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Nutritional and Energetic Effects of Saccharomyces Cerevisiae Leaf Extract and Its Herbal Formulations on Plant Foods

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ABSTRACT

Effect of different physical factors for example heat, sunlight (temperature), pH, storage, etc. was studied by exposing the extract and herbal formulation to changing conditions of the factors selected for a particular time period, and then perceiving the effect as a function of alteration in minimum inhibitory concentration (MIC) of extract against the C. lunata. Tubes containing MIC of extract, herbal formulation and extract free medium were maintained for comparison in each set of experiment against C. lunata. Effect of pH, temperature, and storage on the antioxidant activity of leaf extract of three plants extracts i.e. Mentha spicata, Moringa oleifera and Daucus carota were studied by Arabshahi et al. In this current study, effect of different physical factors for instance sunlight, pH, heat and long-term storage on extract and herbal formulation efficacy have been measured to determine its reliability under the variable physical environmental conditions.

KEYWORDS

Lawsonia inermis; Curvularia lunata; Physical factor; Heat, Sunlight; pH; Storage.

1. Introduction

Plants have been used for treating various diseases. They show promising pharmacological activity because of presence of bioactive chemical substances such as alkaloids, tannins, flavonoids and phenolics [1, 2]. These bioactive compounds are extracted and used to prepare bioformulations for controlling plant diseases in ecofriendly way [3 - 5]. The biological active constituents in the plant material can be affected by various physical factors like climatic conditions, edaphic factors, seasonal variations and diurnal changes [6]. Commercial feasibility of any kind of herbal formulation depends on its capability to sustain consistency at changing physical environments. The perquisite conditions for the consumption of plant extracts in related formulations thus are; its physical and chemical properties should not sustain any drastic change owing to changes in pH, exposure to sunlight, temperature, it should have a prolonged shelf lifespan; as a minimum 6 months and there should be no deteriorating in its antimicrobial activity. Hence, plant extracts or bioformulations are used for disease control it is necessary to check it their stability under different variations of physical factors like temperature, pH, exposure to sunlight, etc.

Several researchers have checked the stability of extracts and bioformulations in varying conditions of physical factors for example temperature, pH, etc. Bonjar [7] reported the effect of temperature on the activity of plant extracts and he observed that all active plant extracts were stable at room temperature in both Methanol: DMSO (1:1, v/v) solvent and in dry state up to 18 months and did not show any reduction of antibacterial activity. Stability of toxicity of Citrus sinensis oil was studied by the Patra et al. [8] using two parameters i.e. temperature and time of storage. The effect of heat, temperature and 0.5 M sucrose on efficacy of turmeric oil was studied by Rath et al. [9]. Ranganathan and Balajee [10] investigated heat stable activity of combination of ethanolic extract of Ocimum sanctum and Cassia alata. Lee et al. [11] evaluated heat and pH susceptibility of Chinese leek extract and observed that heat treatment above 75°C reduce the inhibitory activity of extract while inhibitory activity is stable between pH 2.0 to 8.0. Doughari [12] reported the effect pH of and temperature on root extracts of Carica papaya L. and found that there is significant increase in bioactivity of extract with increasing temperature and decreasing of bioactivity is concomitant with increasing of pH. Similarly, Rakkimuthu et al. [13] testified that anthocyanin extract of Cocculus hirsutus fruit were extremely or moderately resilient to the pH, light factors and temperature. This anthocyanin extract was highly constant at pH 1.0 and 3.0, temperature at 4°C and 37°C both in the existence and inadequacy of light. Destruction of anthocyanin was influence by increase in environmental factors such as pH, light and temperature.

2. Materials and Methods

On the basis of results obtained from in vitro antifungal activity using poison food technique [19], we obtained four best treatments out of thirty treatments (Table 1 & Figure 1). These four best treatments and combinations are as follow:

Figure 1. In vitro antifungal of herbal formulations againt Curvularia lunata.

