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Impact of environmental conditions on antifungal properties of medicinal plants

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Abstract
Fungal infections cause major illness and mortality in humans, animals, and plants, making them a serious to global health. The investigation of alternative treatments has been spurred by the emergence of drug-resistant fungal strains and the drawbacks of existing antifungal medications, including toxicity and the possibility of resistance. Because of their bioactive phytochemicals, medicinal plants have become a promising source of antifungal drugs. These substances, which include phenols, alkaloids, terpenoids, and phytosterols, have a variety of modes of action, including inducing mitochondrial malfunction, disrupting fungal cell membranes, and inhibiting the formation of cell walls. *so we must focus on other alternatives that are biologically safe and nonhazardous*. *In the present investigation,* aqueous *extract of leaf of Calotropis gigantea* and *Dolichandrone falcata* which were found antifungal against *Phytophthora infestans* were exposed to various physical factors such as Autoclave, Temperature, pH, Humidity etc. Specific fungus was used to test the exposed extracts. The influence of physical factors were investigated by subjecting the extract to altering conditions of the parameters chosen for a specified time period and then observing the effect as a function of change in the extract's MIC against the test organism. Three replicates were kept in addition to the control, and the experiments were performed three times. The Finding suggested that efficacy of leaf extracts against tested fungus were affected by physical factors. Results revealed that autoclaving, high temperature, alkaline pH and high humidity decrease the antifungal activity of plant extracts.

Keywords: Phytophthora infestans, Calotropis gigantea, Dolichandrone falcata Temperature, pH etc.

Introduction
The antimicrobial potential of plants is directly related to their secondary metabolites, these metabolites can be affected by various physical factors. Plants' chemical composition, activity, and antimicrobial potential are affected by a variety of physical factors (Mehta and Sharma, 2022) [10]. Medicinal plants are the most important source of life-saving drugs for the huge number of the world's inhabitants. For fungal infections, medicinal plants are becoming useful substitutes and supplemental therapies (Pawar and Ghodke, 2022) [15]. *Dolichandrone falcata* belongs to the Bignoniaceae family and is a deciduous tree while *Calotropis gigantea* belongs to the Apocynaceae family and is a shrub; both are important medicinal plants in the pharmaceutical and medical industries. *D. falcata* showed very effective against *Alternaria alternata*, *Colletotrichum capsici* and *Phytophthora infestans* (Pawar and Nasreen, 2016) [16]. This plant has number of medicinal uses such as antioxidant, anti parasitic, antiallergic, anticonvulsant, analgesic and anti-inflammatory (Kulkarni and Swamy, 2022) [9]. It also used in bloody diarrhea, curing anemia and bark juice is used for menorrhagia and leucorrhoea (Badgujar and Surana, 2010) [1]. *C. gigantea* revealed antimicrobial activities against *Fusarium oxysporum*, *Colletotrichum capsici* and *Xanthomonus axonopodis* (Pawar *et al*., 2019) [17]. Dried whole plant is employed as an effective tonic, anthelmintic, and expectorant in Ayurveda. While the latex is used to cure vertigo, rheumatoid arthritis, baldness, and hair loss, the powdered root is used to treat leprosy, bronchitis and asthma. Additionally, it is frequently used to treat fevers, colds, jaundice, rheumatism, eczema, and dyspepsia (Biswasroy *et al*., 2020). Physiologically active secondary metabolites in the plant extract may be disturbed and their activity reduced by excessive heating during the extraction process (Hada and Sharma, 2017) [6]. Several physical conditions affect the physicochemical properties of plants, changing their chemical makeup, activity, and antifungal capacity (Mehta and Sharma, 2022) [11]. The heterogeneous nature of biologically active plant metabolites can be affected by sunlight, temperature, pH exposure, and storage duration, which may alter their biological activity (Bansal *et al*., 2016) [2]. Meena *et al*., (2022) [10] investigated the effect of temperature, pH and storage on the antioxidant activity of numerous plant extracts. The influence of temperature, autoclave, pH, and humidity on the antifungal activity of an aqueous extract of *Calotropis gigantea* and *Dolichandrone falcata* on its stability under varied physical conditions was investigated in this work.

Materials and Methods
1. Collection of plant Material
Fresh and healthy plant materials were collected from Jalna district, during October 2022. Collected materials of plants were identified by flora of Marathwada (Naik *et al.*, 1998) [12] then wash thoroughly with running tap water and shade dried, powdered and stored in airtight container for further study.

2. Preparation of plant Extract
The Soxhlet extractor was used to prepare the aqueous extract. Soxhlet extraction was applied to approximately 10 grams of powder using 100 ml of water at 60-70 degrees Celsius for up to 72 hours. A rotary evaporator was used to evaporate the solvent at 45–50°C. Dimethyl sulfoxide (DMSO) at a concentration of 10% was used to dissolve the collected powder after it had been weighed. For future study, the extracts were kept in sterile glass bottles at 4^0 C (Handa *et al*., 2008, Subramanian *et al*., 2016) [7, 18].

3. Effect of various physical Factor

A.S Effect of Temperature

All the effective extracts were taken in sterilize conical flask and stored at room temperature (28 ^{0}C) for seven days. The extracts were also exposed to varying temperature such as 45, 55 and 65 ^{0}C for 1 hrs in a hot air oven (Baswa *et al.* 2001) [3]. The antifungal activity was tested after the varying temperature and observation were recorded.

