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Effects of Different Drying Method on Bioactivity of Annona Reticulata Leaves Extract

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***Abstract*— *Anona reticulata* is a semi- evergreen tree with the common name of custard apple. Not much research have been carried out to unleashed the true potential of this plants. The aim of this study is to investigate the effects of different drying methods towards antioxidant, anti-inflammatory and anti-bacterial of *A. reticulata* leaves extract. 4 type of drying methods were selected namely air dry, freeze dry, oven dry 40°C and oven dry 60°C. Extraction process was carried out at 40°C, 20 ratio of solid to solvent (ml:g) and 4 hours extraction time. Antioxidant activity (AO), anti-inflammatory (AI) and anti-bacterial (AB) activity results showed there are no significant differences between freeze dry (AO = 50%, AI = 20% and AB = 15 mm [*B. subtilis*], AB = 23 mm [*E.coli*] and AB = 20 mm [*S. typhi*]) and air dried (AO = 52%, AI=22% and AB = 16 mm [*B.subtilis*], AB = 21 [*E.coli*], AB = 18 mm [*S. typhi*]) and due to economical factor, air dried was selected as the best drying method for *A. reticulata* leaves.**

Keywords*—A.reticulata, bioactivity, extraction, drying method, anti-bacteria

I. INTRODUCTION (*Heading 1*)

Natural remedies from medicinal plants are found to be safe and effective. Many plant species have been used to treat several ailments. Till date compounds from plants continue to play a major role in primary care as therapeutic remedies in many developing countries [1]. Secondary metabolites inside the plants that are being used for their defensive mechanism also has benefit to human due to its interesting biological activities and variety of structural arrangements and properties [2]. Human populations is still depends on traditional medicines for their disease treatments due to easy availability, economic and lesser side effects. Study on the substance constituents of the plants as well as their pharmacological test may offer the basis for developing lead compounds or novel agents.*Annonareticulata* Linn commonly called as bullock's heart or raamphal plant, is widely distributed all over India and are tall, with many branches, and bearing nutritious fruits [3]. The leaves are used as insecticides, anthelmintic and styptic [4]. For morphological, *A. reticulata* is low erect tree, semi- deciduous tree, 3- 7 m in height, with a circular or irregularly spreading branches, which is 22-35 cm thick and light brown bark with visible leaf scars and smoothish to slightly fissured into plates [5]. Most important phytochemicals in *A. reticulata* are alkaloids, tannins, flavonoid and phenolic compounds [6]. The information about effects of drying towards bioactivity of *A. reticulata* is rather scarce although drying is the primary process before extraction being carried out. Drying also has become a widely used way of food processing allowing the extension of shell life. Conventional air drying is the most frequently used because of low- cost drying method in food industry. However, significant quality changes of dried products may occur [7]. Oven drying able to dried up herbs more quickly than conventional air drying technique but have a greater chances to loss in flavour, colour and oils due to over dried [8]. Freeze drying is method of dehydration of frozen materials by sublimation under vacuum and it could produce the high quality dried foods. However, its major problem is the long drying time needed, which leads to high energy consumption and capital costs [7]. *A. reticulata*is known to have medical properties but no scientific research have been conducted to prove the anti-bacterial, anti-inflammatory and anti-oxidant activity on it. Thus, this study might provide a preliminary data on medical properties of *A. reticulata* which can be used in further commercialization of this plant. The aim of this study is to investigate the effect of different drying method towards bioactivity of leaves of *A. reticulata* and suggested the best drying method for drying purposes of *A. Reticulata* leaves.

II. MATERIALS AND METHOD

A. Materials

Fresh Leaves of *A. reticulata* were collected from Kota Bharu, Kelantan. Young leaves were separated and clean by using running tap water prior to different drying method process.

B. Drying procedure

Drying experiment of *A. reticulata* leaves was carried out by using three different method of air drying, oven drying and freeze drying. Air drying was done using room temperature (~20°C) with maintained good air circulation in the room. Air dry process completed after two weeks to achieve it consistent weight. Oven (Memmert, MB 450) drying is done by using two different temperatures, 40 and 60°C until consistent weight was achieved. Freeze drying process was conducted in -80°C by using lyophilisation machine (LabConco Free Zone, 12 Plus) for two days before going through freeze dry process for four days until consistent weight was achieved.

C. Extraction

Dried *A. reticulata* leaves from different drying method were ground by using a grinder. The extraction was carried out by using 40°C extraction temperature, 20 ratio of solvent to solid and 4 hours extraction duration. Petroleum ether was used as a solvent and the experiment for different type of drying were carried out in triplicate. For separation, the extracts of *A. reticulata* were subjected to rotary evaporator (Buchi R-3) to remove solvents from the extracts.

