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ANTIFUNGAL KINETICS ACTIVITY OF PATCHOULI OIL (*Pogostemon heyneanus*) AND ITS MICROENCAPSULATION ON *Aspergillus Flavus*

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ABSTRACT

Research of patchouli oil (*Pogostemon heyneanus*) microencapsulation as antifungal on *Aspergillus flavus* has been carried out. This research aims to find out antifungal activity of microencapsulated patchouli oil on fungi *A. flavus* equipped with kinetics reviews comprising determination of activity and its reaction order. Patchouli oil was obtained from the isolation process using the steam-water distillation method with a yield of 0.88%. Characterization using Gas Chromatography-Mass Spectrometry showed that patchouli oil as the result of isolation process contains 6 main components, which are ß-caryophyllene (8.04%); 6,10,11,11-tetramethyl- tricyclo (17.20%); α-guaiene (17.86%); 1H-3a,7-methanoazulene (11.88%); δ-guaiene (23.49%) and patchouli alcohol (6.68%). Patchouli oil microencapsulation was carried out using spray drying method with several coating material variations:maltodextrin (1:8, 1:10, 1:12) and produced a yellowish-white sticky powder that brings a distinctive aroma of patchouli oil. Inhibition zone of antifungal in patchouli oil and its microencapsulation results toward *A. flavus* are categorized as insensitive, but patchouli oil is 100% sensitive. Resistibility activity of patchouli oil reaction is n = 0.5 with k' = 0,1030; for microencapsulation process is n= 0.7 with k' = 0.1144, causing the difference of curve A_t versus t for those antifungal.

Keywords: Antifungal, *Aspergillus flavus*, Microencapsulation, Patchouli Oil, Kinetics.
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INTRODUCTION

Patchouli (*Pogostemon heyneanus*) is one of the plants producing essential oils widely used as binding agents in the fragrances, cosmetics, medicines and pesticides industries.[1,2] Patchouli oil contains sesquiterpenes including patchouli alcohol (PA), bulnesene, α-guaiene, patchoulene.[3] Sesquiterpenes categorized in terpenoids can be applied as antifungal.[4-6]

Aspergillus flavus is a dangerous fungus for humans, especially people with immune system depression. *A. flavus* is an allergen that causes allergic bronchopulmonary aspergillosis.[7] Also, this fungus can produce aflatoxin B_1 as a secondary metabolite and is naturally the most potent carcinogen. This compound is one of several established mycotoxins as biological weapons.[8] *A. flavus* appears to be more lethal and antifungal resistant than other *Aspergillus*.[9,10]

Many strategies are taken to prevent the growth of the *A. flavus*, whether physically, chemically, or biologically, requiring sophisticated equipment and chemical reagents.[11] An alternative way to overcome the pests before harvesting, which disrupt the crops, is utilizing the essential oil. This is because the compounds in the essential oil can interfere and break down the fungi cell wall structure. The essential oil component from terpenoid class that contained in particular plant can damage the phospholipid layer of cell membrane microorganisms.[12,13]

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Direct application of essential oil has limitations because it is very susceptible to external factors such as light, oxygen, humidity and temperature.[14] One alternative to improving necessary oil stability is the microencapsulation process. The microencapsulation process involves the formation of microparticles in which the active ingredient (the core material) is coated by the encapsulant (the wall) materials.[15] The essential oil microcapsule stability is also highly dependent on the wall material composition. Various polymers such as natural gum, maltodextrin, gelatin and protein can be used as the wall materials.[16] Among them, maltodextrin is considered a good encapsulation because it displays low viscosity at high solid content levels and good solubility.[17] One of the most common techniques used in microencapsulation is spray drying.[18]

Kinetics studies have essential meanings for predicting the optimal treatment of an antibacterial[19], because the reaction order provides information on the rate of dependence on reactant concentrations.[20] A zero-order reaction indicates that a constant reaction rate is independent of the reactant concentration.[21] This means that if the order is close to zero, then the antibacterial use is sufficient in low concentrations because an increase in concentration will not significantly increase the reaction rate. Still, in a relatively large order, an increase in concentration is a practical choice.

Based on these backgrounds, this study's main aims were to manufacture microencapsulated particles containing patchouli oil using a spray-drying technique using maltodextrin as wall material. This work also characterized the particles formed and determined the antifungal activity and its kinetics over *A. flavus*.

