Effect of Physical Factors on Antifungal Efficacy of *Terminalia arjuna* Leaf Extract

Tahira Parveen[1], Tripta Jain[2], Kanika Sharma[3]
[1,2,3]*Microbial Research Laboratory, Dept of Botany, University College of Science, MLSU, Udaipur, Rajasthan, India, 313001*

Abstract **- In the present study partially purified acetone extract of leaf of *Terminalia arjuna* which was found antifungal against *Pythium aphanidermatum* and *Pythium myriotylum* was exposed to various physical factors like heat, temperature, pH and sunlight. The exposed extracts then used against the test fungi. The effect of various physical factors were studied by exposing the extract to varying conditions of the parameters chosen for a specific time period, and then observing the effect as a function of change in MIC of extract against the test organism. Experiments were repeated thrice and three replicates were maintained besides control. In most of the cases there were no changes in the antifungal activity of the extract. Hence from the findings we conclude that the extract can be used in the preparation of bioformulation and have a good shelf life.**

Index Terms **- Pythium aphanidermatum, P. myriotylum, Terminalia arjuna, Physical factors**

INTRODUCTION

The prerequisite conditions for use of plant extracts in herbal formulations are that their physical and chemical properties should not undergo any drastic change due to change in temperature, pH or exposure to sunlight and it should have a long shelf life as well. Hence, studies to check stability of extract in varying conditions of physical factors such as heat, pH, temperature etc. and its effect on MIC should be conducted before using the extract for making any formulations. According to WHO, (1996) guidelines before using any herbal formulation at a commercial level, its viability needs to be checked and if its stability is maintainable at varying physical conditions then it can be considered as commercially viable. Arabshahi *et al.* (2007) studied the effect of pH, temperature and storage on the antioxidant activity of leaf extract of many plants. In the present study, effect of pH, temperature, sunlight etc. on MIC of acetone extract of leaf of *Terminalia arjuna* has been studied to determine its stability under varying physical conditions. It has been reported that the different plant parts (root, stem, bark, leaves, flowers and fruits) of *T. arjuna* vary in composition of various secondary metabolites (Rishi *et al.,* 2009; Sagwan *et al.,* 2010; Talreja, 2011).

MATERIALS AND METHODS

Acetone extract of leaf of *T. arjuna* was exposed to various physical factors like heat, temperature, pH and sunlight. The exposed extracts then used against the test fungi *Pythium aphanidermatum* as they were used before exposure. The effect of various physical factors such as heat, temperature, pH and sunlight etc. were studied by exposing the extract to varying conditions of the parameters chosen for a specific time period, and then observing the effect as a function of change in MIC of extract against the test fungi.

Experiments were repeated thrice and three replicates were maintained besides control.

Tubes containing MIC of extract and extract free medium were maintained for comparison in each sets of experiment against the test fungi *P. aphanidermatum* and *Pythium myriotylum.*

Effect of Sunlight:

Effect of sunlight on the viability of extract was studied according to the method suggested by Wang and Ke-Qiang (2002). Sterile vials containing extracts were placed in direct sunlight for 15 h and 30 h from 11AM to 5 PM and intensity of light was measured by lux meter then the effect on efficacy of extracts was assayed by tube dilution method. Experiments were repeated thrice and three replicates were maintained along with control.

Effect of Heat:

Effect of heat on the efficacy of extract was assayed according to the method suggested by Baswa *et al.* (2001) by exposing sterile glass vials containing the MIC of respective extracts to 40°C and 90°C for 4 hours in hot air oven. While in case of wet heat; extract was kept at 50°C and 100°C in water bath for 4 hours. Effect on activity of extract was then assayed by tube dilution method. One tube containing MIC concentration of untreated extract was maintained at room temperature as control for comparison.

Effect of pH:

Effect of varying pH *i.e.* 4, 7 and 9 on efficacy of extract was studied by method suggested by Dixit *et al.* (1981). Neutral pH of extract was maintained at 7. 0. 0.1 N HCl and 0.1 N NaOH were used to change the pH from 7 to 4 and 9 respectively. Nutrient medium was then added to the tubes which contained the respective extract and the tubes were inoculated with test organisms. Inoculated tubes were incubated at 28±1°C for 72 h and observed for change in MIC of extract.

Effect of Storage:

Effect of storage on antifungal activity of extract was assayed by method suggested by Rath *et al.* (2001). Extract was stored at room temperature and change in the activity was assayed at regular intervals of 6 month up to 24 months by tube dilution method.

RESULTS AND DISCUSSION

The results of effect of different physical factors like sunlight, heat, pH and long-term storage on acetone extract of *T. arjuna* leaf are given in table 1, 2, 3 and 4 respectively.

Table 1 shows a slight decrease in activity of the extract after 30 h exposure to direct sunlight but there was no change in efficacy of the same when exposed for 15 hours against both of the test fungi.

