

In Vitro Antifungal Potential Of Different Combination Of Plant Extract Of Some Common Weed Plants Against Fusarium Oxysporum And Erysiphe Polygoni

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Abstract

*The increasing resistance of phytopathogenic fungi to conventional fungicides has prompted the search for alternative plant-based antifungal agents. This study evaluates the antifungal potential of aqueous extracts prepared from seven commonly occurring weed species: *Datura metel*, *Alternanthera sessilis*, *Calotropis gigantea*, *Argemone mexicana*, *Tridax procumbens*, *Lantana camara*, and *Sida acuta*. Various polyherbal formulations were tested for their efficacy against *Fusarium oxysporum* and *Erysiphe polygoni*, two significant fungal pathogens affecting agricultural crops. Antifungal activity was assessed using the agar well diffusion method on Potato Dextrose Agar (PDA) plates, with inhibition zones measured after 72 hours of incubation at 25 ± 3 °C. Among the tested formulations, F-5 exhibited the highest antifungal activity, producing consistent inhibition zones of 26 mm against both fungal species, suggesting a broad-spectrum fungicidal effect. Formulation F-4 demonstrated moderate activity. The enhanced performance of F-5 may be attributed to synergistic interactions among its phytochemical constituents. These results underscore the potential of specific polyherbal combinations as effective, eco-friendly antifungal agents for agricultural use.*

Key Words :-*Polyherbal formulation, Antifungal activity, *Fusarium oxysporum*, *Erysiphe polygoni*, Weed plant extracts, Agar well diffusion Plant-based fungicide*

INTRODUCTION

Fungi are such a part of the living world that many of its members cause great harm to economically important crops, which impacts the food supply of the country. To reduce this loss, various types of chemical fungicides are used by farmers. This chemical based fungicide protects the food grains from the damage caused by pathogenic fungi, but the damage caused to the environment due to these chemicals is very terrible. Due to these dangerous chemicals, not only various species of pathogenic fungi are being destroyed but also some species of beneficial insects and bacteria are being destroyed, due to which imbalance is being created in the ecosystem.

This imbalance created in the ecosystem is very harmful for the human race and for this earth, hence it is necessary that in place of these chemical fungicides, some other eco-friendly pesticides should be used which can sustainably control these pathogenic fungi and not harm the environment by killing them.

The best substitute of these chemical based pesticides is bio-pesticides, which we all know very well. Due to the damage caused to the environment due to chemical pesticides in the last few decades, the attraction of common people towards bio-pesticides is huge. Biopesticides are obtained from various sources such as micro-organisms, natural chemicals, plant extracts etc. Biopesticides are a very good substitute for chemical pesticides because they do not harm the environment and also control pathogens in a natural way, which will prove to be very effective in maintaining ecological balance. But for this, it is necessary that more and more research can be done on various sources of biopesticides so that more and more biopesticides can be available to the farmers in the market at lower prices.

If we look around us, there is a class of plants which can be used to control these pathogenic organisms and that is weed plants which are found in countless numbers around us and do not require any special protection for their maintenance. Nor are they consumed as food even by herbivorous organisms. There are some special

properties found in these plants due to which they protect themselves from herbivorous organisms and also from pathogenic micro-organisms (Saha and Modi,2024).

In this research article, different combinations of aqueous extracts of seven common weed plants *Dhatura metal*, *Alternanthera sessilis*, *Calotropis genanta*, *Argemone mexicana*, *Tridex procumbens*, *Lantana camera*, *Sida acuta* found nearby have been prepared and their antifungal activity has been studied on two species of fungi, *Fusarium oxysporum* and *Erysiphe polygoni*.

MATERIAL AND METHOD

Collection of plant material

In the present work we have selected a total of seven weed plants, *Dhatura metal*, *Alternanthera sessilis*, *Calotropis genanta*, *Argemone mexicana*, *Tridex procumbens*, *Lantana camera*, *Sida acuta*. These plants have been selected keeping in mind their antimicrobial properties. The antimicrobial properties of these plants are known from previous research work done on them. The weed plants have been collected from the areas around Bhopal. After collection, these plants were identified by Dr. Suman Mishra, CEO, Herbal Testing and Research Laboratory, Bhopal, Madhya Pradesh, India. Information about these seven weed plants is given in Table 1.