EXPERIMENTAL

Material and Methods

The materials used were dried patchouli leaves (*Pogostemon heyneanus*), *Aspergillus flavus*, maltodextrin, ketoconazole 2%, peptone 2%, agar 4%, NaCl 0.1 N, tween oil, anhydrous $MgSO_4$, aquadest, Whatman filter paper, gauze, sterile cotton, and aluminium foil. The equipment used in this research was a set of steam-water distillation apparatus, dryer (LabPlant type SD 05), Gas Chromatography-Mass Spectrometry (GC-MS), Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS) JSM-6510x, spray dryer (LabPlant type SD 05), incubator, Analytical balance (Acis), autoclave (Wisecclave), water bath (HWS24), refrigerator (SHARP), micropipette (DRAGON ONEMED), laminar air flow cabinet, shaker (Ratex), UV lamp, Eppendorf tube, ose needle, petri dish, measuring pipettes, ovens, dropper drops.

Isolation of Essential Oils from Patchouli Leaves by Using Steam-water Distillation

Dried patchouli leaves were weighed 2.5 kg and then placed in a container of steam-water distillation, the container was previously filled with water as much as 28 liters. The process of steam-water distillation was carried out for 3 hours.[22] The distillate was accommodated in a separating funnel that formed a layer of oil and a water layer. The oil layer was then separated and dried using anhydrous $MgSO_4$ to remove residual water.

GC-MS Analyses of the Patchouli Oil

The components in patchouli oil were determined using GC-MS. The operating conditions of GC- MS were programmed from 60 to 250 °C at 10 °C /min and injection temperature was from 70 to 250 °C. The results obtained were in the form of chromatogram and mass spectrum. The data obtained were further interpreted by comparing with NIST MS and WILLEY library.[23]

Microencapsulation

The volatile oil of distillation of water vapor was microencapsulated with maltodextrin. The preparation of microemulsion was done using Tomazelli method with some modifications.[24] The variations in composition between essential oils and maltodextrin were 1:8, 1:10, 1:12. O/W emulsion was mixed with maltodextrin, stirred at 4000 rpm for 3-5 minutes and spray-dried using a laboratory-scale dryer to obtain the microcapsules. The emulsion was fed into spray-dryer at room temperature with a flow rate of 300 mL min^{-1}. The inlet and outlet temperatures were maintained at 110 °C and 68 °C, respectively.

Surface Morphology Analysis with SEM

The Surface Morphology Analysis was performed by placing the sample in a carbon-conductive layer and coated with 60% gold and 40% palladium with sputtercoater at a current of 35 mA for 1 min. Operating

conditions were carried out at a voltage acceleration of 10 kV and 500×, 2000× and 5000× magnifications.

Antifungal Activity of Patchouli Oils

The fungi were grown on potato dextrose agar/PDA (20 ml of potato broth, 0.2 gram of dextrose and 0.4 gram of agar) and were incubated for 3 × 24 hours at 37°C.[25] PDA (20 mL) was inserted into Eppendorf tube and 10 µL inoculum fungi *A. flavus* was added and shaken to make it blend. Afterwards, the mixture was poured on the petri dish in a circular motion until evenly distributed and all of the surfaces of petri dish were covered. Then let it stand for several minutes until it solidified. Next, the disc paper (diameter 0.5 cm) which has been soaked in test solutions of patchouli oil 100%, 50%, 25%, 12.5% and patchouli oil:maltodextrin (1:8, 1:10, 1:12), positive control (ketoconazole 2%), negative control tween (tween oil and distilled water) on the media's surface to make it more compact were prepared. Follow and placed into the petri dish. The petri dish was then closed and wrapped tightly using plastic wrap then was incubated for 3×24 hours at room temperature. The formed inhibition zone was measured. The sensitivity of individual fungi to the patchouli oil was ranked based on the mean of inhibition zone values expressed in millimeters (mm) as follows: not sensitive for total zone diameters of ≤8mm; sensitive for total zone diameters of between 8mm and 14mm; very sensitive for total zone diameters of between 15mm and 19mm; extremely sensitive for total zone diameters of ≤20mm.[26]

Processing and Data Analysis

The obtained data included the separation data with GC-MS, SEM and inhibition zone values in the antifungal test, and antifungal activity kinetics.

