

Potency of ethanolic leaf extracts of *Tithonia diversifolia* (Hemsl.) A. Gray against the plant pathogen *Alternaria brassicicola* (Schwein) Wiltshire

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Research Article

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Abstract

Antifungal potency of *Tithonia diversifolia* leaf extracts against the plant pathogen *Alternaria brassicicola* has been investigated using three solvents: acetone, ethanol and methanol at different concentrations (60%, 80% and 99.9%). Out of the three solvent extracts used, ethanolic extract showed maximum inhibition zone at all concentrations studied, with the inhibition zones ranging from 0.4 to 1.9 mm. Acetone extract showed slight inhibition at 80% concentration as compared to its control whereas, methanolic extract showed slight inhibition at 100% concentration. Zone of inhibition by ethanol as control showed 0.3, 0.5 and 0.8 mm at 60%, 80% and 99.9% respectively, whereas, ethanol plus plant extract showed zone of inhibition measuring 0.4, 0.7 and 1.9 mm respectively, which is higher than their respective control concentrations. This indicates that ethanolic leaf extract of *T. diversifolia* has high potency against the test plant pathogen *A. brassicicola*. There was no inhibition zone observed for 60% acetone, indicating that 60% acetone leaf extract of *T. diversifolia* may not be effective against the test pathogen.

INTRODUCTION

Tithonia diversifolia (Hemsl.) A. Gray, is a notable aromatic medicinal plant, originated from Mexico and Central America. It is commonly known as Mexican sunflower and is classified under one of the flowering family, Asteraceae. The plant is a weed, moderately resistant to draught and is pantropical in its distribution (Kawini et al., 2017). Traditional healing uses of different parts of *T. diversifolia*, particularly leaves and flowers by indigenous people have been well documented. The plant is used though oral administration for treatment of diabetics, malaria, hepatitis, fever and other infectious diseases and several in-vivo and in-vitro studies on the ethno-pharmacological effects of *T. diversifolia* has provided heterogeneous evidence supporting majority of the beneficial therapeutic claims of the plant (Tagne et al., 2018). The presence of phenolic compounds such as tannins and particularly flavonoids enhanced several therapeutic activities including anti-inflammatory, anti-cancer, anti-malarial, antioxidant and antimicrobial activities of *T. diversifolia* (Oyewole et al., 2008; Thongsom et al., 2013; da Gama et al., 2014; Gaicomo et al., 2015; Dlamini et al., 2020). Because of the high potassium content in the leaves, the plant is also used as green manure for enhancement of soil fertility (Jama et al., 2000).

T. diversifolia, like other aromatic and medicinal plants has huge antimicrobial properties with high potency against both bacterial and fungal pathogens. Aqueous and ethanolic extracts of *T. diversifolia* leaves have shown effective inhibitory action against bacterial pathogens *Escherichia coli* and *Salmonella typhi* and fungus *Candida albicans* (Olayinka et al., 2014). Leaf extracts of *T. diversifolia* has also been reported to possess inhibitory effects against destructive fungal pathogens including *Aspergillus niger*, *Colletotrichum gloeosporoides*, *Fusarium oxysporium*, *Gloeotrichum candidum* and *Phytophthora nicotianae* (Rejeki et al., 2017; Ngegba et al., 2018; Awere et al., 2020; Chege and Kimaru, 2021). The plant is also reported to have strong inhibitory effect against potato wilt causal agent *Ralstonia solanacearum* (Opondo et al., 2023). Because of its strong fungicidal and bacteriosidal properties, biofungicidal formulations have been manufactured which has shown promising control

against *Pythium myriotylum*, the causal agent of root rot of *Xanthomonas sagittifolium* (Flore et al., 2023).

