pathogenic (dermatophytic) fungi under study:

Three dermatophytic fungi (animal pathogens) were standardized by the standard protocols given by Clinical Laboratory Standard Institute (NCCLS, 1998).

The standardized inoculum values for these fungi were as follows:

Fungi under study	Standardized Values of the Inocula
▪ <i>Candida albicans</i>	10 <sup>-3</sup>
▪ <i>Microsporum gypseum</i>	10 <sup>-4</sup>
▪ <i>Trichophyton mentagrophytus</i>	10 <sup>-3</sup>

#### **For plant pathogenic fungi under study:**

The plant pathogenic fungi viz; *Alternaria alternata* and *Fusarium oxysporum*, could not form uniform spore suspensions, hence, could not be standardized. Therefore, fungal discs of uniform size, cut out with the help of sterilized cork borer having 4.5mm diameter, from the eight days old pure culture of the fungi, were used for inoculation.

#### **b. Testing Antifungal activity:**

Each weed-extract was screened separately at six different concentration levels against five fungi under study.

#### **For animal pathogenic (dermatophytic) fungi under study:**

Fresh standard inoculum of each dermatophyte under study was prepared separately and used for testing the antifungal activity as follows:

0.1mL of standardized fungal inocula were added to the separate flasks containing 20mL sterilized PDA having moderate temperature and mixed well. The plates were poured and allowed to solidify. The well was scooped out at the centre of each solidified plate with the help of sterilized cork borer having the diameter of 1cm.

Six different concentrations viz; 5%, 10%, 20%, 40%, 80%, 100% of the weed-extract were prepared separately. 100µl of each concentration was added to the wells of six different petriplates indicating the concentrations. Control plate for the dermatophytic fungus was set in similar way except that the extract was replaced by adding 1per cent Tween 80. The plates were kept for incubation at room temperature for two days for *Candida albicans* and four days for the remaining two dermatophytic fungi. The experiments were carried out in triplicates.

At the end of incubation period, the zone of inhibition, if present, was observed and measured. To observe

the persistence of the activity, the plates were incubated further for next two days and the observations were noted. Photographs were taken and the results were tabulated.

#### **For plant pathogenic fungi under study:**

For plant pathogenic fungi under study, flasks containing 20mL of sterilized PDA were prepared. The plates were poured and allowed to solidify. The wells were dug out in similar manner as in case of dermatophytic fungi. The plates were inoculated from three sides, equidistant from the well, by using fungal discs of uniform size (4.5mm diameter) cut out from of eight days old pure cultures of the fungus.

Six different concentrations viz; 5%, 10%, 20%, 40%, 80%, 100% of the weed-extracts were prepared separately. 100µl of each concentration was added to the wells of six different petriplates indicating the concentrations. Control plate for the plant pathogenic fungus was set in similar way except that the extract was replaced by adding 1 per cent Tween 80. The plates were kept for incubation at room temperature for eight days. The experiments were carried out in triplicates.

At the end of incubation period, the zone of inhibition, if present, was observed and measured. To observe the persistence of the activity, the plates were incubated further for next two days and the observations were noted and the results were tabulated.

## **RESULTS AND DISCUSSION**

In general, both the weeds under study, viz., Lantana and Parthenium exhibited wide range of antifungal activity and proved to be effective antifungal agents. Similar reports are available in which antimicrobial activities of different medicinal plants such as *Gyanandropsis gynandra*, *Buchholzia coriacea*, *Sanguisorba officinalis*, *Tussilago farfara*, *Vernonia amygdalina*, *Carum copticum*, *Artemisia afra*, *Backhousia citriodora*, *Callistemone citrinus*, Clove, *Jambolan*, Thyme, Pomgranate, Rosmerry, Guava, Lemongress, Papermint, Bay, Oregano, *Mikania micrantha*, *Ocimum gratissimum*, *Xylopiya aethiopica*, *Acalypha fruticosa*, *Peltophorum pterocarpum*, *Cassia auriculata*, *Momordica charantia*, *Caesalpinia pulcherrima*, *Citrus aurantifolia*, *Parkia biglobosa*,

*Parkia bicolor*, *Catharanthus roseus*, *Polyalthia longifolia* etc. have been reported against the same microorganisms under study (Ajaiyeoba, 2000; Dagmar *et al.*, 2003; Mintesnot and Mogessie, 1999; Cock *et al.*, 2008; Gislene *et al.*, 2000; Hammer *et al.*,

1999; Facey *et al.*, 1999; Ijeh *et al.*, 2005; Veeramuthu *et al.*, 2006; Martinez *et al.*, 1996; Parekh and Chanda 2007; Onyeagba *et al.*, 2004; Ajaiyeoba, 2002; Nayak *et al.*, 2006 and Saha *et al.*, 2005).

