

Research Article

Allelopathic Effect of the Liquid Extract of Parthenium hysterophorus on Microorganism

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A B S T R A C T

Basically in this work study of allelopathic effect of liquid extract of any weed on microorganism like Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Parthenium hysterophorus plants were used for the isolation of liquid and oil extraction, because parthenium has a wide variety of medicinal and adverse effect over microorganism. Basically P. hysterophorus shows allelopathic effect over various crops and some time increases production rate as well as reduces the growth. It was observed that the organic solvent increased the activity of liquid extract of the potent isolates by many times. The best activity of liquid extract in organic solvent, and the liquid extract (distilled water and ethanolic) gives various diameter zones of inhibition in spread plate by well-diffusion methods, and later this liquid extract with the organic solvent Ethanol and acetone gives more valuable zone of inhibition in cultured colonies and we have seen comparative effect of antibiotics, liquid, ethanolic and so this study is helpful for herbal drug formulation and antibiotics as well as vaccine formulation.

Keywords: Herbal Drug Formulation, Allelopathic, Liquid Extract, Well Diffusion

Introduction

Parthenium hysterophorus is a species of flowering plant in the aster family, Asteraceae that is native to the American tropics. Common names include Santa Maria Feverfew and Whitetop Weed. It is a common invasive species in India, Australia, and parts of Africa. P. hysterophorus invades all disturbed land, including farms, pastures, and roadsides. In some areas, outbreaks have been of almost epidemic proportions, affecting crop production, livestock and human health. In India, it is locally known as Congress Grass or Gajar Ghans.

The word Parthenium hysterophorus is derived from the Latin word 'parthenice,' suggesting medicinal uses. The

origin of this obnoxious weed is traced to the Caribbean but its adverse effects are felt largely in African, Australian and Asian countries. The weed was first sighted in Pune in 1956. It was first introduced due to contaminated PL-480 wheat imported from the United States, and is also called as 'Congress Grass' due to the Congress government which imported the wheat.

Parthenium hysterophorus entered India with imported food grains in the mid-1950s. One of the world's seven most devastating and hazardous weeds, P. hysterophorus invaded 14.25 million hectares of farm land during 2001-2007, compared to 2 million hectares in 1991-2000.

Parthenium hysterophorus has invaded 35 million hectares

across the country including crop land, wasteland and forest areas, according to the DWSR, which is preparing a report on this. Initially, the deadly weed occupied largely non-crop areas like wasteland, open forests and roadsides. Now it has spread to cropping land at an alarming rate.

In the Dictionary of Economic Plants in India, *Parthenium hysterophorus* is described as a weed found in Poona and is reported to be used as tonic, febrifuge, and emmenagogue. Root decoction is useful in dysentery (Singh et al. 1996).

Mew et al. (1982) demonstrated that sublethal doses of parthenin exhibited antitumor activity in mice and that the drug could either cure mice completely or increase their survival time after they had been injected with cancer cells. *Parthenium hysterophorus* is also reported as promising remedy against hepatic amoebiasis (Sharma and Bhutani, 1988). South American Indians use a decoction of roots to cure amoebic dysentery (Uphof 1959) whereas parthenin, a toxin of *P. hysterophorus*, is found pharmacologically active against neuralgia and certain types of rheumatism). In Compendium of Indian Medicinal Plants by Rastogi and Mehrotra (1991) parthenin induced dose-dependent damage to human leucocyte chromosomes in vitro and micronuclei formation in polychromatic erythrocytes of mice is reported (Dominguez and Sierra, 1970).

Parthenium hysterophorus is used as folk remedy in the Caribbean and Central America (Nabie et al. 1996). It is applied externally on skin disorders and decoction of the plant is often taken internally as a remedy for a wide variety of ailments (Dominguez and Sierra 1970; Morton 1981). In Jamaica the decoction is used as a flea-repellent both for dogs and other animals (Morton 1981).



Figure 1. Parthenium hysterophorus Plant

Toxicity

Contact with this plant causes dermatitis and respiratory malfunction in humans, dermatitis in cattle and domestic animals, due to the presence of toxin parthenin.

Concept of Allelopathy

The phenomenon of one plant having detrimental effect on

another plant and microorganism by the excretion of toxic chemical compound is called allelopathy and this effect is called allelopathic effect.

Allelopathy is the biochemical interactions between all types of plants including microorganisms.

Important point on allelopathy involves the addition of something to the environment, thus separated from competition, which involves removal of requirements for growth (light, water and nutrients).

There are Various Allelopathic Chemicals; these are as follows:

1. Phenolic acids
2. Coumarins
3. Terpinoids
4. Flavinoids
5. Scopulatens

Ways of Releasing of Allelochemicals

Allelopathic chemicals are released from the plants as:

1. Vapor root and leaf (through stomata)
2. Foliar leachate
3. Root exudates
4. Break down or dead parts of plant body
5. Seed extract

Materials and Methods

The Strain used in this Work is as follows

The microorganism like *E. coli*, *P. aeruginosa* and *B. subtilis* collected from college laboratory of Dr. Ambedkar Institute of Technology for Handicapped, Kanpur, and *Staphylococcus aureus* from LAL Pathology, Kanpur.