(1) Formulations no. 1 [100% alcoholic crude extract (4 ml): 100% clove bud oil cake (4 ml): 100% cow dung (2 ml)]; (2) Formulations no. 2 [100% alcohol crude extract (2 ml): 100% clove bud oil cake (6 ml): 100% cow dung (2 ml)]; (3) Formulation no. 3 [Partially purified acetone extract (4 ml): 100% clove bud oil cake (4 ml): 100% cow dung (2 ml)]; (4) Formulation no. 4 [Partially purified acetone extract (3 ml): 100% clove bud oil cake (4 ml): 100% cow dung (3 ml)].

These extracts and herbal formulations were found to be most potent. Experiments were repeated thrice and three replicates were maintained.

2.1 Effect of Heat

Table 1. In vitro antifungal activity of various herbal formulations against Curvularia lunata.

Formulation No.	Formulation Type	Ratio	Growth Diameter after 7 Days (mm) ± SD	% Mycelial Growth Inhibition
1.	100% alcoholic crude: Clove bud oil cake: cow dung	4:4:2	18.33 ± 0.57	77.82
2.	100% alcoholic crude: Clove bud oil cake: cow dung	2:6:2	16.66 ± 0.57	79.84
3.	Partially purified acetone extract: Clove bud oil cake: cow dung	4:4:2	13.66 ± 0.57	83.47
4.	Partially purified acetone extract: Clove bud oil cake: cow dung	3:4:3	15.33 ± 0.57	81.45
Control	----------	-	82.67	NI*

Efficacy of the extract and herbal formulation was evaluated according to the method suggested by Rath et al. [9]. Effect of dry heat was studied by exposing sterile glass vials containing 100% alcohol crude extract, partially purified acetone extract and herbal formulation (no. 1, 2, 3, 4) to 40°C and 90°C for 4 h in hot air oven

while in case of wet heat; extract and herbal formulation were kept at 50°C and 100°C in water bath for 4 h. Effect on activity of extract and herbal formulation was then assayed by tube dilution method. One tube containing untreated extract as well as herbal formulation (room temperature) was maintained as control for the comparison.

2.2 Effect of Sunlight

Effect of sunlight on viability of the extracts and herbal formulation was studied according to the method suggested by Wang and Ke-Qiang [20]. Sterile vials containing 5 ml of 100% alcohol crude extract, partially purified acetone extract and herbal formulation (no. 1, 2, 3, 4) were placed in sunlight for 15 h and 30 h. Then effect on the efficacy of extract and herbal formulation was assayed by the tube dilution method.

2.3 Effect of pH

Effect of various pH's i.e. 4.0, 7.0 and 9.0 on the efficacy of extract along with herbal formulation was studied by the method suggested by Dixit et al. [21]. Natural pH of extract and herbal formulation is 7.0. 0.1 N HCl and 0.1 NaOH were used to change the pH to 4.0 and 9.0, respectively. Then culture medium was added to the tubes containing extract and herbal formulation and the tubes were inoculated with C. lunata. Inoculated tubes were then incubated at 27 ± 1°C for 72 h and observed for the change in herbal formulation and MIC of the extract.

2.4 Effect of Storage

Effect of storage on the antifungal activity of the extract and herbal formulation was analyzed by method suggested by Rath and coworkers [9]. Extracts and herbal formulations were stored at room temperature and change in their activity was analyzed at regular intervals of 6 month up to 24 months by tube dilution method.

2.5 Statistical Analysis

All the statistical analysis and calculation were accomplished by using IBM SPSS Statistics Ver. 20 software. The statistical data were expressed as the mean of three independent replications ± standard error (SE) of at least three replicates of each experiment. The experiments were performed in triplicates and all the experiments repeated twice via totally randomized design.

3. Results and Observations

The results of effect of different physical factors viz. heat, sunlight, pH, and long term storage on extracts and herbal formulations are given in Tables 2-9. Effect of wet and dry heat on extracts and different formulations are given in Tables 2 and 3, respectively.