B. Effect of Autoclave

The plant extracts were autoclaved at 15 lbs pressure for 20 minutes, autoclaved plant extract of *Calotropis gigantea* and *Dolichandrone falcata* were tested against *Phytophthora infestans*. Both autoclaved and non autoclaved extract were poured with PDA in Petri plate 6 mm diameter disc of fungal pathogen was inoculated on PDA plate and observation was recorded.

C. Effect of pH

Plant extracts were tested at pH ranges from 5 to 9 using 0.1 N HCl and 0.1 N NaOH solutions respectively in series of test tubes for 1 hour and then PDA was added to the tubes and the tubes were inoculated with test organisms. Inoculated tubes were incubated at 27 ± 2 °C for 96 hrs and observations were recorded (Dixit *et al*., 1981) [5].

D. Effect of Humidity

Plant extracts were treated at different Humidity ranges from 60, 70, 80, 90 and 100% respectively in series of 1 hour. For maintained humidity sterile water and glycerol was aseptically poured into sterile plastic containers to create an atmosphere with the required relative humidity (Pardo *et al*., 2005) [13]. Then the extracts were tested for antifungal activity and observations were recorded.

Result and Discussion

Tables 1, 2, 3, and 4 shows the effects of several physical parameters, such as autoclaving, temperature, pH, and humidity, on the aqueous extract of *Calotropis gigantea* and *Dolichandrone falcata* leaves, respectively. Table 1 demonstrates a minor reduction in the antifungal activity of the second autoclaved extracts of both plants after seven days at 15 psi for 20 minutes but there was no change in antifungal efficacy at first Autoclaved immediately after preparation of leaf extracts. Jeffery (2006) and Parveen *et al*., (2022) [8, 14] studied the effect of different physical factors on the antibacterial activity of pepper leaf extracts, such as heat and temperature. The impact of temperature on extract efficacy is shown in Table 2. The findings demonstrate that plant extracts were treated for one hour at several temperatures, including 45, 55, and 65 degrees Celsius. Temperatures as high as 55°C had no effect on plant extract activity for an hour, but at 65°C, the extract's efficiency against the tested fungus was somewhat reduced. Wang and Ke-Quang (2001) [19] examined the impact of temperature on plant extracts and found that extended exposure to temperatures above 90° C reduced the antifungal activity because the acetone extract of *T. arjuna* lost some of its antifungal ability. The effect of pH on plant extract effectiveness is shown in Table 3. Plant extracts treated with pH 5 and 9 slightly decreased their activity, however extracts treated with pH 6, 7, and 8 had no effect. Yen and Duh (1993) [20] investigated that methanol extract from peanut hulls had a superior antioxidant efficacy at neutral and acid pH up to 4.8. Table 4 shows effect of relative humidity for 70, 80, 90 and 100% on the plant extract efficacy, when humidity increase the extracts were showed decrease their antifungal activity respectively.

Table 1: Effect of Autoclave on aqueous *extract against Phytophthora infestans*

Sr. No.	Plant Name	Control	Ist Autoclaved immediately after preparation of plant extracts	IInd Autoclaved of plantextracts after seven days
1	*Calotropis gigantea*	++++	++++	++
2	*Dolichandrone falcata*	++++	++++	++

(-) = No Growth, (++) =Slight Growth, (++++) = Abundant Growth

Table 2: Effect of different temperature on aqueous *extract against Phytophthora infestans*

Sr. No.	Plant Name	Control	Temperatures		
			45	55	65
1	*Calotropis gigantea*	++++	++++	++++	++
2	*Dolichandrone falcata*	++++	++++	++++	++

(-) = No Growth, (++) =Slight Growth, (++++) = Abundant Growth

Table 3: Effect of pH on aqueous *extract against Phytophthora infestans*

Sr. No.	Plant Name	Control	pH				
			5	6	7	8	9
1	*Calotropis gigantea*	++++	++	++++	++++	++++	++
2	*Dolichandrone falcata*	++++	++	++++	++++	++++	++

(-) = No Growth, (++) =Slight Growth, (++++) = Abundant Growth

Table 4: Effect of Humidity on aqueous *extract against Phytophthora infestans*

Sr. No.	Plant Name	Control	Humidity			
			70 %	80 %	90 %	100 %
1	*Calotropis gigantea*	++++	++++	++++	++	++
2	*Dolichandrone falcata*	++++	++++	++++	++	++

(-) = No Growth, (++) =Slight Growth, (++++) = Abundant Growth

Conclusion

Plant-based fungicides are a good substitute for chemical fungicides, according to recent research. The antifungal activity of plant extracts in agricultural areas can be affected by physical factors. To find out how physical characteristics affect plant extracts' antifungal effectiveness for use in agriculture, more research is required. The current study examined how the antifungal activity of aqueous leaf extracts of *Calotropis gigantea* and *Dolichandrone falcata* was affected by a number of physical factors, including autoclave, temperature, pH, humidity, etc. The results indicated that the antifungal activity of plant extracts is somewhat reduced by second-time autoclaving, high temperature, alkaline pH, and high humidity.

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