Processing of plant material for extraction

After collecting the weed plants, the next task is to prepare them for the extraction process, in which firstly the collected plant material is thoroughly washed and cleaned and then dried in the shade. So that the phytochemicals present in the plant are not destroyed. When these plant samples are dried properly, they are ground into fine powder which is used in the next step to obtain the plant extract.

Table 1. List of weed plant selected for antifungal potential

Sr. no.	Local name	Botanical name	Family	Plant parts used
1.	Devil trumpet	<i>Datura metal</i>	Solanaceae	Leaf
2.	Brazilian spinach	<i>Alternanthera sessilis</i>	Amaranthaceae	Twigs
3.	Tridex Daisy	<i>Tridex procumbents</i>	Asteraceae	Twigs
4.	Crown flower or milkweed	<i>Calotropis gigantea</i>	Asclepiadaceae	Leaf
5.	Lantana	<i>Lantana camera</i>	Verbenaceae	Leaf
6.	Mexican prickle poppy	<i>Argemone mexican</i>	Papaveraceae	Twigs
7.	Common wire weed	<i>Sida acute</i>	Malvaceae	Leaf

Extraction procedure

In this study, weed plant extract was obtained from Soxhlation extraction method is used and pure distilled water which is a polar solvent is used for plant extraction

Defatting:-Before obtaining the extract from , these powders of sample are defatted so that the oil, wax, greasy substances etc. present in it are removed and There should not be any hindrance in the further extraction process.Petroleum ether has been used for the defetting process.After the completion of the defetting process, the plant powder is sieved and dried and then used for Soxhlation process of extraction.

Soxhlation extraction method:- All the seven leaf powders prepared which have already been defatted are used for the Soxhlation extraction method In which 20grm of each leaf powder is used in a 250ml capacity Soxhlation apparatus with distilled water solvent at 60C to 80C temperature for 24 hrs.The extract obtained is then concentrated by vaporizing the excess solvent using boiling water bath and The following formula is used to determine the yield of an extract:

$$\% \text{ yield} = \frac{\text{Weight of Extract.}}{\text{Weight of crude subtract take before extraction}} \times 100$$

Preliminary phytochemical test

A.Organoleptic property:-After obtaining phytochemical extracts, the organoleptic properties taste, color and aroma etc.of these extracts are observed. This observation is visual and sensory based. This property play a vital role in assessing the quality and authenticity of herbal product.(Heinrich et al.,2009).Information on the organoleptic properties of the plant extract is given in Table 2.

B. Phytochemical analysis:- In this section, various phytochemicals such as alkaloids, flavonoids, terpenoids and saponins (Harborne.,1998) other trace elements present in the plant extracts were examined by this method.All these phytochemicals have the ability to control pests.(Isman, 2000) Test result of phytochemical analysis is given in Table 3

Table 2 Organoleptic property of plant extracts

S.N.	Aqueous Extract of	% Yield	Organoleptic Properties		
			Colour	Texture	Smell
1.	<i>Datura metel</i>	26.13%	Dark Brown	Greasy texture	extremely strong, often unpleasant
2.	<i>Alternanthera sessilis</i>	27.86%	Chocolate Brown	Crystal Texture,	offensively strong and unpleasant
3.	<i>Tridax procumbens</i>	26.53%	Chocolate Brown	Hard Sticky paste, rough texture	having a heavy, offensive smell
4.	<i>Calotropis gigantea</i>	27.06%	Black	Hard Sticky paste, rough texture	Intense, Aromatic, Unpleasant
5.	<i>Lantana camara</i>	21.06%	Black	Crystal texture	Intense, Aromatic, Unpleasant
6.	<i>Argemone mexicana</i>	32.26%	Chocolate Brown	Hard Sticky paste, rough texture	Intense, Aromatic, Unpleasant