**Table 1: Inhibition Zones exhibiting antifungal activity of Lantana and Parthenium extracts**

<b>Antifungal activity of Petroleum ether extract of Lantana (LP)</b>							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
<i>C. albicans</i>	x	x	x	x	0.3	0.5	0.5
<i>M. gysium</i>	x	x	x	1.0	1.3	1.5	1.5
<i>T. mentagrophytus</i>	x	x	x	x	1.3	1.5	1.5
<i>A. alternate</i>	x	x	x	x	0.5	0.6	0.6
<i>F. oxysporum</i>	x	x	x	x	0.7	0.7	0.9
<b>Antifungal activity of Chloroform extract of Lantana (LC)</b>							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
<i>C. albicans</i>	x	x	x	x	0.5	0.6	0.6
<i>M. gysium</i>	x	x	x	1.2	1.4	1.5	1.5
<i>T. mentagrophytus</i>	x	x	x	1.5	1.5	1.6	1.6
<i>A. alternate</i>	x	x	x	x	0.5	0.5	0.6
<i>F. oxysporum</i>	x	x	x	x	0.8	0.9	0.9
<b>Antifungal activity of Methanol extract of Lantana (LM)</b>							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
<i>C. albicans</i>	x	x	x	x	0.7	1.0	1.0
<i>M. gysium</i>	x	x	2.5	2.6	2.6	2.8	2.9
<i>T. mentagrophytus</i>	x	x	x	2.5	2.6	2.6	2.7
<i>A. alternate</i>	x	x	0.7	0.7	0.7	0.8	0.9
<i>F. oxysporum</i>	x	x	x	0.6	0.7	0.8	0.8
<b>Antifungal activity of Water extract of Lantana (LW)</b>							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
<i>C. albicans</i>	x	x	x	x	x	1.0	1.2
<i>M. gysium</i>	x	x	x	x	1.8	1.8	1.9
<i>T. mentagrophytus</i>	x	x	x	x	1.5	1.6	1.6
<i>A. alternate</i>	x	x	x	x	0.6	0.8	0.8
<i>F. oxysporum</i>	x	x	x	x	0.7	0.9	1.0
<b>Antifungal activity of Petroleum ether extract of Parthenium (PP)</b>							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
<i>C. albicans</i>	x	x	x	x	1.2	1.3	1.3
<i>M. gysium</i>	x	x	x	x	1.0	1.4	1.4
<i>T. mentagrophytus</i>	x	x	x	1.3	1.3	1.5	1.5
<i>A. alternate</i>	x	x	x	x	x	x	x
<i>F. oxysporum</i>	x	x	x	x	x	x	x

**Table 1: Continued...**

<b>Antifungal activity of Chloroform extract of Parthenium (PC)</b>							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
<i>C. albicans</i>	x	x	x	x	1.2	1.4	1.5
<i>M. gysium</i>	x	x	x	1.7	1.8	1.9	1.9
<i>T. mentagrophytus</i>	x	x	x	2.1	2.2	2.4	2.5
<i>A. alternate</i>	x	0.5	0.6	0.6	0.8	0.8	0.8
<i>F. oxysporum</i>	x	0.7	0.7	0.7	0.8	0.9	0.9
<b>Antifungal activity of Methanol extract of Parthenium (PM)</b>							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
<i>C. albicans</i>	x	x	x	1.7	1.7	1.8	1.9
<i>M. gysium</i>	x	2.0	2.3	2.3	2.5	2.5	2.5
<i>T. mentagrophytus</i>	x	2.0	2.2	2.3	2.3	2.5	2.6
<i>A. alternate</i>	x	x	x	X	0.5	0.6	0.7
<i>F. oxysporum</i>	x	x	x	X	0.8	0.8	0.9
<b>Antifungal activity of Water extract of Parthenium (PW)</b>							
Fungi	Zone of inhibition (cm)						
	Ctrl.	5%	10%	20%	40%	80%	100%
<i>C. albicans</i>	x	x	x	x	1.3	1.4	1.4
<i>M. gysium</i>	x	x	x	1.0	1.4	1.5	1.5
<i>T. mentagrophytus</i>	x	1.0	1.0	1.1	1.1	1.3	1.4
<i>A. alternate</i>	x	x	x	x	0.5	0.5	0.6
<i>F. oxysporum</i>	x	x	x	0.8	0.9	0.9	1.0
(x – No inhibition zone and hence No antifungal activity)							

