Original Research Article

Xanthine Oxidase Inhibitory, Antioxidant Activity and Total Phenolic Content of *Momordica charantia L.* Fruit Extracts Prepared using Two Water Temperatures

Nurshazwani Wajiha Zainal Rashid[1], Salfarina Ramli[1,2*]

[1]Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia.
[2]Department of Pharmaceutical Pharmacology and Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia.

Abstract

Momordica charantia L. fruit has good reputation for pharmacology and nutrition. Among the reported biological activities include antihyperlipidemic, blood glucose and serum uric acid reducing potentials. In most studies, *M. charantia* fruit extract was prepared in organic solvents. Nevertheless, water has been a good solvent to extract beneficial compounds with biological activities. Moreover, *M. charantia* fruit has also being consumed in the form of juice. In this study, the aqueous extracts of *M. charantia* fruit were prepared by blending the fruits in two different water temperature (25°C and 100°C). After the extracts were lyophilized, the xanthine oxidase inhibitory (XOI), antioxidant activity and total phenolic content of both extracts, along with allopurinol and ibuprofen were measured. From the results, both extracts exhibit XOI and antioxidant activity. The total phenolic content, XOI and antioxidant activity of the 25°C and 100°C extracts are not significantly different ($p>0.05$), which is in accordance with the negative correlation between total phenolic content and the activities of extracts. Interestingly, the XOI activity of both extracts are not significantly different from allopurinol ($p>0.05$), indicating the extracts effectiveness to inhibit xanthine oxidase enzyme. As a conclusion, it is postulated that the activities and total phenolic content of the *M. charantia* extracts are not affected by the water temperature.

Keywords: Allopurinol, xanthine oxidase inhibitory, antioxidant, *Momordica charantia L.*, fruit

Corresponding author
Salfarina Ramli
Level 11, FF1 Building, Faculty of Pharmacy,
UiTM Puncak Alam, Bandar Puncak Alam, 42300,
Selangor, Malaysia
salfarina2892@uitm.edu.my

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1.0 Introduction

Momordica charantia L., is a tropical plant used in Ayurvedic medicine. The plant belongs to the Cucurbitaceae family and is also known from its common names such as peria, bitter gourd, bitter melon, kugua, balsam pear or karela. It is mainly cultivated in the tropical and subtropical regions of the world (1). The leaves and the fruits of *M. charantia* have been traditionally used for constipation, relieving mild inflammation, respiratory and skin diseases (2). The antidiabetic, antioxidant, antiviral, anti-inflammation, anticancer, immunomodulatory, and anthelmintic activities of *M. charantia* were also reported (3,4).

Gout is an inflammatory disease that resulted from the formation of monosodium urate (MSU) crystals in joints and soft tissues. Apparently, MSU deposit takes time before an actual gouty attack occurs, as evidenced by tophi that grow silently for years. This condition begins with hyperuricemia, where the level of uric acid in the body is high due to overproduction or under-excretion of uric acid (5). The key enzyme for production of uric acid is xanthine oxidase (XO) enzyme that responsible to catalyze the oxidation of hypoxanthine to xanthine to uric acid (6). Allopurinol and nonsteroidal anti-inflammatory drugs (NSAID) such as ibuprofen are medications to stabilize uric acid levels and relieve acute pain and inflammation, respectively (7).

Nevertheless, patient with rare gout attacks have only mild elevated uric acid levels that are amendable by non-pharmacological management such reducing purine diet and alcohol intake (8). Interestingly, it has been widely reported that fruits and vegetables have phytochemicals that are able to inhibit XO enzyme. Therefore, the effectiveness of plant extracts to inhibit XO have been investigated (9), (10). For instance, the xanthine oxidase inhibitory (XOI) activity of *M. charantia* pulp and seed methanolic extracts was previously reported. Both extracts which contained active compounds including flavonoid, tannin, coumarins, and glycoside inhibited 96.5% and 45% of XOI activity, respectively (11,12).