7.	<i>Sida Acuta</i>	26%	Chocolate Brown	Hard Sticky paste, rough texture	Intense, Aromatic, Unpleasant
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Table 3 Test result of phytochemical analysis

S.N.	Tests Conducted	<i>Datura metal</i>	<i>Alternanthera sessilis</i>	<i>Tridax procumbens</i>	<i>Calotropis gigantea</i>	<i>Lantan a camara</i>	<i>Argemone mexicana</i>	<i>Sida acuta</i>
1.	Alkaloid Test	+ve	+ve	-ve	+ve	+ve	+ve	+ve
2.	Flavonoid Test	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3.	Tannins Test	-ve	+ve	+ve	+ve	+ve	+ve	+ve
4.	Glycoside Test	+ve	-ve	+ve	+ve	+ve	+ve	+ve
5.	Terpenoid Test	+ve	+ve	+ve	+ve	+ve	+ve	+ve
6.	Saponins Test	+ve	-ve	-ve	+ve	-ve	+ve	-ve

Preparation of combinations of plant extracts

This aqueous extract is used to prepare the stock solution at 100 mg/ml using distilled water. This stock solution is used to prepare the Binary Tri & Poly herbal extract combination in 1:1 ratio. The combinations thus prepared are used to study antimicrobial activity. Information on these phytochemical extract combinations is given in Table 4

Table 4 combinations of plant extract

S.N.	Combination	Aqueous Extracts Used	Formulation
1.	Triherbal	<i>D.metal</i> + <i>S.acuta</i> + <i>A. sessilis</i>	Formulation – 1
2.	Polyherbal	<i>D.metal</i> + <i>S.acuta</i> + <i>A. sessilis</i> + <i>T. procumbens</i>	Formulation – 2
3.	Polyherbal	<i>D.metal</i> + <i>S.acuta</i> + <i>A.sessilis</i> + <i>T.procumbens</i> + <i>C.gigantea</i>	Formulation – 3
4.	Polyherbal	<i>D.metal</i> + <i>S.acuta</i> + <i>A.sessilis</i> + <i>T.procumbens</i> + <i>C.gigantea</i> + <i>L.camara</i>	Formulation – 4

5.	Polyherbal	<i>D.metal + S.acuta + A.sessilis + T.procumbens + C.gigantea + L.camara + A.mexicana</i>	Formulation – 5
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Test fungus

ATCC microbial cultures, known for their antimicrobial activity, were procured for the present investigation from HiMedia Laboratory Pvt. Ltd. (Mumbai, India) and Molmet Biotech Research Pvt. Ltd. (Bhopal, India). Table 5 presents the list of microorganisms used in the study along with their respective ATCC accession numbers. fungal cultures that are to be used in the present investigation were first revived into the broths. potato dextrose broth was used to revive *Fusarium oxysporum* and *Erysiphe polygoni*. The broths were incubated for 3 to 5 days at 25 ± 2 oC in case of fungal cultures. When broth becomes turbid, using an inoculation loop the taking inoculum from broths the microbial species are streaked onto PDA plates and slants fungi and incubated further under the same conditions to get the microbial colonies on solid surface that could be stored and maintained during the tenure of research work. ThisIt was regarded as stock culture.

Table 5 list of fungal species

S.N.	Pathogen Type	Microorganism	Accession Number
1.	Pathogenic Fungi	<i>Fusarium oxysporum</i>	ATCC-62506
2..	Pathogenic Fungi	<i>Erysiphe polygoni</i>	MBR-0033

Antifungal assay

In present study antimicrobial susceptibility was performed for antifungal activity using agar well diffusion method. For antifungal activity Potato Dextrose agar plates were prepared for working with fungi. Culture media plates were inoculated with pathogenic microbial strain After that 6 mm diameter wells were aseptically punched. For antifungal susceptibility assay 20 μ l of stocks of prospected polyherbal combination were aseptically poured into each well separately. The culture plates with fungal inoculum were incubated at 25 ± 3 oC for 72 hours . Observations were taken in the form of zone of inhibition after incubation which were measured in millimeters (mm).