Alternaria brassicicola (Schwein) Wiltshire is a fungal plant pathogen well-known for causing one of the most devastating diseases called black spot disease in Brassicaceae members, many of which are of agricultural and economic importance. It is spread by wind, rain and insects and has the ability to survive and sporulate even in plant debris under favourable temperature and moisture content. Control of *A. brassicicola* in India hugely relies on chemical fungicides which remain a serious human and environmental threat (Gupta et al., 2020). Till date, convincing measure to bring about effective biological control of *A. brassicicola* diseases such as mustard blight of mustard plant and black spot disease of cabbage remain a huge challenge. Recent researches has focused on the use of plant growth promoting bacteria such as *Pseudomonas fluorescens*, *Bacillus subtilis* and B1 bacterial strains has shown some positive results (Sabry and Ali, 2016; Gupta et al., 2020). Use of the well-known antagonistic, *Trichoderma viridei* and also *T. harzianum* has also shown certain promising result in biocontrol of the pathogen (Sabry and Ali, 2016; Khalse et al., 2017). The present study reveals the potency of *T. diversifolia* leaf extracts against the test pathogen, *A. brassicicola*.

Materials and Methods

Isolation and identification of fungal pathogen from *Brassica oleracea* var. *capitata*: Infected leaves of cabbage were collected from the rural markets, brought to the laboratory and surface sterilized. The sample was then cut into small segments of uniform sizes under sterilized condition and kept ready for inoculation. Potato dextrose agar (PDA) was used for establishment of both initial and pure cultures. The inoculated petri plates were incubated at $25 \pm 2^\circ\text{C}$ for 05 to 08 days. The test fungal pathogen, *A. brassicicola* was identified through cultural and microscopic characterization by consulting Nagamani et al. (2006).

Collection of plant and solvent extraction from the leaves of *Tithonia diversifolia*

Leaves of *T. diversifolia* were collected from Dkhiah East, Khliehriat, Jaintia Hills District, Meghalaya (Fig. 1), located at an average elevation of 717 meters above sea level (masl), with geographical coordinates 25.359°N and 92.367°E . The leaves were rinsed thoroughly with running water to get rid of any soil particles and allowed to dry at room temperature ($25 \pm 2^\circ\text{C}$) in avoiding exposure to direct sunlight. Once completely dried, they were ground into a powder. The powdered material was then weighed and stored under sterile conditions for future use.

Three solvents: acetone, methanol, and ethanol were used. The plant extract and each solvent were combined in a 1:10 ratio. The solutions were thoroughly mixed in a tightly sealed test tubes and left at room temperature for approximately 24 to 48 hours. The solution was then filtered using Whatman filter paper, retaining the filtrate while discarding the residue. The filtrates were left at room temperature for about 3 to 4 days to allow complete evaporation of the unbounded solvents. After total evaporation, any remaining traces of powder extract on the inner surface of the test tubes were scraped off. The obtained

extracts were weighed accurately and then dissolved in their respective solvents at 1:1 ratio. The tightly sealed test tubes were stored in a cold room for further experimentation.

Antifungal activity of *T. diversifolia*

The antifungal activity of *T. diversifolia* leaf extract was assessed using the disc diffusion method according to Benkeblia (2004). Fungal spore cultures were prepared from the pure culture using potato dextrose broth. The petri plates were inoculated with the fungal pathogen, spreading it evenly with a sterilized rod, and allowed to incubate for 02 to 03 days. Sterile diffusion discs inoculated with different solvent concentrations were placed onto labelled petri plates. All procedures were conducted under sterile conditions. Petri plates were incubated for another 02 to 04 days at $25 \pm 2^\circ\text{C}$. After 4 days, the zones of inhibition were observed, measured, and recorded.

Result

The present research work was carried out to investigate the antifungal potency of *T. diversifolia* leaf extracts against the test fungal pathogen *A. brassicicola* (Fig. 2). Out of the three solvent extracts used, ethanolic extract showed maximum inhibition zone at all concentrations studied, with the inhibition zones ranging from 0.4 to 1.9 mm (Table 1, Fig. 3). It was observed that with the increase in the concentration of ethanol from 60–99.9%, the inhibition zone by the ethanolic leaf extract increased. Acetone extract showed slight inhibition at 80% concentration as compared to its control whereas, methanolic extract showed slight inhibition at 99.9% concentration. Zone of inhibition by 60%, 80% and 99.9% ethanol as control showed 0.3, 0.5 and 0.8 mm respectively. On the other hand, ethanol plus plant extract showed zone of inhibition measuring 0.4, 0.7 and 1.9 mm at 60%, 80% and 99.9% respectively, which is higher than their respective control concentrations (Fig. 4). As compared to the respective controls, there is an increased in zone of inhibition for ethanolic extract in all the three replicates studied for each of the concentrations thus giving 100% inhibition. This indicates that ethanolic leaf extract of *T. diversifolia* has high potency against the test plant pathogen *A. brassicicola*. There was no inhibition zone observed for 60% acetone indicating that 60% acetone leaf extract of *T. diversifolia* may not be effective against the inhibition of the test pathogen.