Despite of the good results exhibited by *M. charantia* fruit prepared in organic solvent, water has been a good solvent to extract beneficial compounds with biological activities (13). Thus, the biological activities of aqueous versus organic extract are often being reported. In addition, drinking vegetable juices that are claimed to have health benefits including *M.charantia* fruit juice have been favored by the community. Frequently, homemade juice is prepared by blending the material with water until smooth before being strained. The liquid obtained is called juice. Temperature has effect in the extraction process as it liberates more phytochemical thus resulted in a better biological activity (14). Hence, it is implied that different temperature water to prepare juice may resulted in different activity. In this study, the *M. charantia* fruit aqueous extract were prepared using water at temperature of 25°C and 100°C. This method is similar to preparing a homemade juice. Next, the inhibition activity xanthine oxidase inhibitory, antioxidant activity and total phenolic content of both extracts were also investigated. Results from this study can be used to support the beneficial effect of *M. charantia* juice consumption.

2.0 Materials and methods

2.1 Identification of plant materials

The *M. charantia* fruits were purchased from a local supermarket in Puncak Alam, Selangor. The fruits were identified and authenticated by Dr. Salfarina Ramli from Faculty of Pharmacy, UiTM.

2.2 Chemicals

Allopurinol, xanthine and xanthine oxidase, methanol, phosphate buffer (pH 7.5), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid, ethanol, dimethyl sulfoxide (DMSO) 2,2-diphenyl-1-1picrylhydrazyl (DPPH), ethanol. All chemicals and reagents were purchased from Sigma-Aldrich, Malaysia distributor.

2.3 Preparation of plant extract

M. charantia fruits (500 g) were washed with distilled water and the seeds were removed. The fruits were sliced thinly before being blended in a blender with distilled water (25 ºC) or boiled distilled water (100 ºC) for 5 minutes until smooth. The mixture was filtered using Whatman No. 1 filter paper. The extraction was repeated for four times. The extracts were initially frozen at -80ºC for 3 days before being freeze-dried at -50ºC to remove the water.

2.4 Xanthine oxidase inhibitory assay

The xanthine oxidase inhibitory activity was measured spectrophotometrically at 290 nm in 96 well plates, with some modification (15). The extracts were initially dissolved in dimethyl sulfoxide (DMSO), before being dissolved in phosphate buffer (pH. 7.5). Final concentration of DMSO was 1%, which did not affect the enzyme assay. The activity of drugs; allopurinol and ibuprofen were also carried out. The assay mixture consisted of 100 µL extract or drug at 100 µg/ mL in phosphate buffer (pH 7.5) and 50 µL of 0.01 unit/mL of xanthine oxidase enzyme solution. The mixture was pre-incubated at 37ºC for 5 minutes. Then, 50 µL of 0.5 mM xanthine solution was added. The reaction mixture was incubated at 37 ºC for 10 minutes. A control reaction was prepared by replacing the extract with dimethyl sulfoxide (DMSO) in phosphate buffer (pH 7.5). The assay was run in triplicate. The XO inhibitory activity was calculated as follows:

Inhibition percentage (%) = (1-B/A) X 100

Where; A is the control reaction, B is the activity of an enzyme in the presence of extracts or drug.

2.5 Total phenolic content (TPC)

Quantification of the TPC extracts was determined by using Folin-Ciocalteu reagent with modifications (16). Briefly, 40 µL of extract in ethanol was pipette into 96 well microplate, followed by 50 µL of 15% Folin Ciocalteau. Distilled water was added to adjust the volume to 100 µL. The mixture was left for 5 minutes before the addition of 50 µL of Na2CO3 aqueous (0.105 g/mL). The absorbance of the extract was measured at 756 nm after incubation at 30 ºC for 30 minutes. All determinations were performed in triplicate. Different concentrations of gallic acid (0.03, 0.06, 0.12, 0.25, 0.5 and 1.0 mg/mL) were used to prepare a standard graph. The concentration of total phenolic compounds in all extract was expressed as mg of gallic acid equivalents per g dry weight of extract using a linear equation.

2.6 DPPH radical scavenging assay

The activity was determined by using 2,2-diphenyl-1-1picrylhydrazyl (DPPH) with modifications (16). The antioxidant activity of Allopurinol and ibuprofen were also carried out. A 160 µL of DPPH in ethanol (0.4 mM) was pipetted into 96 well microplate, followed by 40 µL of extract prepared in ethanol. The reaction was incubated at room temperature for 30 minutes and absorbance was measured at 517 nm using microplate reader. All extracts were analyzed in triplicate. The control was a reaction mixture with ethanol substituting the extract. The DPPH radical scavenging activity was calculated by the following equation:

Radical scavenging activity (%) = (Absorbance $_{control}$ − Absorbance $_{sample}$)/ Absorbance $_{control}$ x 100

2.7 Statistical analysis

The experimental measurements for determination of xanthine oxidase inhibitory activity, antioxidant and total phenolic content were carried out in triplicate. The results were expressed as the mean ± standard deviation (SD). Differences between extracts and drugs (allopurinol and ibuprofen) were analyzed by one-way ANOVA followed by post-hoc Tukey's multiple comparison test. Next, results obtained from all assays were subjected to correlation analysis. The statistical program SPSS version 25.0 was used.

