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In – vitro Antifungal Evaluation of *Partheniumhysterophorus* and Characterization of its Extract by GCMS Analysis

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Abstract—*Botanical fungicides are emerging as potential option for having environmental friendly management. Chemical and biochemical advances had helped in extraction and biological characterization of main active ingredients of currently used botanical control agents. These botanicals are of immense importance as they are ecofriendly in nature and cost effective. The present work was an approach towards the development of ecofriendly antifungal compound for controlling plant diseases caused by Alternariaalternata. Antifungal potential was analysed by MIC and MFC and characterization of extract was done by GCMS analysis. Ethanolic extract of Partheniumhysterophorus was used for MIC and MFC analysis. The MIC was found to be 12.5 mg/ml and MFC 50 mg/ml respectively for all isolated strains of Alternariaalternata. GCMS analysis revealed the presence of 1, 2-Benzenecarboxylic acid, bis (2-methylpropylacid) bis (2-methylpropyl) ester (9.95%), Azuleno (4,5-b) furan-2,9-dione, 9a-(acetyloxymethyl) (8.39%), 1,7,7,7B-tetramethyldecahydrocyclopropane (8.06%), 1,2-Benzenedicarboxylic acid (7.17%), n-hexadecanoic acid (7.14%), 2-hexadecen-1-OL,3,7,11,15-Tetramethyl (5.20%) as major compounds. The present study provides the insight that Partheniumhysterophorus can be used for the development of novel herbal fungicidal formulations after in-vivo and field trial.*

Keywords: *Partheniumhysterophorus, Antifungal activity, MIC, MFC, GCMS*

1. INTRODUCTION

The plant comprises a rich storehouse of biochemical that could be tapped for use as biofungicide. The toxic principles in plants represent the secondary metabolites and have only significant role in primarily physiological in plants [1]. Several attempts were made to screen out the antifungal properties of some plants against the pathogenic microbes [2, 3]. Extracts isolated from several plants have been reported to have biological activity such as antimicrobial, antifungal, anti-inflammatory, and antioxidant activities [4, 5]. The potential biological activity of extracts and essential oils from a wide range plants on various microorganisms has been assessed [6-14]. Plant extracts have played significant role in the inhibition of seed-borne pathogens and in the improvement of seed quality and field emergence of plant seeds.

Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides [15]. 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 [16]. Natural plant products are biodegradable, exhibit structural diversity and complexity and rarely contain halogenated atoms. These can act directly as pesticides or may provide structure lead for pesticidal discovery. Because ofenvironmental and economic considerations, plant scientists are involved to find the cheaperand more environmental friendly bio-compounds for the control of plant diseases usingdiffusates from different plants [17-20].Nowadays there is a clear tendency towards the utilization of natural products, especially allelochemicals, as alternative compounds for pest and plant disease control, safe for humans and environment. Therefore, the search of new natural products including plant extracts, which might substitute synthetic agrochemicals or contribute to the development of new agents for pest control, seems to be important.The objective of the present investigation was to acertain the antifungal potential of ethanolic leaf extracts of *Partheniumhysterophorus* to control the phytopathogenic fungi *Alternariaalternata*.

2. MATERIALS AND METHODS

Collection of leaves: Fresh and healthy leaves of *Partheniumhysterophorus*were collected from BHEL area, Haridwar for screening their antifungal activity. Identity of plant species was authenticated by referring taxonomic literature.