Table 1
Inhibition zones of different solvent extract concentrations against *A. brassicicola*.

Control along with % of treatments	Zone of inhibition (mm) against various concentration (mg/ml)								
	60%			80%			99.9%		
	R1	R2	R3	R1	R2	R3	R1	R2	R3
Acetone	0.00			0.4			0.8		
A + PE	-	-	-	0.6	0.5	0.5	0.7	0.6	0.6
Ethanol	0.3			0.5			0.8		
E + PE	0.4	0.4	0.5	0.7	0.5	0.4	1.9	1.6	1.5
Methanol	0.6			0.8			0.9		
M + PE	0.5	0.6	0.5	0.3	0.2	0.2	1.1	0.8	0.8

Where, Acetone, ethanol and methanol were controls at their respective concentrations; **R1**, **R2** and **R3**: Replicates 1, 2 and 3; **A**: Acetone, **E**: Ethanol; **M**: Methanol; **PE**: Plant extract.

Discussion

There are reports showing that aqueous extracts of medicinal plants such as *Azadiracta indica*, *Ricinus communis*, *Aloe vera*, *Ocimum sanctum* and *Cassia alata* possess effective inhibitory property against *A. brassicicola* (Das et al., 2023). Khalse et al. (2017) also reported the inhibitory effects of leaf extracts from *Eucalyptus*, *Lantana* and *Datura* plants against *A. brassicicola* with percentage inhibition of 58.69%, 54.96% and 52.17%, respectively. A study conducted by Ho et al. (2007) have shown that out of seven organic solvents studied, ethanolic extract of *Polygonum perfoliatum* showed most effective spore germination inhibition against *A. brassicicola* followed by methanol extract however very less or no spore germination inhibition has not been observed for acetone, petroleum ether and chloroform. The effectiveness of ethanolic plant extracts of medicinal plants such as *Coscinium fenestratum*, *Piper bettle*, *Syzygium aromaticus* and *Zingiber cassumunar* showing complete inhibition of mycelial growth against *Alternaria brassicicola* has also been reported by Dethoup et al. (2018). These results are in conformity with the present finding where ethanolic extract is the most effective solvent, even at 60% concentration. Thus, investigations on the efficacy of medicinal plants for biological control of plant diseases including *A. brassicicola* have shown promising results.

On the other hand, there is also an existing threat where a large number of aromatic and medicinal plants having antimicrobial properties have become threatened because of their illegal and unscientific exploitations. Conservation of such plants remains a huge challenge in spite of the latest tissue culture advancements because of their selective behaviour in natural habitat, decrease in yield of secondary metabolites *in vitro* and also due to unlimited demand and are now in urgent need of sustainable exploitation (Pandey et al., 2005; Shukla et al., 2020). Thus, alternate search for potent plants to fulfil

such shortcomings is the need of hour. *T. diversifolia* has become an invasive weed in Africa and Asia which have become an ecological, agricultural and economic burden (Omokhua et al., 2018). With this status and the light shed from the present finding, *T. diversifolia* serves as a good option. Ho et al. (2007) also reported the effective potency of ethanolic extract of another weed, *Polygonum perfoliatum*, which, along with *T. diversifolia*, can be considered for future formulations of biofungicides against *A. brassicicola*. In-depth study on various other solvent extraction methods and the effect of phytochemical constituents of *T. diversifolia* and their roles in inhibition of pathogen growth *in vitro* and *in vivo* is much encouraged so as to meet an eco-friendly and effective biological control measure against *A. brassicicola*.

Declarations

Conflict of Interest

The authors have no conflict of interest to declare.

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N/A

Author Contribution

The research work was proposed and initiated by the first author. Survey and collection of the plant was done by the second author. Both the authors were actively involved in the laboratory experimental works. The first author prepared and wrote the manuscript.