3.0 Results and Discussion

3.1 Extract yield

Table 1 shows the yield of extract obtained for both *M. charantia* fruit aqueous extracts. It was found that blending the *M. charantia* fruit with boiled water (100 °C) result in 2.03 gram higher of extract yield than 25 °C water. The temperature of the water used gave difference to the extraction yield.

Table 1: The extract yields of *M. charantia L.* fruit

Extract	Total yields (g)
25 °C	20.58
100 °C	22.03

3.2 Xanthine oxidase inhibitory activity

Spectrophotometric determination of XOI activity is based on measuring uric acid production from xanthine or hypoxanthine substrate at 295 nm. The assay mixture contained extract and xanthine as a substrate. Reaction is initiated by adding the xanthine oxidase enzyme. Higher absorbance values indicate a higher uric production. Frequently, the inhibitory activities of plant extracts and their constituents are compared with the activity of allopurinol as standard (17). The XOI activity of XOI activity of both extracts and drugs at concentration of 100 µg/ml in 0.5 mM of xanthine and 0.01 unit xanthine oxidase enzyme are shown in Figure 1.

Overall, the XOI activity of both extracts and drugs are between 3.6-9.7%. It was unlooked for that ibuprofen which is a cyclooxygenase (COX) inhibitor, exhibits the highest XOI activity. The 25°C and 100°C extracts demonstrate lower inhibitory activities compared to those drugs (Figure 1). The activity of 25 °C extract is significantly different ($p \leq 0.05$) compared to allopurinol and ibuprofen, but were not significantly different with 100°C extract ($p > 0.05$). Interestingly, the activity of 100°C extract is not significantly different from allopurinol ($p > 0.05$). Allopurinol is a strong competitive inhibitor for XO, so this result is inferred as the effectiveness of 100°C *M. charantia* extract as XO inhibitor.

3.3 DPPH radical scavenging activity

The antioxidant activity of the both *25*°C, 100°C *M. charantia* extracts, allopurinol and ibuprofen were determined by its ability to scavenge DPPH free radical. In this assay, the antioxidant activity of extracts is evaluated by its ability to donate electron or hydrogen to the DPPH· radical. The antioxidant activity of extracts and drugs (400 µg/mL) are shown in Figure 2. At the concentration of 400 µg/mL, the activity of extracts is less than 20% (Figure 2), allopurinol demonstrated the highest percentage of antioxidant activity followed by ibuprofen. Among the extracts, the 25°C extract has slightly higher antioxidant activity than 100°C extract. But the scavenging activity of both extracts is not significantly different ($p > 0.05$).