There is use of two types of antibiotics disks, i.e., Ampicillin and Tetracycline of 10 µg and 30 µg respectively. These disks are available in the market and we can form them in laboratory also.

Plant Material

Fresh plant material was collected in the month of September in 2013 from Lakhanpur Kanpur roadside regions-1 to 1.5 mt plants-and harvested the plant material removal of leaves from stem and flower, shade drying the leaves into plastic bags; after that powdered the leaves for liquid extract.

Formulation of Distilled Water Extract

Basically, formulation of liquid extract was made in distilled water, in distilled water extract there is use of grounded and harvested leaves and form. One-gram extract in 1 mL of water was mixed well in Eppendorf's and quantity maintained according to use. First it was mixed well on vortex shaker, after that centrifuged up to 8000 rpm for

5-8 min and kept in the tube for overnight; after that it was ready for use.

Formulation of Alcoholic Extract

To make alcoholic extract, first plant material is harvested. Then the leaves are grounded and 16 gm of leaves are mixed with 1 ml of 70% Ethyl alcohol filled in Eppendorf. After that these Eppendorf's shakes are placed on vortex shaker. Then it is centrifuged up to 12000 rpm for 10 min, after which Eppendorf's is kept open for drying the supernatant for 1-2 days. After drying it, TrisHCl is added to the dried mixture and is mixed well by vortex shaker. Now complete alcoholic liquid extract is formed and is ready for use on cultured Petri dishes.

- Now turn off the UV and turn on the filter of laminar and lighten the spirit lamp.
- Clean hands by 70% ethanol and sterilize the spreader by cotton swab.
- Name the plates.
- Now pouring of media into petri dishes after that leave the plate to solidify.
- After 15-20 min solidify the nutrient agar in plates, now transfer the broth of stored culture of various microorganisms into laminar.
- After transferring stored culture broth of microorganism, there is spreading of culture of microorganism by use of 100 μ L pipette.

Table I. Disk Potency of Antibiotics and Zone of Inhibition in mm according to References

Antibiotics	Disk Potency	Inhibition Zone Diameter to Nearest mm		
		Resistant	intermediate	susceptible
Ampicillin (AM)	10 μ g	11 or less	12-13	14 or more
Tetracycline (TM)	30 μ g	14 or less	15-18	19 or more

Note: In primary screening, there are various zones of inhibition present in plate. We measure them and compare with above table by which we can say which microorganism is resistant or intermediate or susceptible.

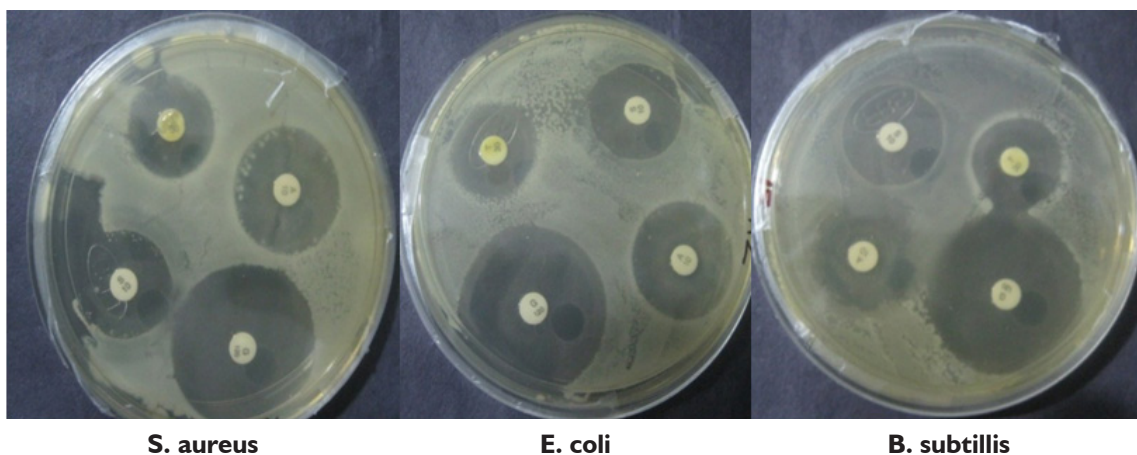


Figure 2. Disk Potency in all the Three Bacterial Cultures

Secondary Screening of Distilled Water and Ethanolic Extract on Test Microorganism

In this section, we perform disk diffusion method or well diffusion method for screening of test sample like distilled water and ethanolic extract of *P. hysterothorus* on culture plate of microorganisms.

General steps for test of distilled water, ethanolic extract are

- Weigh 2.8 gm readymade nutrient agar on analytical balance, add 100 mL distilled water in it.
- Make 100 mL nutrient agar media in 200 mL conical flask after autoclaved 15 lbs and 121°C for 15 min.
- Transfer the 5 petri dishes and autoclaved media into clean laminar air flow, then after UV on for 10-15 min.