D. Antioxidant activity

The DPPH radical scavenging activity test of A. reticulata leaves extract was carried out according to a method described by [9]. Briefly, 300 µg/mL concentration of extract were added to 1.5 x 10^{-4} mL solution of methanolic DPPH solution and mix vigorously. Absorbance was determined at 520 nm using microplate reader (Tecan Infinite 200) and radical scavenging activity was calculated using formula as follows:

$$\%inhibition = \left[\frac{A_0 - A_1}{A_0}\right] \times 100 \qquad (1)$$

Where A_0 is the absorbance of DPPH solution and A_1 is the absorbance of the samples.

E. Anti- inflammatory activity

300 µg/mL concentration of extract were mixed with 1% aqueous solution of bovine albumin fraction. pH of reaction mixture was brought to 7 by using 1N HCl. The mixture were incubated at 37°C for 20 minutes and then heated at 57°C for 20 minutes. After cool down at room temperature, the turbidity of the samples was measured by using spectrophotometer (Thermo Scientific, Genesys 20) at 660 nm. The experiments conducted in triplicate. Percentage of inhibition of albumin denaturation was calculated as follows:

$$\%inhibition = \left[\frac{Abs_0 - Abs_1}{Abs_0}\right] \times 100 \qquad (2)$$

Where Abs_0 is the absorbance of control and Abs_1 is the absorbance for samples.

F. In-vitro Anti- bacterial activity

Disc diffusion test were conducted by using three types of bacteria consisting of one gram- positive bacteria namely Bacillus subtilis and two gram- negative bacteria namely Salmonella typhi and Escherchia coli. Each bacterial strain were grown and maintained on nutrient agar (NA) plate. Each strain was pre- cultured in nutrient broth (NB) at 37°C and 150 rpm prior using in the diffusion test. Standard drug or anti- biotic namely Kanamycin was put on the nutrient agar to identify the strength of anti- bacterial activity possessed by A. reticulata leaves. Briefly, 10 µg/ mL of Kanamycin was used as positive control and 10 µg/ mL of methanol solvent was used as negative control. The anti- bacterial plates were incubated at 37°C for 24 hours before measuring the inhibition zone. The experiment was conducted in triplicate.

III. RESULTS AND DISCUSSION

A. Antioxidant Activity

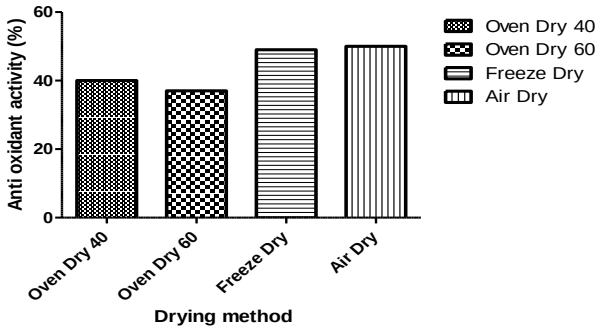

Figure 1: Inhibition by DPPH assay for different drying method of *A. reticulata* leaves extract

Figure 1 shows the percentage of inhibition for DPPH ranging from 40 – 50% inhibition for different type of drying methods of *A. reticulata* leaves extract. Statistical analysis shows that there are no significant difference ($p < 0.05$) between freeze dry method and air dried method. This is probably because of low temperature during drying process thus secondary metabolites for both methods is preserved. However, oven dry method (40°C and 60°C) yield low antixodant activity as both of them operated at high temperature and the secondary metabolite in *A. reticulata* leaves extract is denatured. According to this finding, air dry method is selected in antioxidant activity determination by considering economical factors. A study done by [7] stated that freeze drying method could produce high quality dried materials however due to its long drying time needed, which in turn leads to high energy consumption and capital cost, air drying is favourable to choose.

B. Anti- inflammatory activity

Figure 2 indicated that highest anti-inflammatory activity of albumin denaturation is by using air dry method which is 22%, follows by freeze dry (20%), oven dry 60°C (17%) and oven dry 40°C (10%). Oven dry shows low percentage of inhibition probably because of high temperature during drying process may denatured secondary metabolites presence in *A. reticulata* leaves extract. Statistical analysis by using SPSS V 17.0 shows that there are no significant difference between oven dry 60°C, freeze dry and air dry. However, by taking economical consideration and quality, air dry is selected as suitable drying method based on anti-inflammatory activity. Similar results was found by [8] where the suitable drying method in vegetables were air dry and freeze dry as compared to oven dry because of denaturation of secondary metabolites can occurred at high temperature of drying.