RESULTS AND DISCUSSION

The Isolation and Characterization of Volatile Oil in Patchouli Plants

Isolation of volatile oil on dried patchouli leaves in this study used a method of steam-water distillation. During the distillation process, moisture will penetrate the leaf oil gland tissue. The volatile oil was removed through the hydro diffusion process. The mixture of oil in the water diffuses outward with the event of osmosis through the membrane that is blooming up to the surface of the material and subsequently evaporated by the vaporpassed to the condenser. The distillation is collected into a separating funnel, oil and water layers were separated for volatile oil. The essential oil that still contains water molecules is dried by adding anhydrous $MgSO_4$. Its additional function is to bind the water remained in the oil. This study produced clear coloured with a yield of 0.88%. According to Hussin *et al* (2012) this low yield is affected by cultivation technique, post-harvest handling and the condition of distillation process.[27] The result of GC-MS analysis patchouli oil using steam-water distillation shows 6 main elements with retention time of 23.198, 23.769, 23.857, 24.224; 25.513; and 29.107 minutes (Table-1 and Fig.-1).

Table-1:Components of the Main Compound of Patchouli Oil

No.	Compound	Retention Time (Minute)	Area (%)
1	ß-caryophyllene	23.198	8.04
2	6,10,11,11-tetramethyl-tricyclo	23.769	17.20
3	α-guaiene	23.857	17.86
4	1H-3a,7-methanoazulene	24.224	11.88
5	δ-guaiene	25.513	23.49
6	patchouli alcohol	29.107	6.68

A standardized grade PEO must have a Patchouli Alcohol (PA) between 26-40%, as required by Essential Oil Associations, to enter the global markets.[3, 27] However, in this study, the highest grade from patchouli plant is δ-guaiene compound. According to Hussin *et al* (2012) the different kinds of patchouli plants (pogostemon) is highly influence the contents of the compounds.[20] This variation is caused by the chemical relation from the chemical compound inside essential oil with the secondary metabolic process occurring

inside the plant. This process is affected by the ecosystem, natural conditions such as climate, weather, and soil mineral.

Surface Morphology Analysis with SEM

The morphology of the microcapsules was studied by scanning electron microscopy (SEM). Patchouli oil microencapsulation shows the form of a maltodextrin coating capsule that has been coated by patchouli oil. The spray drying method is based on the principle of producing small particles through atomization and solvent removal.[28] As the solvent is evaporated during the spray-drying process, the droplets lose most of their volume, allowing small particles to be formed.[29] Most of the particles exhibited a spherical shape with the occurrence of shrinkage (Fig.-2).

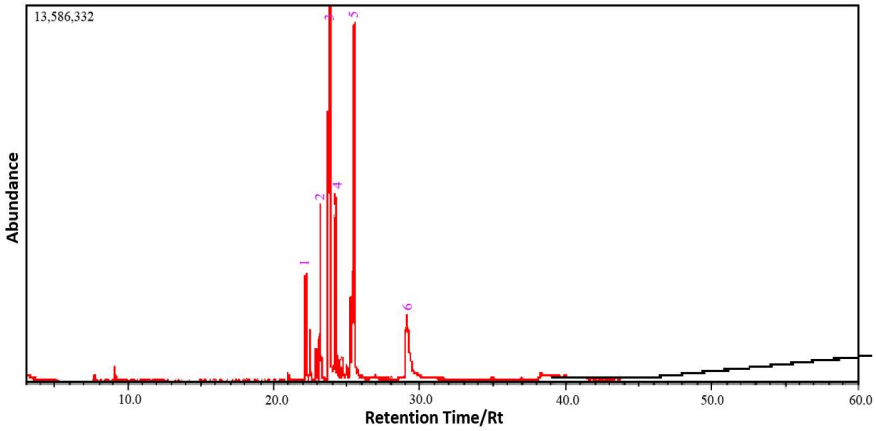

Fig.-1: Chromatogram of Patchouli Oil

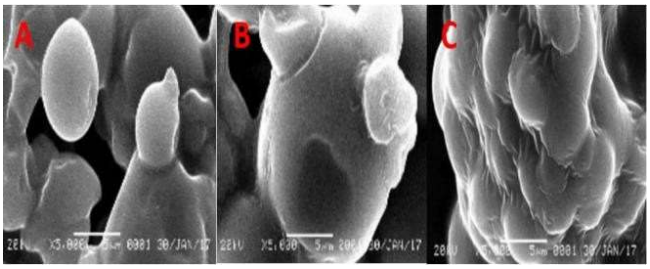

Fig.-2: SEM Photographs of Patchouli Oil:Maltodextrin (A. 1:8; B.1:10; C. 1:12)