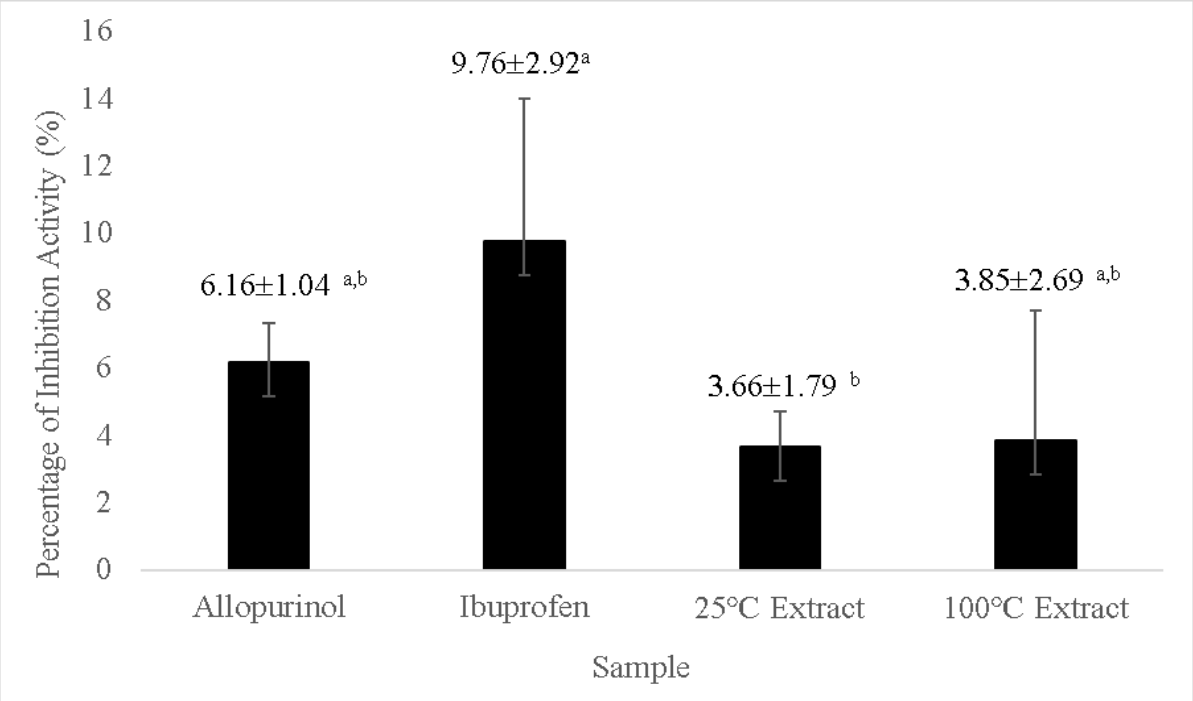

Figure 1: XOI activity of allopurinol, ibuprofen, *M. charantia* extracts (100 µg/mL). The value was expressed as mean ± SD, n=3. Different letter on the graph obtained by ANOVA indicates significantly difference ($p<0.05$).

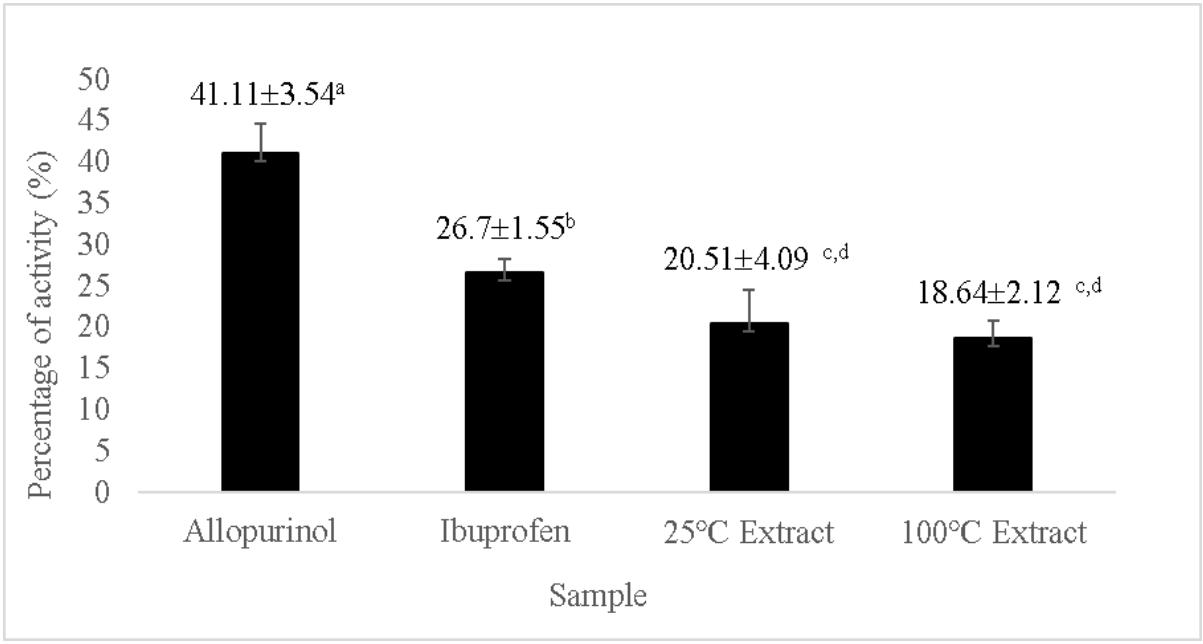

Figure 2: Antioxidant activity of allopurinol, ibuprofen and *M. charantia* extracts. The value was expressed as mean ± SD, n=3. Different letters obtained by ANOVA indicates significant differences ($p<0.05$).

