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Original Article

Pharmaceutical Formulation of Garlic and Turmeric Dried Crude Extract and Their Synergistic Antifungal Activity and Safety

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Abstract

Candidiasis is a fungal infection caused by *Candida albicans*. *Allium sativum* (garlic) and *Curcuma longa* (turmeric) have been used as antifungal agents. The main aim of this study was to identify the effectiveness of these natural products towards *C. albicans* and on their pharmacological and toxicity aspects. Thus, agar disc diffusion method was used to study the antifungal activity of the ethanolic extracts. Fluconazole served as the positive control while solvent (ethanol) served as the negative control. Minimum inhibitory concentration (MIC) of the plant extracts were tested by using two fold agar dilution method at concentrations ranging from 0.390g/L to 100g/L. As delivery agents, cream and gel formulations demonstrated good stability test results. Furthermore, both plants showed synergistic effects. Active compound of garlic and turmeric which is allicin and curcumin were observed through Thin Layer Chromatography (TLC). Moreover, all the formulation resulted in optimum MIC at pH 5.5 and temperature 25.5ºC. Toxicity test using Brine Shrimp Lethality Test (BSLT) showed that ethanolic extracts of both plants displayed LC_{50} values at 77.93µg/mL and 31.97µg/mL. Whereas, LC50 value of synergistic experiments of extract was 10.77µg/mL. Besides, synergistic cream formulation was most potent against brine shrimp larvae compared to others with LC50 value of 5.35µg/mL. Synergistic experiment using gel formulation also showed potency against brine shrimp larvae with LC50 value of 3.58µg/mL. As a conclusion, both plant extracts and preparations showed significant effectiveness against *C. albicans* and potency on shrimps.

Keyword: *C. albicans*, Anti-fungal, *A. sativum*, *C. longa*, Ethanolic extract, Brine Shrimp Lethality

1. Introduction

Types of candidiasis are included invasive, vulvovaginal, and oropharyngeal [1]. The infection normally occurs in patients who have immunocompromised and prone to develop both superficial and life threatening *cadida* infection [2]. The pathogenesis of candidiasis is multifactorial such as intact mucocutaneous

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barriers, phagocytic cells, monocytic cell, broad spectrum antibiotics, solid organ transplant and mechanical ventilation. Current antibiotic, fluconazole used to treat candidiasis has been used for long periods with a resulting development of resistance to fluconazole. [3]. Compounds such as curcumin and allicin investigated isolated from *Allium sativum* (garlic) and *Curcuma longa* (turmeric) which effective against antifungal. This study is to comfort the sensitivity of microbes to ethanolic extracts of these plants and also to investigate its potential for cytotoxic activity [4].

2. Materials and Methods

2.1. Preparation

Both garlic and turmeric were cleaned with tap water, then cut into small pieces and dried in the hot-air oven for three days at 50ºC [5]. Then, the grounded into powder form and soaked with absolute ethanol with the ratio (1:2) and concentrated by using rotary vacuum evaporator [6].

2.2. Antifungal Activity

Disc diffusion was conducted for each of concentration from 50g/L to 500g/L with the ethanolic extract of garlic and turmeric. Fluconazole act as a positive control while ethanol as negative control [4].

2.3. Determination of Minimum Inhibitory Concentration (MIC)

The MIC was done using concentration of extract from 0.390g/L to 0.100g/L. The experiments were carried out in triplicates [7]. After 24 hours, the turbidity of the mixture was recorded. Synergy of the extracts was evaluated based on 100g/L [11]. There totally six combination of extraction were done. After 24 hours, the turbidity of all the mixture was recorded [5].

2.4. Separation of Active Fractions from Extract of Plant

This test was done to identify the active compounds of garlic and turmeric. The R_f values were calculated by the given formula [9].

$$\text{Retention factor } (R_f) = \frac{\text{Distance travelled by solvent from origin}}{\text{Distance travelled by solut from origin}}$$

2.5. Formulation Preparation

The main ingredients for cream is emulsifying ointment whereas for gel are carbopol 934 and prophylenegycol. After that, the cream and gel were prepared by using ethanolic extract of garlic and turmeric [8].