Isolation of test fungus: Different isolates of the phytopathogenic fungi *Alternariaalternata* were isolated from diseased leaf of Potato (*Solanumtuberosum*), Tomato (*Lycopersiconesculentum*), Onion (*Alliumcepa*) and Mustard (*Brassicacampestris*). The standard tissue isolation procedure was followed to isolate the pathogen. The infected leaf bits were surface sterilized with 1:1000 mercuric chloride ($HgCl_2$) solution for 30 sec and repeatedly washed separately in sterilized distilled water to remove the traces of mercury if any and then transferred to sterilized petri plate (4 leaf bits per petri plate) containing potato dextrose agar. Thepetriplates were incubated at 28±2°C and observed periodically for the growth. As a bit of fungal growth developed from infected tissue, it was transferred to PDA slants and incubated at 28±2°C for 7 days. Then these slants with pure culture were used for further studies. The pathogens were subcultured on PDA slants as well as in PD broth and allowed to grow at 28±2°C for 7 days and such slants were preserved in refrigerator at 5°C and renewed once in 30 days.

Identification of pathogen: Identification of pathogen was done by lactophenol cotton blue staining and further observing them under microscope (40X). The conidial body, beak and septa were observed. The observation was compared with those of the standard characteristics given by Barnett to identify the pathogen [21]. Further, the identity of the pathogenic isolates was confirmed by the division of pathology, IARI, New Delhi.

Extract preparation: Extract was prepared by cold soxhlet extraction method. The leaves were thoroughly washed 2-3 times with tap water and then with distilled water and were shade dried. After getting dried up they were converted into powdered form with the help of mixer grinder. 200 mg/ml concentration was selected, so 20 g of powdered leaf material was used for extraction using 100 ml of ethanol. The obtained extracts were further filtered through Whatmann filter paper no.1. The supernatant was collected and was evaporated in vacuum rotavapour to make final volume $1/5^{th}$ of the original volume. Then the final extract was stored at 4˚C in airtight bottles for further study [22].

Minimum inhoibitoy concentration (MIC): The minimum inhibitory concentration (MIC) was determined by microdilution method using serially diluted plant extracts according to the NCCLS protocol [23]. The ethanolicextracts was diluted to get a series of concentration from 0.39 mg/ ml to 200 mg/ ml, in sterile PD broth in 96 well microtiter plate. Mancozeb Fungicide used was also diluted. The fungal suspension of 50 µl was added to the broth dilutions. These were incubated for 7 days at 28±2°C. MIC of each extract was taken as the lowest concentration that did not give any visible fungal growth.

Minimum fungicidal concentration (MFC): Minimum fungicidal concentration is the lowest concentration of antifungal agent that will prevent the growth of an organism after subculture on an antifungal free media. To determine MFC 50 µl mixture from the well showing MIC and from wells having extract concentration more than MIC was placed on PDA plate without extract and the plates were incubated at 28±2°C for 7 days. After incubation the plates were examined for the growth of fungal isolates to determine the concentration of the extract at which no growth occurred.