3.4 Total phenolic content (TPC)

Plant extracts are known to contain phenolic compounds. However, the content of phenolic compound in an extract can be affected by the method of extraction. Therefore, the TPC of *M. charantia* fruit extracts were compared in Table 2. Total phenolic content was expressed as mg gallic acid equivalent / g extract. The 25°C extract exhibit the higher total phenolic content than 100°C extract. However, TPC of both extracts are not significantly difference ($p>0.05$).

Table 2: TPC and anti-antioxidant activity of *M. charantia* extract

M. charantia fruit	Total phenolic content (mg GAE/g)
25°C extract	0.42 ± 1.32^{a}
100°C extract	0.35 ± 1.13^{a}

Result was expressed as mean ± SD, n=3. Superscript letter indicates TPC of both extracts are not significantly difference ($p>0.05$).

3.5 Correlation between total phenolic content (TPC), antioxidant activity, and XOI activity

In order to establish a correlation between the TPC and biological activities of *M. charantia*, results obtained from all assays were subjected to a statistical analysis. The results are presented in Table 3.

Generally, the relationship between two variables is considered strong when their r value is larger than 0.7. Based on the r value (Table 3), this correlation indicates that there is a relation between TPC and antioxidant activity. However, the antioxidant activity of extract is not related with XOI activity, whereas a strong negative correlation indicates that concentration of TPC is not related with XOI and antioxidant activity of *M. charantia* extract.

Table 3: Correlation between TPC, antioxidant activity, and xanthine oxidase inhibitory activity

Assay	R^2	r
Between total phenolic content and antioxidant activity	1	1
Between antioxidant activity and xanthine oxidase inhibition	0.15	0.39
Between total phenolic content (TPC), antioxidant activity, and xanthine oxidase inhibition	1	-1

The R^2 value denotes the regression value and the r value denotes Pearson's correlation value.

Generally, the relationship between two variables is considered strong when their r value is larger than 0.7. Based on the r value (Table 3), this correlation indicates that there is a relation between TPC and antioxidant activity. However, the antioxidant activity of extract is not related with XOI activity, whereas a strong negative correlation indicates that concentration of TPC is not related with XOI and antioxidant activity of *M. charantia* extract.

Results from all assays in this study indicated the antioxidant, XOI activity and TPC of both *M. charantia* extracts. However, the temperature of water used in the preparation of extract have no effect on the activities and TPC of *M. charantia* extracts. It is well established that the effect of extraction process and its parameters are reflected in the extract yield, extract content and the biological activity of the extract. The effect of solvent polarity, temperature been widely discussed (18). Apparently, time is also an important factor that needs to be considered (19). For instance, a study reported that the antioxidant activity of *M. charantia* after being boiled for 5 minutes exhibited higher activity (51.62%) compared to non-boiled extract (13.22%) (20). Whereas, another study reported that *M. charantia* demonstrated the highest value of

TPC when boiled and microwaved for up to 10 minutes (21). The TPC values from studies (20) and (21) were higher compared to TPC values obtained in this study which was reported as approximately 0.88 and 4 mg GAE/g sample respectively.

Therefore, from this study it is apparent that method employed in the extraction resulted in no difference in TPC, antioxidant and XOI assay. Although different water temperature was used, it is likely that the time of extraction was insufficient to allow difference in the extract hence it is reflected in the activity and total phenolic content. Thus, a juice of the same *M. charantia* fruit can be prepared in water either 100 or 25 °C. Future study will be emphasizing on the time spend to extract the plant material in the temperature. In addition, this study evaluated the inhibitory activity of extracts and drugs at 100 µg/ml only, therefore future study will be carried out to investigate the dose dependent effect of *M. charantia* extract as an XO inhibitor.

5.0 Conclusion

Both *M. charantia* aqueous extracts exhibited XOI and antioxidant activity. Interestingly, the XOI activity of both extracts were not significantly different from allopurinol ($p>0.05$) indicating their effectiveness as xanthine oxidase enzyme inhibitor. Moreover, the XOI, antioxidant activity and total phenolic content of both *M. charantia L.* extracts were not significantly different, which in accordance with the negative correlation between TPC and extracts activities. Therefore, it can be postulated that the two temperatures used do not cause the activities and total phenolic content of the two extracts to differ significantly.