RESULT

Table 6 presents a comparative evaluation of the antifungal efficacy of various polyherbal formulations against *Fusarium oxysporum* and *Erysiphe polygoni*. Among the tested formulations, F-5 emerged as the most potent, exhibiting a uniform inhibition zone of 26 mm against both fungal species, indicative of a broad-spectrum fungicidal effect. In contrast, F-4 demonstrated moderate activity, with inhibition zones of 22 mm and 18 mm against *Fusarium oxysporum* and *Erysiphe polygoni*, respectively. The marked difference in inhibition zones suggests that the composition of F-5 may contain synergistic phytoconstituents contributing to its enhanced antifungal performance. These findings underscore the potential of specific polyherbal combinations, particularly F-5, in developing effective antifungal agents.

Table 6 Effect of plant extract combination on fungal species

S.N.	Sample Stock (100 mg/ml)	Zone of inhibition (Φ in mm) against test microbes	
		<i>Fusarium oxysporum</i>	<i>Erysiphe polygoni</i>

1.	F-1	12 mm	0 mm
2.	F-2	15 mm	12 mm
3.	F-3	17 mm	16 mm
4.	F-4	22 mm	18 mm
5.	F-5	26 mm	26 mm

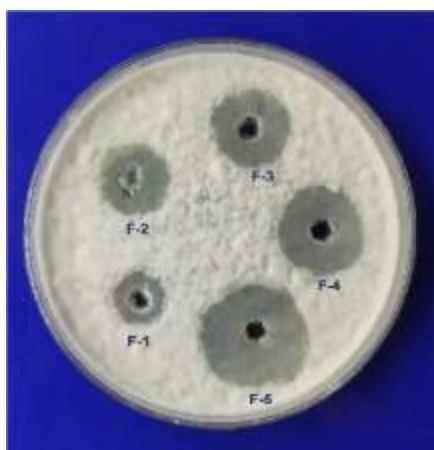
A. *Fusarium oxysporum*B. *Erysiphe polygoni*

Image 1. In vitro effect of polyherbal combination on fungal sample. **A.** *Fusarium oxysporum*, **B.** *Erysiphe polygoni*

DISCUSSION

The antifungal screening of five polyherbal formulations revealed notable differences in their efficacy against *Fusarium oxysporum* and *Erysiphe polygoni*. Among them, formulation F-5 showed the highest inhibitory effect against both pathogens, indicating strong fungicidal potential. This enhanced activity is likely due to synergistic interactions among its phytochemical constituents, which aligns with findings from previous studies suggesting that combinations of plant extracts can produce greater antimicrobial effects than individual components alone (Kong et al., 2019).

Formulations F-4, F-3, and F-2 exhibited moderate antifungal activity, while F-1 showed minimal to no activity, particularly against *Erysiphe polygoni*. These results emphasize the importance of the specific plant extract combinations used in each formulation. The lack of activity in F-1 also suggests possible antagonistic interactions or insufficient concentrations of active compounds, highlighting the need for careful formulation design. Overall, the results support the potential of polyherbal formulations as natural alternatives to synthetic fungicides. Their plant-based nature offers advantages such as reduced toxicity, environmental safety, and lower risk of resistance development. Formulation F-5, in particular, holds promise for further development as an effective biopesticide for sustainable agricultural practices.

CONCLUSION

Based on the findings, it can be concluded that polyherbal formulations possess significant antifungal potential, with formulation F-5 demonstrating the highest efficacy against both *Fusarium oxysporum* and *Erysiphe polygoni*. The results emphasize the role of synergistic interactions among phytochemicals in

enhancing antifungal activity and underscore the importance of selecting optimal plant extract combinations. This study supports the use of natural, plant-based formulations as promising alternatives to synthetic fungicides, which are often associated with environmental and health risks. The superior performance of F-5 suggests its potential for further development into a commercial biopesticide. Continued research, including phytochemical profiling, mechanism of action studies, and field trials, will be essential to validate its practical application and establish its role in sustainable and integrated plant disease management systems.

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