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Data Availability

The data available in the manuscript are authentic. The pure cultures of the test pathogen were deposited to the Department of Botany, University of Science & Technology Meghalaya. Additional data requirement may be provided by the corresponding author if reasonable.

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Figures

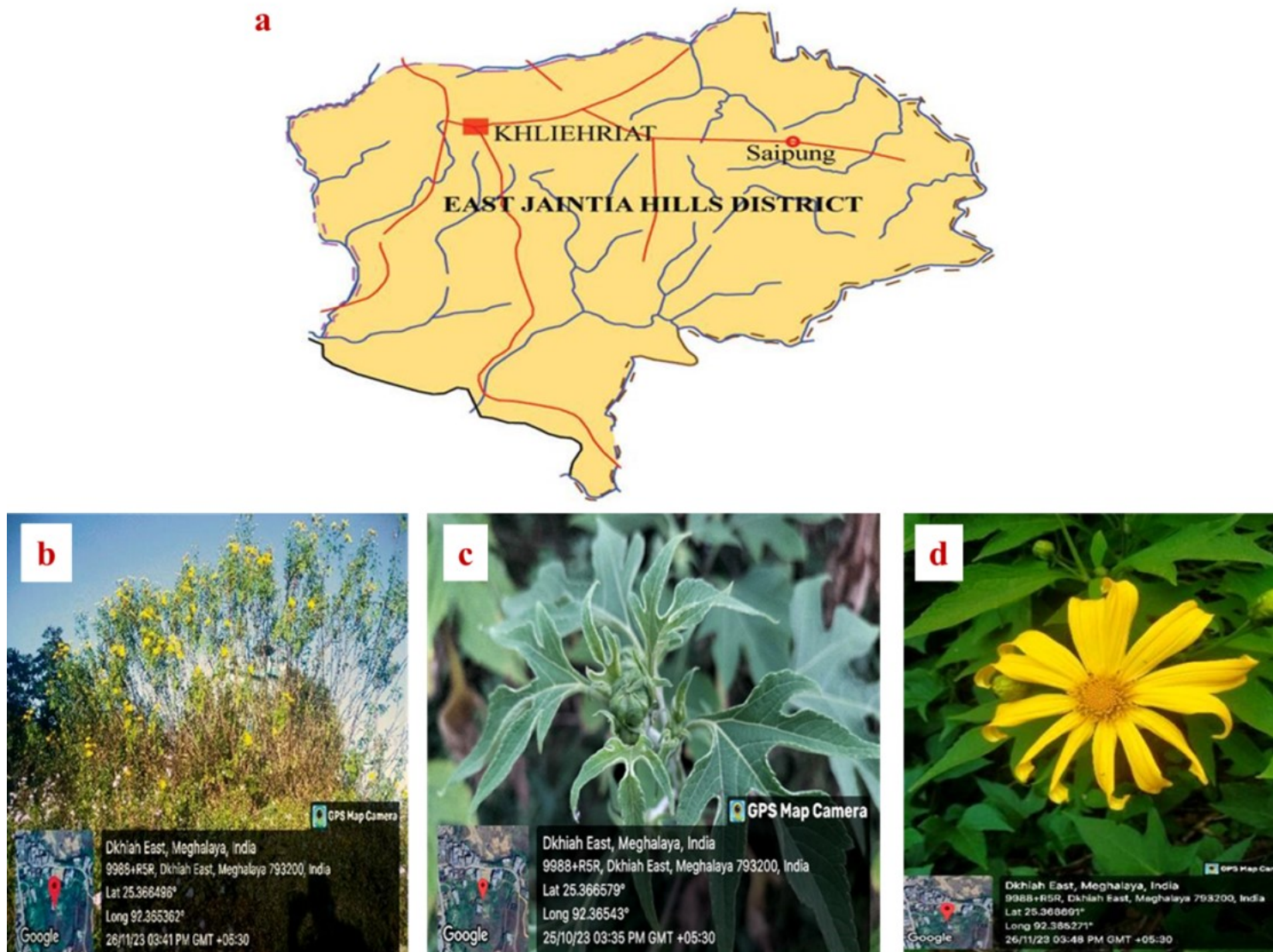


Figure 1

Tithonia diversifolia: **a.** Map of collection site; **b.** Plant growing in wild; **c.** Leaves; **d.** Flower.