Different morphologies of particles can be generated by changing process conditions. Spray-dried particles are usually spherical in shape; however, this depends substantially on the carrier used. The comparison 1:12 reveals that the spherical capsule is still lumpy and has not maximally expanded. In the ratio 1:10 shows that the capsule has been more coated yet is not fully maximal. On the other hand, 1:8 ration exposes that the capsule has been fully coated because there is no capsule stick to each other, it has been completely round.

Antifungal Kinetics Activity of Patchouli Oil

The patchouli oil antifungal activity and its microencapsulation results were determined by measuring the inhibition zones produced after applying the samples to *A. flavus*. Ketoconazole 2% was used as a positive control. Whereas negative control uses tween oil for patchouli oil and distilled water for microencapsulation results. After around three days, several inhibition zones are treated differently presents that patchouli oil allows the activity of antifungal as the insensitive category because of its diameter ≤ 8mm; excluding the 100% concentration, which can be categorized as sensitive inhibition (+) with the diameter between 8 to 14mm. Meanwhile, all of the microencapsulation results produced insensitive inhibition antifungal (-) because its diameter is ≤ 8 mm.[26] The complete data of inhibition zones diameter each treatment are shown in Fig.-3 and Fig.-4.

The measurement for kinetics oil and patchouli microencapsulation result is carried out to find the data of reaction order and the constant rate of material inhibitory activity against *A. flavus*. The approach to determine the order is done by using the linear regression equation. It has been known the general kinetic form to connect reaction rate of activity with antifungal concentration as follows:[30]

$$r = k\,[A]^n$$

The process of inhibition by antifungal occurs due to the process of cell wall destruction[31] which means that when antifungal interacts with a test fungus then there are stages that occur. The faster the process takes place in a certain incubation period, the greater the inhibition zone formed. This shows that the inhibition zone is a function of rate and can be mathematically written as:

$$\text{Inhibition Zone (iz)} \approx r$$

Hence:

$$iz = k_{iz}\, r$$
$$iz = k_{iz}\, k\,[A]^n$$

then:

$$iz = k'\,[A]^n$$

If both sides are logarithmic then:

$$\log iz = \log k' + n \log [A]$$

Which is a linear regression form with general form:

$$y = a + bx$$

Where y = log iz; a = log k'; b = n dan x = log [A].

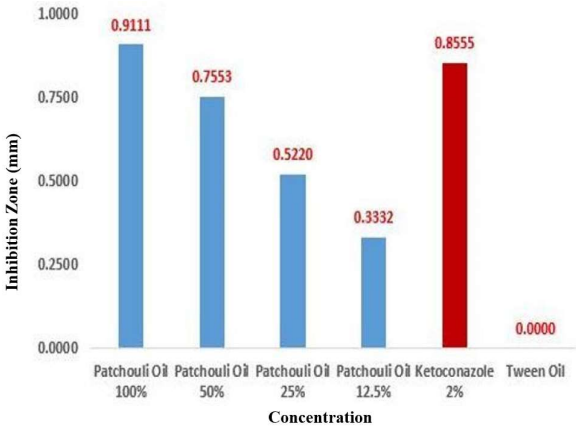

Fig.-3: Growth Inhibition of Antifungal patchouli oil on *A. flavus*

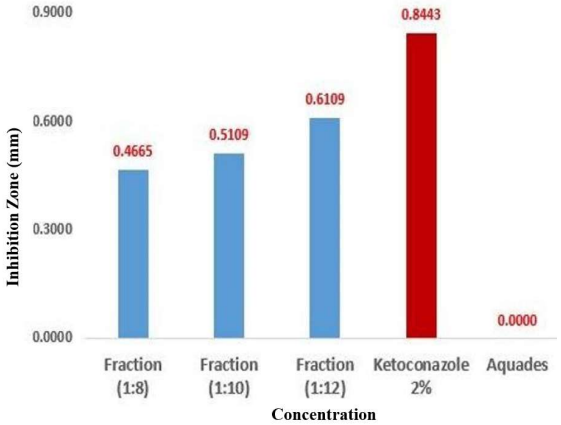

Fig.-4: Growth Inhibition of Antifungal microencapsulated of patchouli oil on *A. flavus*

