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Effect of physical factor on antifungal efficacy of plant extracts

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Abstract

Medicinal plants contain number of important secondary metabolites which are able to control fungal diseases. Even it is safer and alternative to synthetic fungicides. As we all know, chemical fungicides are harmful not only to humans but also to animals, other vegetation, and the entire ecosystem, so we must focus on other alternatives that are biologically safe and nonhazardous. In the present investigation, aqueous extract of leaf of *Madhuca indica* and *Eucalyptus alba* which were found antifungal against *Phytophthora infestans* were exposed to various physical factors such as Autoclave, Temperature, pH, Humidity etc. The exposed extracts were tested against given fungi. The effect of physical factors were investigated by subjecting the extract to varying circumstances 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. Experiments were repeated thrice and three replicates were maintained besides control. Result suggested that efficacy of leaf extracts against tested fungus were slightly affected by various physical factors. Hence from the results we finding autoclaving, high temperature, alkaline pH and high humidity decrease the antifungal activity of plant extracts.

Keywords: *Phytophthora infestans*, *Madhuca indica*, *Eucalyptus alba*, temperature and pH

1. Introduction

Plant compounds such as alkaloids, phenols, tannins, steroids and terpenoids are responsible for the beneficial pharmacokinetic properties of medicinal plants. Medicinal plants are the primary source of life-saving pharmaceuticals for the vast majority of the world's population. *Madhuca indica* is an evergreen plant belonging to the Sapotaceae family, it is very important in Ayurveda, Sidhha, and Unani systems of traditional medicine. Flowers and seeds are important parts which are used to make alcoholic beverages and oil. This is used as stimulants, demulcents, emollients, heating agents, and astringents (Kirtikar and Basu, 1987) [3]. Mahua oil is used to cure skin conditions, rheumatism, headaches, and as a laxative. Fruits are astringent and are commonly used as a lotion to treat chronic ulcers, pharyngitis, acute and chronic tonsillitis (Krishnamoorthy *et al.*, 2014) [2]. The oil contained in the leaves of Eucalyptus trees is widely known for its therapeutic effects. It is a very potent antibiotic that is especially efficient against certain kinds of bacteria (Sani *et al.*, 2014) [1]. The oil is used to treat wounds and fungal infections. Decoction prepared from *Eucalyptus* leaves was also used to treat fevers. Secondary metabolites in plants are directly related to their antimicrobial capability and can be influenced by a variety of physical factors (Mehta *et al.*, 2016) [4]. These secondary metabolites are responsible for alterations in microbial biochemistry and cytology. Several plants' crude or various solvent extracts have been tested for antimicrobial activity against pathogenic viruses, bacteria, fungus and protozoa (Gurjar *et al.*, 2012) [5]. Excessive heating during the extraction process may cause physiologically active secondary metabolites in the plant extract to be disrupted and reducing their activity (Hada and Sharma, 2017) [6]. A number of physical circumstances impact on plant physicochemical, altering their chemical composition and activity, as well as their antifungal ability (Mehta and Sharma, 2022) [7]. Sunlight, temperature, pH exposure and storage time can impact the heterogeneous character of biologically active plant metabolites, potentially changing their biological activity (Bansal *et al.*, 2016) [8]. Arabshahi *et al.* (2007) 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 *Madhuca indica* and

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Eucalyptus alba on its stability under varied physical conditions was investigated in this work.

2. Materials and Methods

2.1 Collection of plant Material

Fresh and healthy plant materials were collected from Mantha Tahsil, during September 2021. The identification is done with the help of standard floras (flora of Marathwada by Naik *et al.*, 1998). The collected, fresh plant materials were washed and shade dried, powdered and stored in airtight container for further study.

2.2 Preparation of plant Extract

Aqueous extract was prepared by using Soxhlet extractor. About 10 gm of powder were subjected to soxhlet extraction with 100 ml of ethanol at 55-65 °C up to 72 hours. Solvent was evaporated at 40-50 °C by using Rotary evaporator. The collected powder was weighted and dissolved in Dimethyl sulfoxide (DMSO) with 10% concentration. The extracts were preserved in sterile glass bottles at 4 °C temperature for further study.

2.3 Effect of various physical Factor

A) Effect of Temperature

All the effective extracts were taken in sterilize conical flask and stored at room temperature (28 °C) for 10 days. The extracts were also exposed to varying temperature such as 45, 55 and 65 °C for 1 hrs in a hot air oven. 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 *Madhuca indica* and *Eucalyptus alba* 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 of 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) ^[9].

D) Effect of Humidity

Plant extracts were treated at different Humidity ranges of 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) ^[10]. Then the extracts were tested for antifungal activity and observations were recorded.

3. Result and Discussion

The results of effect of various physical factors including Autoclave, Temperature, pH and Humidity on aqueous extract of leaf of *Madhuca indica* and *Eucalyptus alba* are given in table 1, 2, 3 and 4 respectively. Table 1 shows a slight decrease antifungal activity of the IInd Autoclaved of leaf extracts of both the plants after seven days at 15 lbs pressure for 20 minutes but there was no change in antifungal efficacy at first Autoclaved immediately after preparation of leaf extracts. Jeffery (2006) ^[13] and Parveen *et al.*, (2022) ^[15] investigated the effect of various physical factors on the antibacterial activity of pepper leaf extracts, such as heat and temperature. Table 2 illustrates the effect of temperature on extract efficacy. The results show that plant extracts were treated at various temperatures such as 45, 55 and 65 °C for 1 hrs. Plant extract activity was unaffected by temperatures as high as 55 °C for 1 hour; however, extract efficacy against tested fungus was somewhat reduced at 65 °C for 1 hour. Wang and Ke-Quang, (2001) ^[12] investigated that the effect of temperature on plant extracts and they showed prolonged exposure of extract with more than 90 °C reduce the antifungal activity due to shattered some of the antifungal potential of acetone extract of *T. arjuna*. Table 3 indicates the impact of pH on the efficacy of plant extracts. Extracts treated with pH 6, 7, and 8 had no effect on their activity; however pH 5 and 9 marginally reduced the activity of plant extracts. Yen and Duh (1993) ^[11] studied 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	I st Autoclaved immediately after preparation of leaf extracts	II nd Autoclaved of leaf extracts after seven days
1.	<i>Madhuca indica</i>	++++	++++	++
2.	<i>Eucalyptus alba</i>	++++	++++	++

(-) = 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.	<i>Madhuca indica</i>	++++	++++	++++	++
2.	<i>Eucalyptus alba</i>	++++	++++	++++	++

(-) = 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.	<i>Madhuca indica</i>	++++	++	++++	++++	++++	++
2.	<i>Eucalyptus alba</i>	++++	++	++++	++++	++++	++

(-) = 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.	<i>Madhuca indica</i>	++++	++++	++++	++	++
2.	<i>Eucalyptus alba</i>	++++	++++	++++	++	++

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

4. Conclusion

Recent study has demonstrated that plant-based fungicides are effective alternatives to chemical fungicides. Physical factors can impact the antifungal activity of plant extracts in agricultural fields. Further research is needed to determine how physical parameters impact the antifungal efficacy of plant extracts for use in agriculture. The present study investigated the effect of various physical factors such as Autoclave, Temperature, pH, Humidity etc. on the antifungal activity of aqueous leaf extracts of *Madhuca indica* and *Eucalyptus alba*. Result suggested that IInd time autoclaving, high temperature, alkaline pH and high humidity slightly decrease the antifungal activity of plant extracts.

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