2.6. Optimization

The stability for each formulation was pH, temperature and storage duration were tested. Each of parameter was conducted three tests which are effectiveness, viscosity, and appearance. Sabouraud dextrose agars were streaked with Candida strain and leave it for few minutes to absorb. Cut approximately 0.5cm diameter of holes from agar (six holes). Thus, the holes were filled with respective preparation of cream and gel [6, 7].

2.7. Brine Shrimp Lethality Test (BSLT)

38g of sea salt was used to prepare 1L of artificial seawater. Artificial seawater was used to hatch the *Artemia* cysts in a plastic container which set-up with an aspirator and table lamp. After 48 hours, the hatched shrimps were collected by using plastic pipette [8].

2.8. Preparation of Vials for Testing

10mg of the extracts and the formulations were each dissolved in 10ml of 1% ethanol diluted with artificial seawater to make a 1000µg/mL of stock solution. The stock solution was used to prepare six different concentrations, 1000µg/mL, 500µg/mL, 100µg/mL, 50µg/mL, 10µg/mL and 5µg/mL; 10mL, 5mL, 1mL, 0.5mL, 0.1 and 0.05mL of the stock were transferred into the six vials respectively. This method was calculated by the given formula. Fluconazole solution served as positive control, while ethanol solution was served as negative control [10].

Formula, $C_1V_1 = C_2V_2$
V_1= The volume of stock will start (x= unknown)
C_1= Stock solution's concentration (1000µg/ml)
V_2= Total volume needed at the new concentration (10ml)
C_2= New concentration (5, 10, 50, 100, 500, 1000µg/ml)

2.9. Bioassay

Each test tube vials containing different concentrations of the extract were placed ten brine shrimp larvae. Thus, there were a total of 30 brine shrimps per concentrations. After that, the test tubes were left under the lamp to maintain the temperature. The survivors were recorded after 24 hours [11].

2.10. Lethal Concentration Determination

After 24 hours, the lethal concentration (LC_{50}) of both plant extract and formulation were determined by calculated the mortality percentage of the shrimps. Then, used MS Excel to plot the graph by means of a trend line fit linear regression analysis. From the linear equation obtained the value of LC_{50} [6, 7].

3. Results and Discussion

In disc diffusion, ethanolic extract of garlic and turmeric showed larger zone of inhibition at 26mm and 30mm against *C.albicans*. The ethanolic extracts were found to be effective against C.albicans with low MIC of 3.125g/L. Besides that, both plants showed the

synergistic effect with lowest MIC of 0.390g/L for the turbidity of combination plant extract. In thin layer chromatography (TLC), separation of active compound from the ethanolic extracts of garlic and turmeric which are allicin and curcumin was observed. Moreover, the optimal formulation pH is 5.5 which showed large zone of inhibition as 30.67mm turmeric cream and 29mm turmeric gel. In the optimal temperature, 25.5ºC showed large zone of inhibition and viscosity more than 30 Pa in all range from 100 Pa to 600 Pa. The storage period for all formulation was stable from 30 minutes till 2 days. At the same time, the effectiveness and viscosity remain same through the storage period conducted [12].

The results of BSLA using ethanolic extracts of garlic and turmeric and positive control, fluconazole and about detail of LC_{50} values were given below (Figures 1, 2, and 3). After 24 hours, the surviving brine shrimp larvae were recorded and LC_{50} values were determined. Results demonstrated that the ethanolic extract of *A.sativum* and *C.longa* were potent (toxic) toward the brine shrimp with LC_{50} values of 77.93 and 31.97µg/mL. Whereas, LC_{50} value of synergistic extracts was 10.77µg/mL. Besides that, synergistic cream formulation was most potent against brine shrimp larvae compared to others with LC_{50} value of 5.35µg/mL. Moreover, synergistic gel formulation also showed potency against brine shrimp larvae with LC_{50} value of 3.58µg/mL [13].