The calculation results show that the reaction order of patchouli oil accounts for n = 0.5 with k' = 0.1030. As for the calculation result of patchouli microencapsulation result is n = 0.7 and k' = 0.1144. Reaction order is within zero and one, which means there is an influence of reagents concentration toward inhibition activity even though it is not too substantial.[21] Proposed that if reaction order is zero, the reaction rate will be independent of reactants. Meanwhile, if the reaction order is one, the reactants will be consumed and changed into the product. The charts of calculation results can be seen in Fig.-5 and Fig.-6.

Reaction order value (n) and k', which has been found will be used to calculate the rate of reduction in antifungal ingredients both in oil form and patchouli oil microencapsulation result to compare its activity pattern by portraying the relation chart [A]t versus t and obtain results as shown in Fig.-7. The obtained activities pattern shows that inhibitory performance of both antifungal ingredients in oil and patchouli microencapsulation result have a difference in the steepness of the curve.

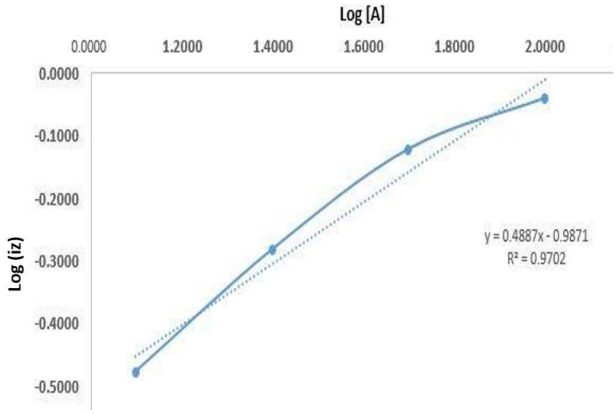

Fig.-5: log [A] vs log (iz) curves of patchouli oil

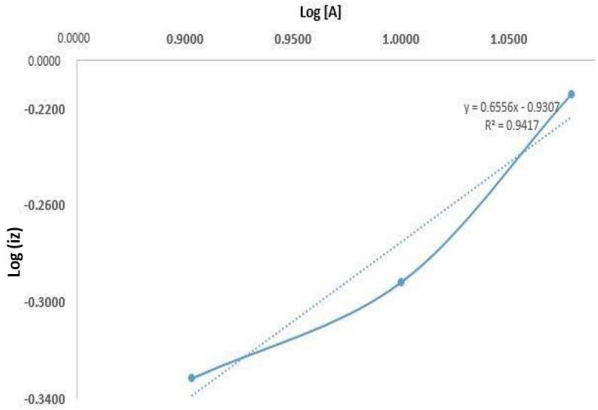

Fig.-6: log [A] vs log (iz) curves of microencapsulated patchouli oil

Microencapsulation result's curve is steeper than patchouli oil which means microencapsulation run out faster. Meanwhile, the oil needs more time or slower to run out. These are affected by the reaction order of patchouli oil n = 0.5 with activity rate k' = 0.1030 lower than microencapsulation result with the order n= 0.7; activity rate k' = 0.1144. Moore and Pearson (1981) proposed that every reaction with different order will bring a different curve.[32] On the other side, Dybkov (2013) exposes that two reactions will have a different steep curve in the concentration versus time if the number of k is different.[30]