3.1. Effect of Heat

Results indicated that wet heat at room temperatures 50°C and 100°C were not affected the activity of 100% alcoholic crude extract and partially purified acetone extract in comparison to control. However, dry heat up to 100°C showed slight reduction in the antifungal activity of 100% alcoholic crude extract and partially purified acetone extract in comparison to control (Table 2).

Table 2. Effect of heat on crude and partially purified acetone leaf extract of Lawsonia inermis against Curvularia lunata.

Extracts	Dry heat			Wet heat		
	R. T.	50°C	100°C	R. T.	50°C	100°C
100% Alcoholic crude	No growth	No growth	Slight growth	No growth	No growth	No growth
Partially purified acetone	No growth	No growth	Slight growth	No growth	No growth	No growth

Control (without extract)	Abundant growth

Similarly, dry heat up to 100°C showed slight reduction in the antifungal activity of different herbal formulation (no. 1, 2, 3, 4) in comparison to control. While, wet heat at room temperature, 50°C and 100°C not affected the activity of these herbal formulations as compare to control (Table 3).

Table 3. Effect of heat on herbal formulations against Curvularia lunata.

Herbal formulations number	Dry heat			Wet heat		
	R. T.	50°C	100°C	R. T.	50°C	100°C
1	No growth	No growth	Slight growth	No growth	No growth	No growth
2	No growth	No growth	Slight growth	No growth	No growth	No growth
3	No growth	No growth	Slight growth	No growth	No growth	No growth
4	No growth	No growth	Slight growth	No growth	No growth	No growth
Control (without extract)	Abundant growth					

3.2. Effect of Sunlight

Effect of sunlight results revealed that there were no any changes in the activity of leaf extracts (crude and partially purified) (Table 4) and different herbal formulations after the exposure of 15 h, 30 h and unexposed conditions (Table 5).

Table 4. Effect of sunlight exposure on crude and partially purified acetone leaf extract of Lawsonia inermis against Curvularia lunata.

Extracts	15 h	30 h	Unexposed condition
100% Alcoholic crude	No growth	No growth	No growth
Partially purified acetone	No growth	No growth	No growth
Control (without extract)	Abundant growth		

Table 5. Effect of sunlight exposure on herbal formulation against Curvularia lunata.

Herbal formulation number	15 h	30 h	Unexposed condition
1	No growth	No growth	No growth
2	No growth	No growth	No growth
3	No growth	No growth	No growth
4	No growth	No growth	No growth
Control (without extract)	Abundant growth		

3.3. Effect of pH

Change in pH of extracts and herbal formulation showed reduction in activity. Results indicated that pH 9.0 and pH 7.0 (neutral) were not affected the activity of 100% alcoholic crude extract and partially purified acetone extract in comparison to control. But, pH 4.0 showed slight reductions in the antifungal activity of 100% alcoholic crude extract and partially purified acetone extract in comparison to control (Table 6).

Table 6. Effect of pH on crude and partially purified acetone leaf extract Lawsonia inermis of against Curvularia lunata.

Extracts	pH 4	pH 9	pH 7 (Control)
100% Alcoholic crude	Slight growth	No growth	No growth
Partially purified acetone	Slight growth	No growth	No growth
Control (without extract)	Abundant growth		

Similarly, pH 9.0 and pH 7.0 showed no any growth in the antifungal activity of different herbal formulation in comparison to control. While, pH 4.0 slightly affected the activity of these herbal formulations as compare to control (Table 7).

Table 7. Effect of pH on herbal formulations against Curvularia lunata.

Herbal formulation number	pH 4	pH 9	pH 7 (Control)
1	Slight growth	No growth	No growth
2	Slight growth	No growth	No growth
3	Slight growth	No growth	No growth
4	Slight growth	No growth	No growth
Control (without extract)	Abundant growth		

3.4. Effect of Storage

Effect of storage results exposed that there were no any alterations in the activity of leaf extracts (crude and partially purified) (Table 8) and different herbal formulations against C. lunata subsequently exposure of 6 months, 12 months and fresh extracts.