- Take stored culture and drop out 20 μ L carefully and spread through spreader uniformly on plates.
- Now after spreading use of gel puncture, make two or three wells into plate and cut the wells carefully.
- After formation of wells filled the wells with test sample like distilled water ethanolic and oil simultaneously according to screening.
- After that incubate the plates 37°C for overnight or 48 hr, and measure the zone of inhibition by the use of stationary scale in millimeters.
- Write down the result.

Effect of Distilled Water Extract and Ethanolic Extract on Pure Culture

- According to above general method of screening

follows, wells of petri dishes filled with 50 µL distilled water and ethanolic extract respectively into the wells and seen the comparative effect.

- Incubate the plates at 37°C for overnight and observe the plates and take measurement of the zone of inhibition if found.

Here both the liquid extracts were used in a single plate and we found various zones of inhibitions. Following figures show them.

Table 2 shows the allelopathic effect of extracts against various microorganisms. The maximum inhibition zone is of 16 mm.

Results

According to above project work, we have seen that we found various types of zones of inhibition which is due to allelochemicals effect and show there potency against the microorganism/ pathogens. The comparative analysis shows following result.



Figure 2. Disk Potency in all the Three Bacterial Cultures

Table 2. Result of the Antibiogram after Treatment of Distilled Water and Ethanolic Extract

Microorganism Strain	Zone of Inhibition Diameter in mm	
	Distilled Water Extract	Ethanolic Extract
E. coli	6 mm	14 mm
B. subtilis	10 mm	14 mm
S. Aureus	12 mm	16 mm

Comparative Analysis of Zone of Inhibition

Table 3. Comparative Analysis of Zones of Inhibition/ Susceptibility by the Treatment of Antibiotic (Ampicillin and Tetracycline), Distilled Water Extract, Ethanolic Extract of P. hysterosporus

Microorganism Strains	Antibiotics		Distilled Water Extract mm	Ethanolic Extract mm
	Ampicillin	Tetracycline		
E. coli	32 mm	25 mm	6	14 ^b
B. subtilis	22 mm	18 mm	10	14 ^b
S. aureus	30 mm	19 mm	12	16 ^a

Comparative Analysis of Antibiotics, Distilled Water and Ethanolic Extract

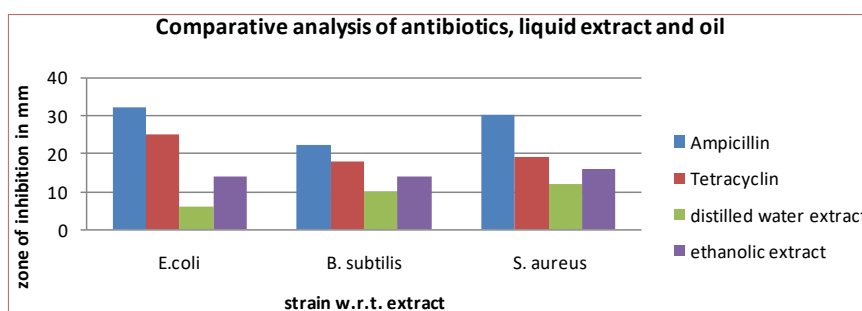


Figure 4. Comparative Analysis of Antibiotics, Liquid Extract and Oil

- We get ethanolic extract of *P. hysterophorus* gives maximum 16 mm zone of inhibition in *Staphylococcus aureus*.
- Distilled water extract not more potent and gives only maximum 12 mm zone of inhibition in *S. aureus*.

Statistical Analysis

From the above experimental data, ethanolic extract of *P. hysterophorus* shows much better result. The data were analyzed by JMP4 software used for data analysis. The generated result is significantly different at $p > 0.05$.

Discussion

The result of this conceptual study shows that this weed has inherent ability to induce allelopathic effect on growth of microorganism and similar effect shows on various plant and seed germination were reported by other authors, in present study shows that *P. hysterophorus* shows better inhibitory effect in ethanolic extract in comparison with distilled water extract and oil which were less effective on the basis of data obtained in the present investigation, conclusion may be drawn that the ethanolic extract of *P. hysterophorus* can be used as novel bactericidal against *S. aureus* but large-scale bioactive component identification is important.

Conclusion

This study shows the allelopathic effect and antimicrobial actions and medicinal potentials of the aqueous and ethanolic extracts of the leaves of *P. hysterophorus*. This approach to the management of this plant or weed can be economically viable if adopted.

Finally, the study gives a broad idea about new vaccine and herbal drug formulation through this plant.

The extract made from the leaves of young *Parthenium* plants had the strongest overall inhibitory effects on the growth of microorganism. Based on these results it can be deduced that the allelochemicals are released from the *Parthenium* plants through leaching from leaves also in nature. This is further supported by the fact that the *Parthenium* roots and stems did not have significant inhibitory effects so the author did not use stem and root extracts.

Conflict of Interest: None

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