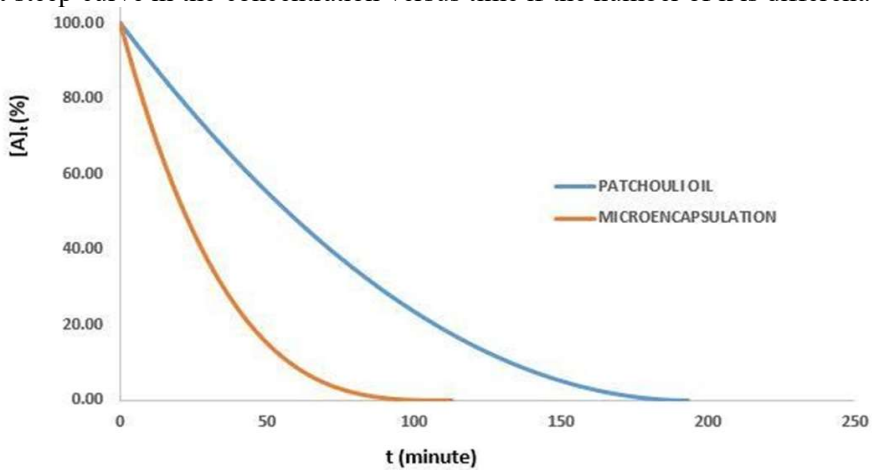

Fig.-7: Comparison Curves of Patchouli Oil and Microencapsulated

CONCLUSION

The main component of volatile oil from patchouli plant (*Pogostemon heyneanus*) based on characterization using GC-MS are ß-caryophyllene, 6,10,11,11-tetramethyl-tricyclo, α-guaiene,1H-3a,7-methanoazulene, δ-guaiene and patchouli alcohol. Inhibition zones of antifungal in patchouli oil and microencapsulation result toward *A. flavus* are categorized into insensitive excluding patchouli oil 100% which the inhibition zones included into a sensitive category. The performance for inhibition of patchouli oil is n = 0.5 with k' = 0,1030; for microencapsulation result is n= 0.7 with k' = 0.1144, causing plot A_t Vs t for both antifungal and it is obtained a chart that microencapsulation has a steeper curve than patchouli oil.