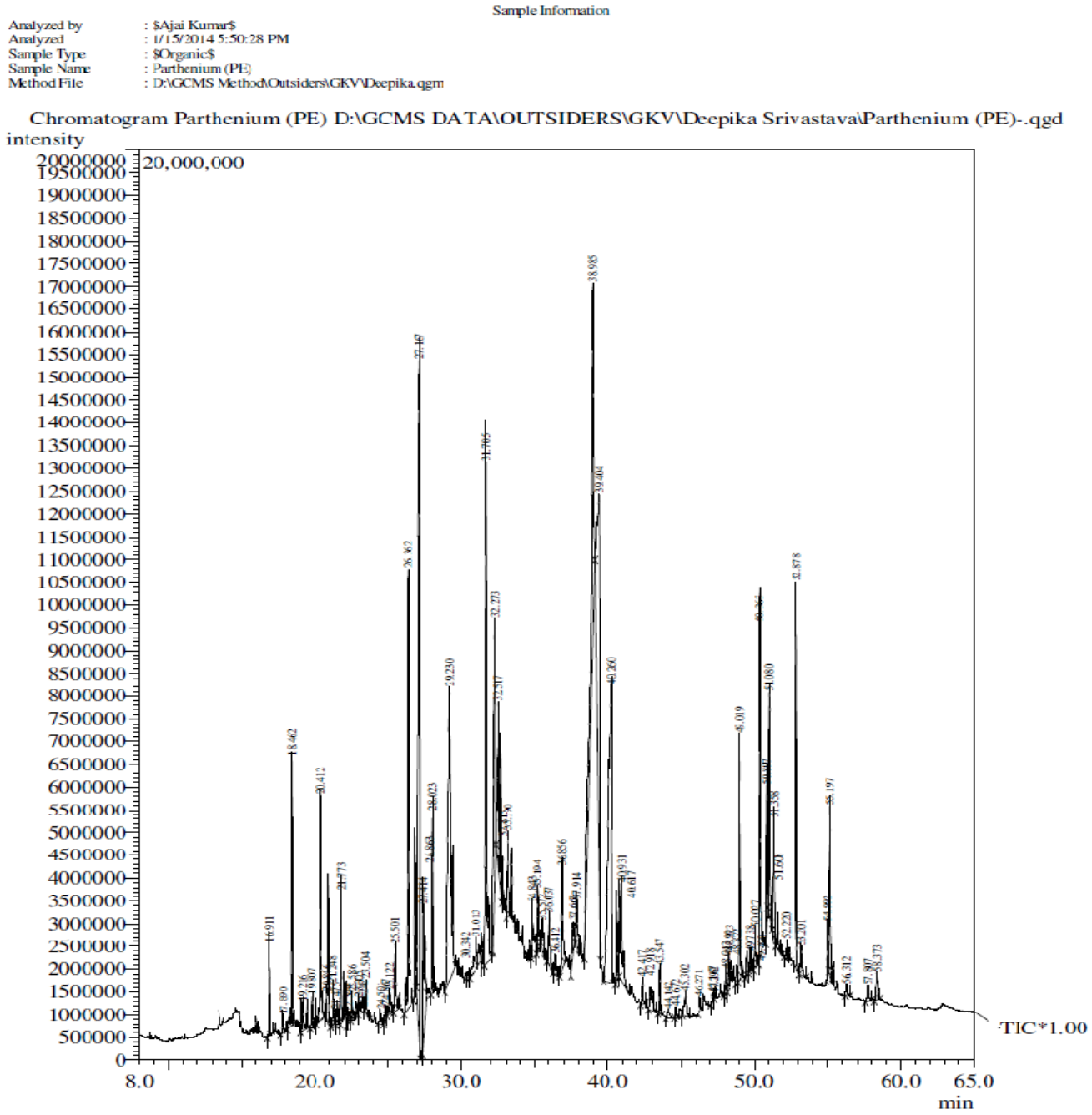
GCMS analysis: Phytochemical screening was done by standard procedures and for Chromatographic analysis Gas Chromatography Mass spectroscopy (GCMS) was selected. The GCMS analysis was done by using GC-MS-QP 2010 Plus (Shimadzu Mass Spectrometer- 2010 series system) from AIRF, JNU, New Delhi equipped with Rtx_5Ms column (30 m x 0.25 mm id), 0.25 µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1.21 ml/min. Injection temperature was set at 260°C. The oven temperature was programmed at 60°C with 2 min hold time, 250°C with 5 min hold time and 310°C with 14 min hold time. 2.5 µl of sample was injected through autoinjector with split mode. Identification of the compounds of the sample was based on GC retention time on Rtx_5Ms column. The total running time was 65 minutes. The software used for analyzing the compounds was GCMS solution software by the help of Nist-08 and wiley-08 libraries. The relative percentage amount of each component was calculated by comparing its average peak area to the total area.