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Conflict of interest

Authors declare no conflict of interest in the present work.

References

1. Kulip J. An ethnobotanical survey of medicinal and other useful plants of Muruts in Sabah, Malaysia. Telopea. 2003;10(1):81–91.
2. Joseph B, Jini D. Antidiabetic effects of Momordica charantia (bitter melon) and its medicinal potency. Asian Pac J Trop Dis. 2013;3(2):93–102.
3. Ezuruike UF, Prieto JM. The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. J Ethnopharmacol. 2014;155(2):857–924.
4. Jia S, Shen M, Zhang F, Xie J. Recent advances in Momordica charantia: Functional components and biological activities. Int J Mol Sci. 2017;18(12):2555.
5. Becker MA, Chohan S. We can make gout management more successful now. Curr Opin Rheumatol. 2008;20(2):167–72.
6. Grassi D, Ferri L, Desideri G, Di Giosia P, Cheli P, Del Pinto R, et al. Chronic Hyperuricemia, Uric Acid Deposit and Cardiovascular Risk. Curr Pharm Des. 2013;19(13):2432–8.
7. Klinenberg JR, Goldfinger SE, Seegmiller JE. The Effectiveness of the Xanthine Oxidase Inhibitor Allopurinol in the Treatment of Gout. Ann Intern Med. 2016;62(4):639–47.
8. Mikuls TR, Saag KG. New insights into gout

epidemiology. Curr Opin Rheumatol. 2006;18(2):199–203.

9. Unno T, Sugimoto A, Kakuda T. Xanthine oxidase inhibitors from the leaves of Lagerstroemia speciosa (L.) Pers. J Ethnopharmacol. 2004;93(2–3):391–5.
10. Boumerfeg S, Baghiani A, Messaoudi D, Khennouf S, Arrar L. Antioxidant Properties and Xanthine Oxidase Inhibitory Effects of Tamus communis L. Root Extracts. Phytother. 2009;23:283–8.
11. Alsultanee IR, Ewadh MJ, Mohammed MF. Novel Natural Anti Gout Medication Extract from Momordica charantia. J Nat Sci Res. 2014;4(17):16–24.
12. Kong LD, Cai Y, Huang WW, Cheng CHK, Tan RX. Inhibition of xanthine oxidase by some Chinese medicinal plants used to treat gout. J Ethnopharmacol. 2000;73(1–2):199–207.
13. Tambunan AP, Bahtiar A, Tjandrawinata RR. Influence of extraction parameters on the yield, phytochemical, TLC-densitometric quantification of quercetin, and LC-MS profile, and how to standardize different batches for long term from Ageratum conyoides L. leaves. Pharmacogn J. 2014;9(6):767–74.
14. Access O. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength, and Limitation. Med Aromat Plants. 2015;04(03).
15. El-Rahman H. Xanthine Oxidase Inhibitory Activity and Antigout of Celery Leek Parsley and Molokhia. Adv Biochem. 2015;3(4):40.
16. Ramli S, Harada K, Ruangrungsi N. Antioxidant, Antimicrobial and Cytotoxicity Activities of Acacia farnesiana (L.) Willd. Leaves Ethanolic Extract. Pharmacogn J. 2011;3(23):50–8.
17. Abdullahi A, Hamzah R, Jigam A, Yahya A, Kabiru A, Muhammad H, et al. Inhibitory activity of xanthine oxidase by fractions Crateva adansonii. J Acute Dis. 2013;1(2):126–9.
18. Azwanida NN. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength, and Limitation. Med Arom Plants. 2015;04(196): 2167-0412.
19. Yeo YL, Chia YY, Lee CH, Sow HS, Yap WS. Effectiveness of maceration periods with different extraction solvents on in-vitro antimicrobial activity from fruit of Momordica charantia L. J Appl Pharm Sci. 2014;4(10):16–23.
20. Subramaniam S, Rosdi MHB, Kuppusamy UR. Customized Cooking Methods Enhance Antioxidant, Antiglycemic, and Insulin-Like Properties of Momordica charantia and Moringa oleifera. J Food Qual. 2017;2017:1–9.
21. Kubola J, Siriamornpun S. Phenolic contents and antioxidant activities of bitter gourd (Momordica charantia L.) leaf, stem and fruit fraction extracts in vitro. Food Chem. 2008;110(4):881–90.