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REFERENCES

1. H.G. Ramya, V. Palanimuthu, S. Rachna, *Agricultural Engineering International: CIGR Journal,* **15**, 243 (2013).

2. H.S. Kusuma, M.E. Syahputra, D. Parasandi, A. Altway, M. Mahfud, *Rasayan Journal of Chemistry,* **10(3)**, 861 (2017), **DOI:**10.7324/RJC.2017.1031763
3. J. Gokulakrishnan, E. Kuppusamy, D. Shanmugam, A. Appavu, K. Kaliyamoorthi, *Asian Pacific Journal of Tropical Disease,* **3**, 26 (2013), **DOI:** 10.1016/S2222-1808(13)60006-7
4. M. Russo, F. Suraci, S. Postorino, D. Serra, A. Roccotelli, G.E. Agosteo, *Revista Brasileira de Farmacognosia,* **23**, 239 (2013), **DOI:**10.1590/S0102-695X2013005000017
5. I. Dammak, Z. Hamdi, S. Kammoun El Euch, H. Zemni, A. Mliki, M. Hassouna, S. Lasram, *Industrial Crops and Products,* **128**, 85 (2019), **DOI:**10.1016/j.indcrop.2018.11.006
6. P. An, X. Yang, J. Yu, J. Qi, X. Ren, Q. Kong, *Food Control,* **98**, 42 (2019), **DOI:**10.1016/j.foodcont.2018.11.013
7. I.S. Sehgal, H. Choudhary, S. Dhooria, A.N. Aggarwal, S. Bansal, M. Garg, D. Behera, A. Chakrabarti, R. Agarwal, *Medical Mycology Journal,* **57**, 270 (2018), **DOI:**10.1093/mmy/myy012
8. J.W. Bennett, M. Klich, *Clinical Microbiology Reviews,* **16**, 497 (2003), **DOI:**10.1128/CMR.16.3.497-516.2003
9. J. Mosquera, P.A. Warn, J. Morrissey, C.B. Moore, C. Gil-Lamaignere, D.W. Denning, *Antimicrobial Agents and Chemotherapy,* **45**, 1456 (2001), **DOI:**10.1128/AAC.45.5.1456-1462.2001
10. S.M. Rudramurthy, R.A. Paul, A. Chakrabarti, J.W. Mouton, J.F. Meis, *Journal of fungi Basel Switzerland,* **5**, (2019), **DOI:**10.3390/jof5030055
11. K.R.N. Reddy, S.B. Nurdijati, B. Salleh, *Asian Journal of Plant Science,* **9**, 126 (2010), **DOI:**10.3923/ajps.2010.126.133
12. M.K. Swamy, M.S. Akhtar, U.R. Sinniah, *Evidence-based Complementary and Alternative Medicine,* **2016**, 3012462 (2016), **DOI:**10.1155/2016/3012462
13. M.B. Karo, T. Tambaip, M. Hatta, T. Simanjuntak, L. Irmawaty, T. Rina, E. Kamelia, F. Rahmawati, M. Bintang, *Rasayan Journal of Chemistry,* **10**, 1280 (2017), **DOI:**10.7324/RJC.2017.1041887
14. C. Turek, F.C. Stintzing, *Comprehensive Reviews in Food Science and Food Safety,* **12**, 40 (2013), **DOI:**10.1111/1541-4337.12006
15. E.A. Soliman, A.Y. El-Moghazy, M.M. El-Din, M.A. Massoud, *Journal of Encapsulation and Adsorption Sciences*, **3**, 1 (2013), **DOI:**10.4236/jeas.2013.31006
16. Y.F. Wang, J.J. Shao, C.H. Zhou, D.L. Zhang, X.M. Bie, F.X. Lv, C. Zhang, Z.X. Lu, *Food Control,* **27**, 113 (2012), **DOI:**10.1016/j.foodcont.2012.03.008
17. A. Gharsallaoui, G. Roudaut, O. Chambin, A. Voilley, R. Saurel, *Food Research International,* **40**, 1107 (2007), **DOI:**10.1016/j.foodres.2007.07.004
18. D.A. Botrel, R.V. de Barros Fernandes, S.V. Borges, 2015, Microencapsulation of essential oils using spray drying technology in: (Eds.), Microencapsulation and microspheres for food applications, Elsevier, pp. 235-251.
19. W.H. Mohammed, W.K. Ali, M.J. Al-Awady, *Journal of Pharmaceutical Sciences and Research,* **10**, 11, (2018).
20. J.E. House, Principles of chemical kinetics, Academic Press, (2007).
21. A. Cornish-Bowden, Fundamentals of enzyme kinetics, John Wiley & Sons, (2013).
22. X. Yang, X. Zhang, S.-P. Yang, W.-Q. Liu, *Iranian Journal of Pharmaceutical Research,* **12**, 307 (2013), **DOI:**10.22037/IJPR.2013.1320
23. W.-R. Li, Q.-S. Shi, Q. Liang, X.-B. Xie, X.-M. Huang, Y.-B. Chen, *PLOS ONE,* **9**, e110983 (2014), **DOI:**10.1371/journal.pone.0110983
24. O. Tomazelli Júnior, F. Kuhn, P.J.M. Padilha, L.R.M. Vicente, S.W. Costa, A.A. Boligon, J. Scapinello, C.N. Nesi, J. Dal Magro, S.L. Castellví, *Brazilian Journal of Biology,* **78**, 311(2018), **DOI:**10.1590/1519-6984.08716
25. F.C.d. Silva, S.M. Chalfoun, V.M.d. Siqueira, D.M.d.S. Botelho, N. Lima, L.R. Batista, *Revista Brasileira de Farmacognosia,* **22**, 1002 (2012), **DOI:**10.1590/S0102-695X2012005000052
26. A.J. Babu, A.R. Sundari, J. Indumathi, R.V.N. Srujan, M. Sravanthi, *Veterinary World,* **4**, 311 (2011), **DOI:**10.5455/vetworld.4.311
27. N. Hussin, L. Mondello, R. Costa, P. Dugo, N.I.N. Yusoff, M.A. Yarmo, A.A. Wahab, M. Said, *Natural Product Communications,* **7**, 1934578X1200700732 (2012), **DOI:**10.1177/1934578X1200700732

28. R.A.R. Teodoro, R.V. de Barros Fernandes, D.A. Botrel, S.V. Borges, A.U. de Souza, *Food and Bioprocess Technology,* **7**, 2560 (2014), **DOI:**10.1007/s11947-014-1302-1
29. A.B.D. Nandiyanto, K. Okuyama, *Advanced Powder Technology,* **22**, 1 (2011), **DOI:**10.1016/j.apt.2010.09.011
30. V.I. Dybkov, Chemical Kinetics, IPMS Publications Kyiv, (2013).
31. R. Vila, B. Freixa, S. Cañigueral, *Recent Advances in Pharmaceutical Sciences,* **3**, (2013).
32. J.W. Moore, R.G. Pearson, Kinetics and mechanism, John Wiley & Sons, (1981).

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