Based on the results, the disc diffusion step using ethanolic extracts of garlic and turmeric showed highest zone of inhibition. This could be as a result of better extraction with alcohol solvents. Besides that, turmeric extract has the lowest MIC that is at 3.125 g/L, followed by garlic extract at 25 g/L. Whereas the MIC of

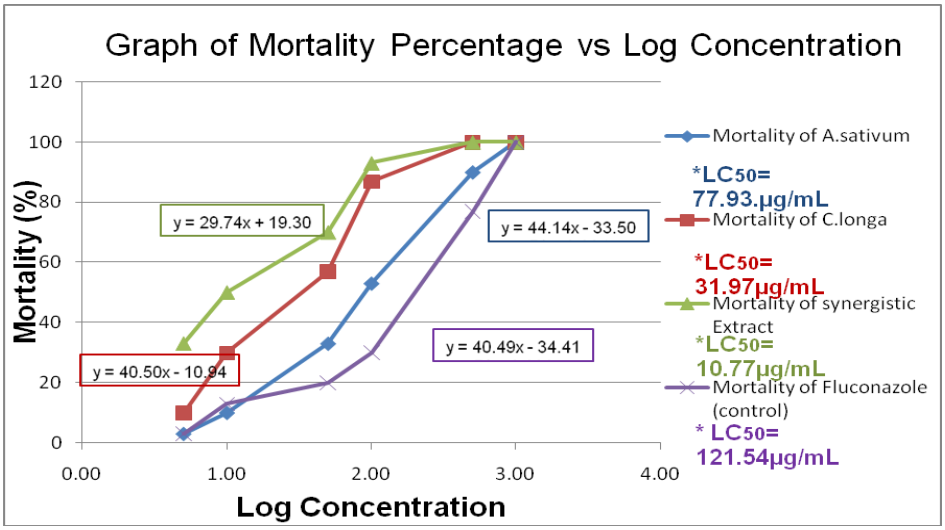


Figure 1. The number of shrimp larvae that survived after test with three samples of plant extracts and the percentage mortality.

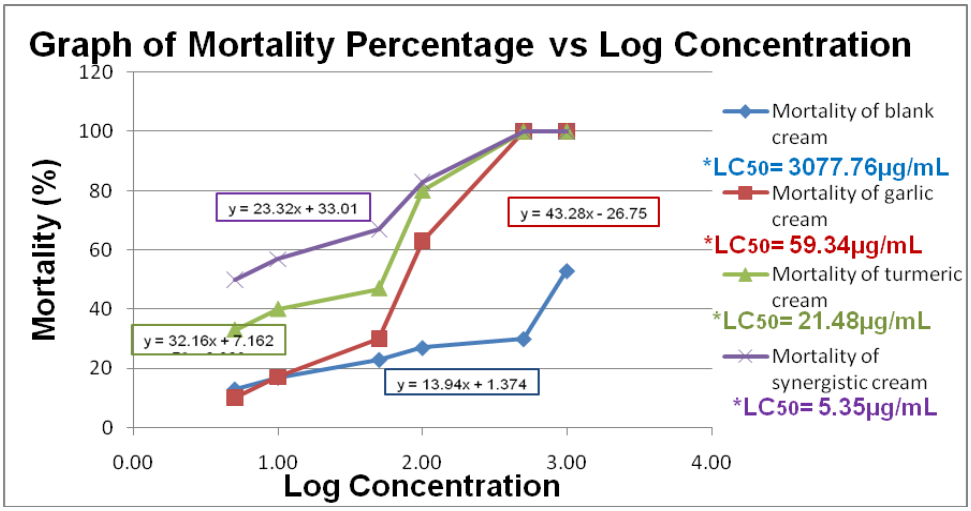
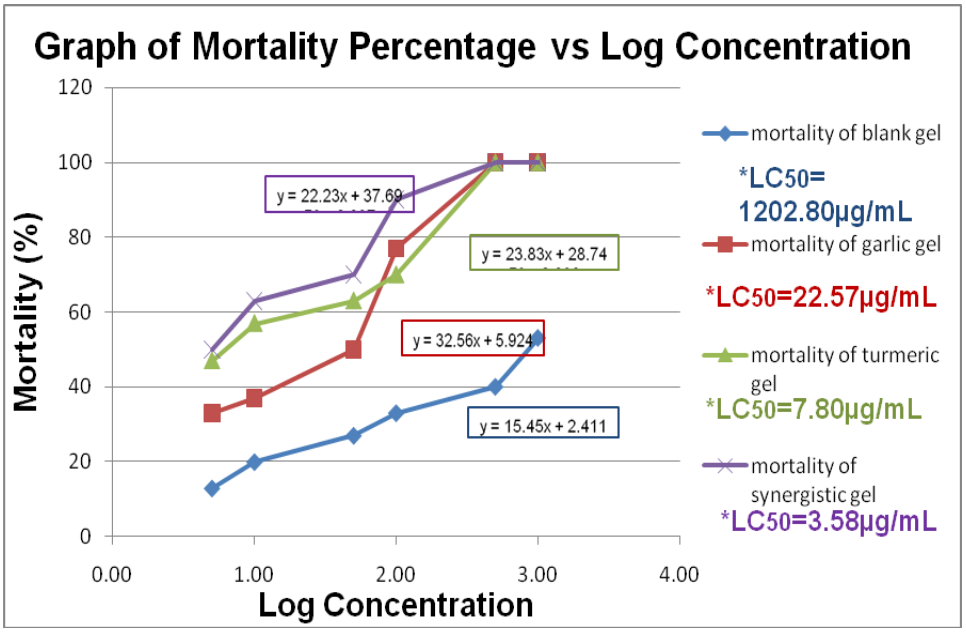