**Table 2: Best antifungal concentrations of Lantana and Parthenium extracts and their persistence**

Microorganisms Tested	<b>Lantana Extracts</b>											
	LP			LC			LM			LW		
	C	Z	D	C	Z	D	C	Z	D	C	Z	D
<i>C. albicans</i>	40%	0.3	3	40%	0.5	4	40%	0.7	5	80%	1.0	5
<i>M. gysium</i>	20%	1.3	5	20%	1.2	5	10%	2.5	7	40%	1.8	7
<i>T. mentagrophytus</i>	40%	1.3	5	20%	1.5	5	20%	2.5	7	40%	1.5	7
<i>A. alternate</i>	40%	0.5	7	40%	0.5	7	10%	0.7	8	40%	0.6	8
<i>F. oxysporum</i>	40%	0.7	7	40%	0.8	7	20%	0.6	8	40%	0.7	8
	<b>Parthenium Extracts</b>											
	PP			PC			PM			PW		
	C	Z	D	C	Z	D	C	Z	D	C	Z	D
<i>C. albicans</i>	40%	1.2	3	40%	1.2	4	20%	1.7	5	40%	1.3	5
<i>M. gysium</i>	40%	1.0	6	20%	1.7	6	5%	2.0	7	20%	1.0	7
<i>T. mentagrophytus</i>	20%	1.3	6	20%	2.1	6	5%	2.0	7	5%	1.0	7
<i>A. alternate</i>	NA			5%	0.5	7	40%	0.5	8	40%	0.5	8
<i>F. oxysporum</i>	NA			5%	0.7	7	40%	0.8	8	20%	0.8	8
<b>LP:</b> Lantana – Petroleum-ether, <b>LC:</b> Lantana – Chloroform, <b>LM:</b> Lantana – Methanol, <b>LW:</b> Lantana – Water, <b>PP:</b> Parthenium – Petroleum-ether, <b>PC:</b> Parthenium – Chloroform, <b>PM:</b> Parthenium – Methanol, <b>PW:</b> Parthenium – Water, <b>C:</b> Best antifungal concentration, <b>Z:</b> inhibition zone produced (cm), <b>D:</b> Number of days for which activity persisted, <b>NA:</b> No Activity.												

Petroleum ether, chloroform, methanol and water extracts of Lantana were effective in controlling the growth of all the dermatophytic fungi under study viz., *Candida albicans*, *Microsporum gypsum* and *Trichophyton mentagrophytus*. This is in accordance with Dabur *et al.* (2007); Prashantkumar *et al.* (2006) and Bhakuni *et al.* (1969). However, some of the reports show that *Lantana camara* did not exhibit antifungal activity against *C. albicans* (Ahmad and Beg, 2001 and Rajakaruna *et al.*, 2002). All the extracts of Lantana possessed antifungal activity against both the plant pathogenic fungi under study viz., *Alternaria alternata* and *Fusarium oxysporum*. These findings are in line with the observations made by Mamatha and Ravishankar Rai (2004), Tiwari *et al.* (2004) and Bhatnagar *et al.* (2004). Besides these, there are also reports available where in antimicrobial activity of Lantana oil is reported (Deena and Thoppil 2000; Tania *et al.*, 2000 and Juliani *et al.*, 2002).

The survey of literature revealed that antifungal activity of Parthenium is well documented as compared to its antibacterial activity. Petroleum ether, chloroform, methanol and water extracts of Parthenium proved to be effective against all the dermatophytic fungi under study. These findings are in accordance with Rai *et al.* (1999) and Dhawan *et al.* (1980).

All the extracts, except the petroleum ether extract of Parthenium restricted the growth of plant pathogenic fungi viz., *Alternaria alternata* and *Fusarium oxysporum*. Similar kinds of observations were also reported by Rai *et al.* (1999); however, Bajwa *et al.* (2004), obtained surprising results by testing effect of aqueous extract of Parthenium shoot on *Drechslera hawaiiensis*, *Alternaria alternata*, *Fusarium moniliformae*. According to their findings, lower concentrations of the extract (10 to 50 per cent) proved to be antifungal while that of higher concentrations like 60, 70 per cent stimulated the fungal biomass production.