Figure 2

Alternaria brassicicola: **a.** infected host; **b.** fungal culture; **c.** pure cultures; **d.** ascospores of test pathogen.

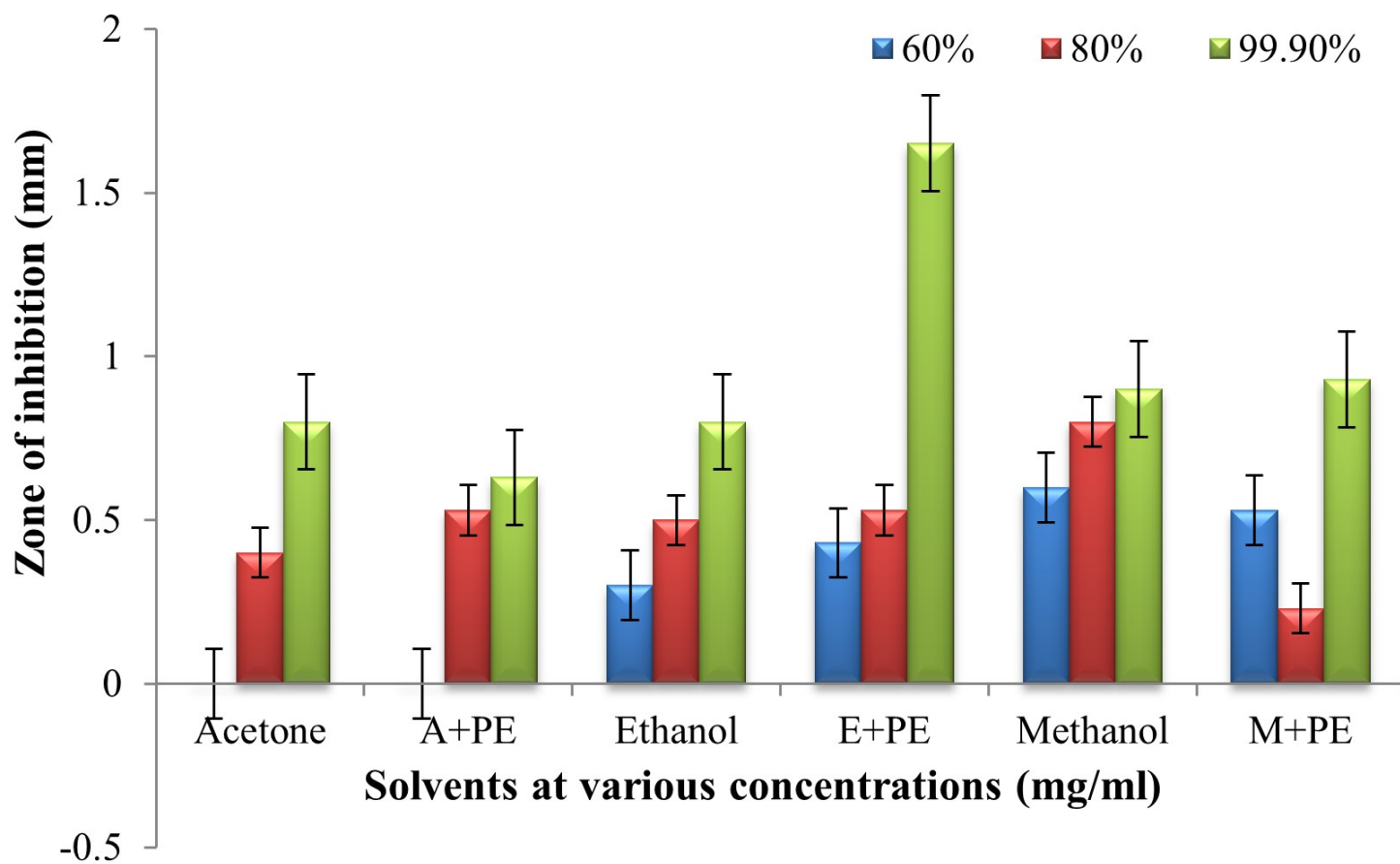


Figure 3

Average zone of inhibitions for different solvent at 60%, 80% and 100% concentrations in mg/ml (error bars represent standard error).