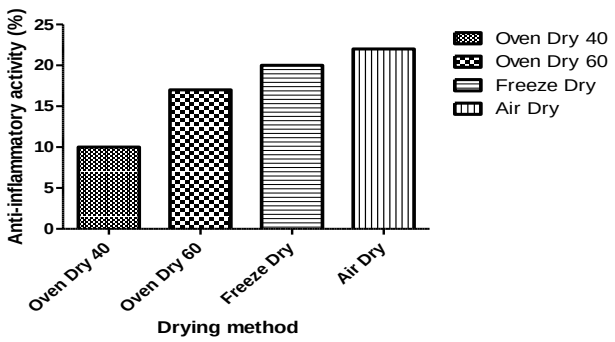

Figure 2 :Inhibition of albumin denaturation for different drying method of *A. reticulata* leaves extract

C. In-vitro antibacterial activity

Table 1 : Diameter of inhibition zone for different extracts by 3 types of bacteria

Bacteria	Diameter of inhibition zone(mm)				
	Air dry	Freeze dry	Oven dry 40°C	Oven dry 60°C	Kanamycin (positive control)
B. subtilis	16.0	15.0	-	-	17.5
E. coli	21.0	23.0	5.0	-	17.0
S. typhi	18.0	20.0	-	-	17.5

Table 1 shows the diameter of inhibition zone (mm) of *A. reticulate* leaves extract by using *B. subtilis, E. coli and S. typhi* for different drying method. The result shows that the largest inhibition zone was produced by freeze dry sample (23mm) is in *E.coli*. Kanamycin is used as positive control for this study which the diameter of inhibition zone is measured 17.5 mm for *B. subtilis* and *S. typhi* while 17.0 mm for *E. coli*. Statistical analysis shows there are no significant difference of diameter of inhibition for all types of bacteria to air dry and freeze dry method. This is probably because both of them are operated at low temperature condition. However, for oven dry 40°C and 60°C, no inhibition zone is reported probably because of denaturation of anti- bacterial property in *A. reticulata* leaves extract due to high temperature during drying process.

IV. CONCLUSION

From this study, it is concluded that compounds in *A. reticulata* is not a thermal stable compounds and cannot stand with high temperature of process. Four different type of drying method is used to determine the most suitable method for drying of *A. reticulata* leaves. The result indicated that freeze drying is the most suitable method since it operated at lower temperature and conserve all compounds and secondary metabolites in *A. reticulata* leaves extract. However, statistical data shows there are no significant difference ($p < 0.05$) between air dried method and freeze dry method for all tested sample and due to economical factors being considered, air dried was selected as the best drying method for *A. reticulata* in this study.

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REFERENCES

[1] V. Vanitha, K.J. Umadevi and K. Vijayalakshmi (2011). Determination of Bioactive Components of *Annona squamosal* L Leaf by CG-MS Analysis. International Journal of Pharmaceutical Sciences and Drug Research, 4: 309 – 312.

[2] A. Fatima, L.V. Modolo, L.S. Conegero, R.A. Pilli, C.V. Ferreira, L.K. Kohn and J.E. Carvalho (2006). Lactones and their derivatives : Biological activities, mechanisms of action and potential leads for drug design. Curr.Med. Chem. 13: 3371 – 3384.

[3] R. Rajini and M.R. Jothi Nisha, (2013). Potential bioactivity of *Annona reticulata* L. leaf extract and its compound identification by GCMS. International Journal of Science Innovation and Discoveries, 1: 202 – 211.

[4] H.M. Suresh, B. Shivakumar, K. Hemalatha, S. Heroor, D.S. Hugar and K.R.S. Sambasiva Rao (2011). In vitro anti- proliferativeactivity of *Annona reticulata* roots on human cancer cell lines. Pharmacognosy Research, 1: 9 – 12.

[5] S. D. Raj, J.J. Venila and Aiyavu (2009). The hepatoprotective effect of alcoholic extract of *Annona reticulata* leaves on on experimentally induced liver injury in swiss albino mice. International Journal of Intergrative Biology., 5(3), pp: 182-186.

[6] N. Krishdanev and A.D. Meleth (2010). Nutritional supplements for age-related macular degeneration. Current Opinion in Ophthalmology, 21 : 184 – 189.

[7] Liuping Fan, Jinwei Li, Kequan Deng and Lianzhong Ai (2012). Effects of drying methods on the antioxidant activities of polysaccharides extracted from *Ganoderma lucidum*. Carbohydrate Polymers, 87: 1849 – 1854.

[8] P. V. Hung and T. L. Duy (2012). Effects of drying methods on bioactive compounds of vegetables and correlation between bioactive compounds and their antioxidants. International Food Research Journal., 19: 327 – 332.

[9] E.W.C. Chan, Y.Y. Lim, S.K. Wong, K.K. Lim, S.P. Tan, F.S. Lianto and M.Y. Yong (2008). Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. Food Chemistry, 113: 166 – 172.