Table 2 depicts the effect of dry as well as wet heat on extract efficacy. Results indicate that wet heat treatment of leaf extract up to 50°C did not affect the activity of extract; however, heating at 100°C for 4 hours resulted in slight decrease in extract efficacy as a slight growth of the test fungi was observed. Dry heat treatment (40°C for 4 hrs) had no effect on leaf extract but heating at 90°C resulted in decreased activity similarly as in case of wet heat against both of the test fungi.

Table 3 shows the result of effect of pH on the efficacy of plant extract. No inhibitory effect on efficacy of leaf extract at neutral and acidic pH upto 4 but there was decrease in antifungal activity at pH 9.

Table 4 shows effect of long-term storage on the extract efficacy when extract was stored at room temperature. According to the results observed there was no effect on MIC upto 12 months and the antifungal activity was same as the fresh extract against both of the test fungi.

Table-1 Effect of Sunlight on Acetone Leaf Extract of *T. arjuna*

S. No	Test Organisms	Control	Leaf Extract	
			15 h	30 h
1.	*Pythium aphanidermatum*	++++	-	++
2.	*Pythium myriotylum*	++++	-	++

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

Table-2 Effect of Heat on Acetone Leaf Extract of *T. arjuna*

S. No.	Test Organisms	Control	RT	Wet Heat		Dry Heat	
				50° C	100° C	40° C	90° C
1.	*Pythium aphanidermatum*	++++	-	-	++	-	++
2.	*Pythium myriotylum*	++++	-	-	++	-	++

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

Table-3 Effect of pH on Acetone Leaf Extract of *T. arjuna*

S. No.	Test Organisms	Control	Leaf Extract	
		pH 7	pH 4	pH 9
1.	*Pythium aphanidermatum*	++++	-	++
2.	*Pythium myriotylum*	++++	-	++++

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

Table-4 Effect of Storage on Acetone Leaf Extract of *T. arjuna*

S.No.	Test Organisms	Control	6 Months	12 Months
1.	*Pythium aphanidermatum*	++++		-

2.	*Pythium myriotylum*	++++	-	-

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

- * Upto 40% loss in fresh biomass of test fungi.
- ** Full growth (No change in biomass weight)

In the present study there was no change in the efficacy of extract when exposed for 15 hours. Decrease in activity of the extract after 30 hrs. exposure to direct sunlight was observed, against test fungi. No change in antifungal activity of leaf extract after exposure to direct sunlight for 15 h indicates that acetone extract of *Terminalia ajuna* leaf is photo stable for about 15 hours and does not undergo photo oxidation. Wang and Ke-Quang, (2001) also reported that active antifungal molecules of leaf extract are not photosensitive. Effect of heat on the extract showed that the active principle of extract can withstand the dry heat and wet heat upto 50°C and 90°C respectively. While prolonged exposure of extract with 100°C wet heat and 90°C dry heat destroyed some of the antifungal potential of acetone extract of *T. arjuna.* Singh *et al.* (2006) also reported similar results for antifungal and antioxidative potential of acetone extract of *Foeniculum vulgare*. The temperature resistance studies indicate that the phytoconstituents are thermostable but decrease/loss in the antimicrobial activity of natural products by heating to 120°C may be due to volatilization of components and/or due to some physical and chemical changes in those molecules during heating. The antifungal activity of acetone extract of leaf of *T. arjuna* was found to be stable at the pH 4 and 7. Decrease in the activity of the same at pH 9 was observed. This result suggests that the active principles of the extract are better active at neutral and acidic pH in comparison to alkaline.

Results of the storage studies suggest that efficacy of leaf extract of *T. arjuna* did not change upto 6 months, but loss in antifungal activity against both test fungi was observed after 6 months storage. After 12 month storage leaf extract retained its antifungal potential as previous against both of the test fungi. These results indicate that the active molecules present in leaf are sensitive to temporal changes or undergo slight structural changes during storage. They may retain their efficacy as the time passes. Griggs *et al.*,(2001) also reported the similar results from the extracts of medicinal plants. He reported decrease in their antifungal potential after 6 months storage and they regain their antifungal potential when assayed after one year. Different physical factors during storage accelerate the aging process of plant extract and chemical decomposition of active components which results into decrease in antifungal potential. These effects vary from species to species hence degree of changes in biological activity and chemistry of secondary metabolites due to storage is species-specific (Stafford *et al.*,2005).The present results suggests that the active principle/s present in *T. arjuna* are susceptible to varying degrees of change in physical environment. However it is found that it can be stored for at least 6 months, remains stable at acidic pH, can with stand exposure to sunlight and high temperature. Hence a little favorable manipulation of physical conditions could improve its shelf life. In addition purified active compound might have different scenario in terms of storage and other physical conditions.

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