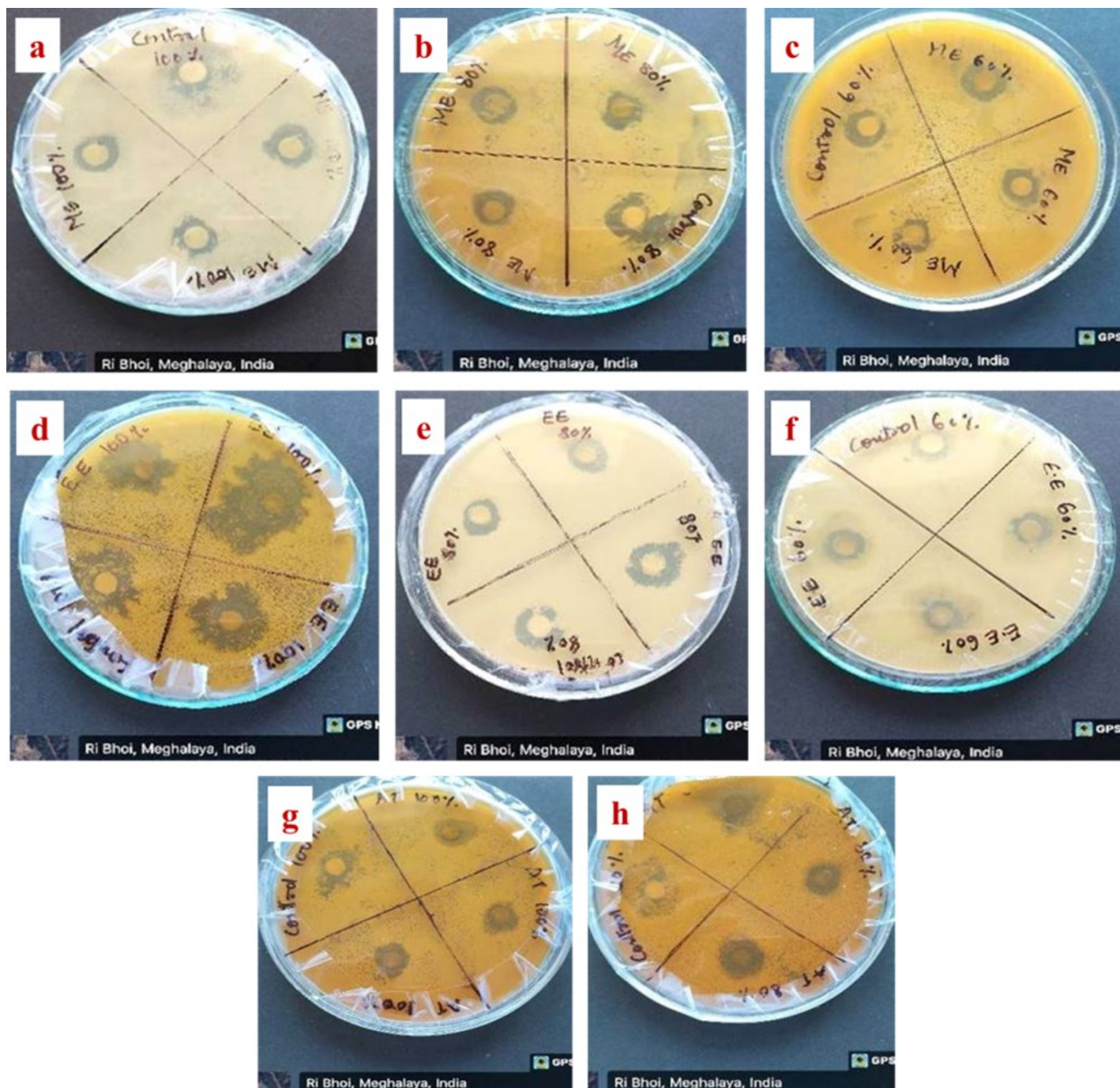


Figure 4

Formation of inhibition zones showing antifungal activity against the test pathogen. **a – c**: 99.9%. 80% and 60% Methanolic extracts; **d – f**: 99.9%. 80% and 60% Ethanolic extract; **g & h**: 99.9% and 80% Acetone extracts.