Figure 2.The number of shrimp larvae that survived after test with the plant extract's formulation (Cream) and the percentage mortality.



Figures 3. The number of shrimp larvae that survived after test with the plant extract's formulation (Gel) and the percentage mortality.

synergy of turmeric ethanol with garlic ethanol was at 0.390 g/L. According to the formula of Fraction Index Concentration (FIC) synergy means ≤ 0.5 while indifferent > 0.5 to ≤ 4.0. At the end of result, the ethanol extracts of garlic and turmeric showed high synergy activity which mean the turmeric ethanol with garlic ethanol is 0.281 and these combinations are less than 0.5 that indicates synergy. The synergy possibly had higher antifungal potential when mixed, as they were prepared in MIC, compared to that is found on individually. The viscosity of room temperature, 25.5ºC on each of formulations were within high range which more than 30 Pa while other temperature viscosity were low

range which was less than 30 Pa. According to the viscosity, in appearance and effectiveness test of two formulations it was found that the room temperature 25.5ºC is suitable to store the formulations. Thus, the storage periods of each formulation were conducted from 30 minutes to 2 days. At the end of the results, all the formulation reached the optimized pH (pH 5.5) and the temperature (25.5ºC) was effectively towards *C.albicans*.

Results demonstrated that the effects of the ethanolic extracts of garlic and turmeric on the mortality of the brine shrimp *Artemiasalina* depended on its concentration. In the ethanol, most of the brine shrimps died upon exposure to 500µg/mL and higher concentrations (1000µg/mL) of such extracts. The brine shrimps mortality rate was found directly proportional to the increasing concentration of the samples. Thus, the result clearly showed that theethanolic extraction of garlic and turmeric were a better way to obtain the bioactive compounds of garlic and turmeric with the LC_{50} values of 77.93 and 31.97µg/mL. Besides that, synergistic of both formulations (cream and gel) showed the most potent effect with LC_{50} values of 5.35 µg/mL and 3.58 µg/mL. According to Meyer et al., crude plant extract is toxic active) if it has an LC_{50} value of less than 1000µg/mL while non-toxic (inactive) if it is greater than 1000µg/mL. This suggests that the ethanolic extract of these both plants and its formulations could have compounds that are cytotoxic as the LC_{50} value was lower than 1000µg/mL.

4. Conclusion

The results of ethanolic extracts of turmeric, garlic, and its synergistic effect of both plant extracts showed antifungal activity in all the studies. In the current study, both plant extract and its formulations were found to show potent activity against brine shrimps larvae. As a conclusion, all the plants extract and formulations are consider active since their LC50 values <1000µg/mL and have antifungal activities towards candidiasis inducing *C. albicans*.

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