There are other evidences showing antifungal activity of Parthenium against other plant pathogenic fungi which further confirm the antifungal potential of Parthenium (Polyanna *et al.*, 2008; Bajwa *et al.*, 2003; Sinha *et al.*, 2004; Hsieh *et al.*, 2005 and Patel *et al.*, 2007). Besides these, there is also a report on antimycotic activity of Parthenium oil in which the oil is proved to be the best antimycotic agent against *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp.,

*Microsporum* sp., *Trichophyton* sp. etc. (Sharma and Sharma, 1989).

Over all, all the extracts of Lantana and Parthenium except petroleum ether extracts of Parthenium inhibited the growth of 100 per cent of the fungi tested. Petroleum ether extract of Parthenium found to be least effective amongst all as it inhibited the growth of only 60 per cent of the fungi tested.

The dermatophytic fungi tested found to be more susceptible than the plant pathogenic fungi tested. This variation in susceptibility of the fungi tested may be due to varying permeability of their mycelial wall as well as spore wall.

It was also observed that the diameter of inhibition zone may increase with the increase in concentrations which may be due to increase in the antifungal principles of the extract at higher concentrations. However, after certain limit, the diameter of inhibition zone remains constant. With very few exceptions, almost in all cases the inhibition zone produced at 80 per cent is similar to that of 100 per cent. This may be because of the reduced fluidity of the extracts and thereby that of the active antifungal principle of the extract at higher concentrations. Hence, even being more potent, the highest concentrations of the extracts can not produce larger inhibition zones but gives the inhibition zones similar to that of the immediate lower concentrations.

It was also noted that though the same concentration of the extract is used against different fungi, the diameter of the inhibition zone varies with respect to the fungi tested. This suggests that different antifungal constituents of the same concentration of the extract may be responsible to arrest the growth of different fungi. The difference in the qualitative and quantitative levels of the antifungal constituents of the extracts may be responsible to produce difference in the inhibitory effect.

The persistence of the antifungal activity is checked in terms of the number of days for which the activity persisted. It was observed that the persistence of the antifungal activity of the extracts was varying depending upon the fungi tested. The minimum antifungal concentration of the extract which produced large inhibition zone and remained active

for maximum number of days was considered to be the best antifungal concentration. (Table no. 2).

For both the weeds, the methanol extract gave best antifungal results by producing large inhibition zones at low concentrations and remained active for comparatively more number of days, against maximum number of the fungi tested. Next to methanol, water and chloroform extracts proved to be the best for Lantana and Parthenium respectively.

Though, methanol extract of Lantana and Parthenium proved to be the best extracts, it should not be overlooked that the water extract of both the weeds also exhibited overwhelming antifungal results by producing more or less similar persistent inhibition zones but at higher concentrations. (Table no. 1)

Scanning of literature revealed that variety of research work has been carried out on isolation of chemical constituents of the plants to check its antimicrobial activity. However, in the present investigations, the author has reported the significant antifungal activity of the whole extract of the weeds. Our traditional Ayurvedic medicinal system also supports the concept of using whole plant extract instead of using isolated individual phyto-constituent, for treating various diseases. It is a bitter fact which one should not overlook that individual phyto-constituents many times show side effects, however, whole plant preparations like Ayurvedic preparations are always reported to be more effective and safe. Besides this, the isolation and identification of phyto-constituents involve complicated methods which are time consuming and expensive.

It was very interesting to note that the water extracts of both the weeds obtained, by famous Ayurvedic hot decoction method, exhibited significant antifungal activity. Besides that, its persistence was also praiseworthy. These results are encouraging, because for a common man, it is more feasible to use safest and cheapest water extract than using isolated phyto-constituents by tedious and costly extraction methods. Similarly, these findings have further confirmed and also justified the folkloric uses of these weeds by traditional healers in the treatment of skin disorders or infections.

## CONCLUSION:

Conclusively, most of the extracts of Lantana and Parthenium displayed wide range of antifungal activity. There is need for the development of new antibiotics due to acquired resistance, more importantly from natural sources as this delays resistance (Ajaiyeoba, 2000). The weeds, *Lantana camara* Linn. and *Parthenium hysterophorus* Linn. screened in present investigations provide very good opportunities for drug development in this area.

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