Table 8. Effect of storage on crude and partially purified acetone leaf extract of Lawsonia inermis against Curvularia lunata.

Extracts	6 Months	12 Months	Fresh extract
100% Alcoholic crude	No growth	No growth	No growth
Partially purified acetone	No growth	No growth	No growth
Control (without extract)	Abundant growth		

4. Discussion

The effectiveness of plant extracts or herbal formulations depend on the supply of the active compounds without any kind of change in its physical and chemical properties. Therefore, it should distribute the vital active component at an adequate concentration throughout the comprehensive treatment, and focused toward the preferred target. Herbal formulations are prepared by using plant extracts having active constituents which may be useful for the management of various sorts of diseases because they have no side effects as compared to synthetic antimicrobial drugs [22 - 29]. Herbal formulations may be viable only when they have capability to sustain the strength, immobility and physical factors such as temperature, pH, sunlight exposure, etc. do not interrupt the antimicrobial activity of these formulations [30 - 38].

Results suggest that there was no change in antifungal activity of 100% alcohol crude and partially purified acetone extract and all herbal formulations subsequently exposure to the direct sunlight. This shows that active principles constituents of extracts and herbal formulations are stable in sunlight and they do not undergo photo oxidation. Wang and Ke-Qiang [20] have been reported similar kind of results. Possibly, sunlight exposure does not detriment the active molecules of extract of L. inermis. Results of the effect of heat on

efficacy of extracts and formulations indicates that wet heat up to 100°C did not affect the activity of leaf extracts and different formulations where as dry heat treated L. inermis leaf extracts and formulations showed slight reduction in the antifungal activity at 100°C. Temperature resistance studies reveals that the phytochemical constituents are thermo stable but heating at 100°C with dry heat leads to decline/loss in the antifungal activity this may be due to volatilization of active components or due to some chemical and physical alterations in the molecules of natural products during heating. Sharma and Sharma [17] also reported the similar pattern when they treated the L. inermis leaf extract with dry heat and studied its effect against Aspergillus flavus and Aspergillus parasiticus.

The present study revealed that the antifungal activity of extract and herbal formulation of L. inermis was observed to be balanced at the pH 7.0 and 9.0. However, it was observed that the activity of the same was decreased at pH 4.0. These results recommend that the active principle compounds of the extract are maximum active at neutral pH. Nishihara et al. [39] reported that the existence of a high concentration of salt obstruct with the binding of cationic peptides to the cell surface of Bacillus subtilis, which are prerequisite for its growth enlargement. Yen and Duh [40] revealed that methanol extract of peanut hulls have higher antioxidant activity at neutral and acid pH. The activity of the phytoconstituents has been increased in the presence of acidic medium [12]. The antioxidant activity of various extracts from cocoa by-products was found higher at alkaline pH [41]. Kirca et al. [42] reported that the stability of black carrot anthocyanins was degraded with increasing solid content and pH (4.3 to 6.0) during heating (80°C), but it reduced during storage (18.7, 30.8 and 35.9 weeks at 20°C). Arabshahi et al. [14] reported that antioxidant activity of extract of the mint, drumstick and carrot leaves differs with the variation in pH. Similarly, Srinivasan et al. [43] described that reduction in the antimicrobial activity of Allium sativum extract depend on increasing pH value and it was minimum at pH 9.0. Bayliak et al. [44] revealed that the antioxidant activity of aqueous extracts of Rosa canina, Hypericum perforatum, Rhodiola rosea and Gentiana lutea is declined at alkaline pH but pro-oxidant activity rise at equivalent pH.

Storage investigates outcomes suggested that there is no effect of long-term storage on efficiency of extract and herbal formulation [45 - 49]. During storage combinations of physical factor not as much affect the efficacy of extract along with herbal formulation than individually affect. Arias et al. [50] examined ethanolic and aqueous extract of the Acacia aroma against gram (-) ve and gram (+) ve bacteria and also considered that stored extracts have same antibacterial activity as the fresh extracts. Hada and Sharma [18] have reported similar results.

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