3. RESULTS

Four different isolates of *Alternariaalternata*were isolated from selected diseased plants of potato (*Solanumtuberosum*), tomato (*Lycospersiconesculentum*), Onion (*Alliumcepa*) and mustard (*Brassicacampestris*) denoted as A1, A2, A3 & A4 respectively. The cultures were grown on PDA. The pure culture of all the four fungal isolates appeared to be grayish white at first and became black later on. Microscopic study revealed that fungus produced abundant conidia having 3-8 transverse septations and 1-2 longitudinal septation. Conidia were solitary and also in short chains, mostly ovoid with a short conical or cylindrical apical beak and smooth walled. Hyphae were branched, and septate. Septateconidiphores were present that were variable in length. Based on these characters the isolated fungus were identified as *Alternariaalternata* [21]. The fungal isolates were further confirmed as *Alternariaalternata* from pathology department, IARI (Indian Agricultural Research Institute) New Delhi, they also assigned laboratory code numbers to these fungal isolates. A standard ITCC culture *Alternariaalternata* (6203) was also procured from pathology department, IARI, New Delhi.

For A1,A2, A3 and A4 the MIC of ethanolic extract of *Partheniumhysterophorus*was recorded as 12.5 mg/ml and MFC was recorded 50 mg/ml for all isolates.Interpretation of mass spectrum GC-MS was conducted using the database of National Institute standard and technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test material was ascertained. The analysis of the organic compounds present in the ethanolic extract of leaf of *Partheniumhysterophorus* by GC-MS analysis revealed the presence of 75 compounds. The 10 major compounds with their retention time (RT) and area (%) are presented in **Table-1**and the chromatogram is presented in **graph-1**. The identified compounds belong to different phytochemical groups such as terpenoids, fatty acid, sterols, glycosides, hydrocarbons, phenolics and heterocyclic compounds.

Graph 1: GCMS analysis of ethanolic extract of Partheniumhysterophorus



4. DISCUSSION

The results of this conceptual study clearly reflects that this weed has inherent ability to induce allelopathic effects on mycelium growth rate and consequently on proliferation of this fungi. Similar effects of various other plant products effective against *Alternaria spp.* were reported by several authors [24-26].Antifungal activity of *Partheniumhysterophorus* was reported against different bacteria and fungi [27-30]. The leaves extracts of *Partheniumhysterophorus* using ethanol as extracting solvents presented a better inhibitory effect on the test organisms. This could be ascribed to the alcoholic aqueous environment which promotes easy extraction as reported by Nostro*et al*., 2000 [31]. The ethanolic extracts give best antifungal effect which is in agreement withHassanein*et al*., (2008) who screened ethanol, ethyl acetate and aqueous extracts of neem and chinachery against two tomato fungal pathogens and found that ethanol and ethyl acetate extracts of these plants to suppress growth of *F. oxysporum*and inhibited *A. solani*in comparison to aqueous extracts which were less effective [32]. On the basis of the data obtained in the present investigation, conclusion may be drawn that the ethanolic extract of *Partheniumhysterophorus* can be used as a novel fungicide against *Alternariaalternata*but for large scale use bioactive component identification is important.

Table 1: GCMS analysis of ethanolic extract of *Partheniumhysterophorus*

S. No.	R. time	Area	Area%	Name
1	27.167	117268451	9.95	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
2	40.26	98901162	8.39	Azuleno[4,5-b]furan-2,9-dione, 9a-[(acetyloxy)methyl]decahydro-6-
3	39.404	94965387	8.06	1,7,7,7B-TETRAMETHYLDECAHYDROCYCLOPROPA[3,4]NAPHTHO[
4	38.985	84464614	7.17	1,2-BENZENEDICARBOXYLIC ACID
5	29.23	84128474	7.14	n-Hexadecanoic acid
6	31.705	61297829	5.2	2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-[R*,R*-(
7	26.362	47241729	4.01	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE
8	32.273	37342519	3.17	2(3H)-NAPHTHALENONE, 4,4A,5,6,7,8-HEXAHYDRO-4,4A-DIMETHYL-
9	50.363	35668758	3.03	STIGMASTA-5,23-DIEN-3-OL, (3.BETA.)-
10	32.517	32394592	2.75	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-

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