

PHYTOCHEMICAL ANALYSIS AND IMMUNO-MODULATORY EFFECT OF *MORINGA OLEIFERA* FLOWERS

VENKATASTHYA SAI APPALA RAJU VELAGA^a, NAGARAJA SURYADEVARAB^{*}, LIM LI CHEE^a,
NAHLAH ELKUDSSIAH ISMAIL^a

^aFaculty of Pharmacy, MAHSA University, Malaysia, ^bDepartment of Biomedical Sciences, Faculty of Medicine, MAHSA University, Malaysia
Email: nagaraja@mahsa.edu.my

Received: 22 Nov 2016 Revised and Accepted: 20 Apr 2017

ABSTRACT

Objective: This study evaluates the anti-inflammatory activities of different solvent extracts of *Moringa oleifera* flowers using carrageenan-induced mice paw edema.

Methods: Soxhlet extraction method was employed in this study to extract the crude phytochemicals. Phytochemical analysis testing of *Moringa oleifera* extracts was performed to identify the presence of various phytoconstituents based on the standard procedures. The anti-inflammatory activity was evaluated using acute inflammatory model carrageenan-induced paw edema. Three different flower extracts (petroleum ether, chloroform and methanol) of *Moringa oleifera* at the dose level of 500 mg/kg body weight. The anti-inflammatory activity on the different extracts of *Moringa oleifera* was determined through the α -carrageenan induced left hind paw oedema method in albino mice. 0.05 ml of 1% w/v carrageenan suspension was reconstituted with normal saline (0.9% NaCl) to give a homogenous solution which then be injected into the subplantar tissue of the left hind paw of each mice to induce acute inflammation.

Results: Treatment with three different solvent extracts showed significant ($p < 0.05$) inhibition in carrageenan-induced paw edema. Petroleum ether and chloroform extracts were found to be less effective than methanolic extracts when compared to (Indomethacin) reference standard at the dose of 10 mg/kg body weight. The phytochemical results obtained indicates that anti-edematous action of *Moringa oleifera* flowers exhibited in this study is due to the presence of potent anti-inflammatory phytoconstituents (flavonoid, alkaloid, tannin) in impeding arachidonic acid metabolism and production of reactive free radicals. A significant ($p < 0.05$) increase of left hind paw thickness after the drug injection was noticed in the negative control mice group as time persisted. It showed the highest paw thickness at the fifth hour with $4.72 \text{ mm} \pm 0.07$. Whereas the indomethacin treated group showed the highest percent oedema inhibition amongst all experimental group with 38.60% at the fifth-hour post-carrageenan induction. It exhibited a significant inhibition of 29.02% against the oedema after the third hour of carrageenan injection.

Conclusion: The methanolic extract of *Moringa oleifera* flowers extract has anti-inflammatory activity. This activity was related to the dose and these results collaborate the potential traditional use of the plant in folk medicine.

Keywords: *Moringa oleifera*, Soxhlet extraction, Carrageenan-induced paw edema model, Paw thickness, Percentage of inhibition

© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)
DOI: <http://dx.doi.org/10.22159/ijpps.2017v9i6.16285>

INTRODUCTION

Moringa oleifera Lamarck (syn. *Moringa pterygosperma* Gaertner.), one of the most underutilized species in the genus *Moringa* [1] belongs to the monogeneric family *Moringaceae*, is a deciduous monoecious soft wood plant. It is commonly referred by regional names as drumstick tree, horseradish tree, 'La-mu', 'kelor', 'sajiwani', 'murungai' and 'sajhan' [2], among which it is best dubbed as the miracle tree [3] or natural gift or the mother's best friend [4].

Moringa oleifera is a short, slender and perennial evergreen tree that grows up to 10 to 12 m, but occasionally attains heights up to 15 m in cultivation [5]. It prefers well-drained sandy or loamy soil with a slightly acid pH of 6.2 to neutral 7.0, but able to tolerate in clay soil in the wide range of pH 5 to 9.

Flowers: The inflorescence is borne on slender, hairy stalks in spreading auxiliary panicles 10-25 cm long [6]. Flower buds are in ovoid shape. Flowers are yellowish-white, lightly fragrant and about 2.5 cm in diameter, with five unequal thinly veined, spatulate petals, five stamens with five smaller staminodes, and a pistil composed of a one-celled ovary and slender style. Apart from the ethnomedicinal benefits, *Moringa oleifera* has served as a crucial daily diet since ancient times in various cultures. In India, people use immature pods, fresh leaves and flowers for culinary purposes [6].

According to Fuglie [7], *Moringa oleifera* has been highly valued by the Romans, Greeks and Egyptians for thousands of years for its nutraceutical properties. Inflammation is a consecutive and intricate

pathophysiological process alerting organisms in the host via protective and defensive responses. By recruiting inflammatory cells to the site of damage, a series of cellular signalling mediators such as proinflammatory cytokines, interleukins IL-1, IL-6, IL-12, IL-18, tumour necrosis factor (TNF), interferon (IFN)- γ and the granulocyte-macrophage-colony-stimulating factors (GM-CSF) are secreted [8] with the goal of promoting tissue breakdown and strengthening host defence in response to invading infection of various microbial pathogens and noxious chemical or physical insults. Among these, nuclear factor-kappa B (NF- κ B), a transcription factor, plays a vital role in the inflammatory response by regulating the expression of various genes encoding pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes [9], of which both inducible cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), constitutively trigger the production of large quantities of pro-inflammatory mediators [10]. The anti-inflammatory study of the flowers has not been screened before and therefore the authors had selected to conduct this research on the experimental animals.

MATERIALS AND METHODS

Procurement of plant materials

The fresh flowers of *Moringa oleifera* Lam. were collected at an oil palm estate at the outskirts of Klang, Johor, Malaysia, between the month of August and October. The taxonomic features of herbarium were authenticated by taxonomist at forest research institute of

Malaysia (FRIM) located in Kepong, Selangor, Malaysia. The voucher specimen was deposited in the lab of herbarium unit in FRIM with a number of PID 010116-01. The flowers were air dried under shade and coarsely powdered via grinder. The powdered materials were kept in well-closed containers and protected from direct sunlight for the extraction process.

Experimental animals

Animal ethics approval was granted by the pharmacy research ethics committee of MAHSA University (PREC/MU/0216), prior to the execution of the study. Both sexes of healthy Swiss albino mice weighing 25-30 g were purchased from the animal supplier (Chenur supplier 001801135-A, 4300 Kajang, Malaysia). The animals, acclimatized for one week were housed in standard cages and left free to access rodent diet and water *ad libitum*, at a constant temperature of 22±1 °C, relative humidity 65±5% with 12 h light-dark cycle. Animals used in this study were kept and cared in accordance with the NIH guide for the care and use of laboratory animals. All animals were fasted for at least 12 h prior to experimentation.

Chemicals and reagents

Organic solvents used for extraction of the plant material included methanol, chloroform and petroleum ether (RandM Chemicals, U. K.). The chemicals and reagents used were Hager's reagent, Mayer's reagent, Benedict's reagent, Biuret reagent, Barfoed's reagent, Sudan Red III reagent, acetyl chloride, acetic anhydride, concentrated H₂SO₄, zinc chloride, glycerine, sodium carbonate, 5% ferric chloride solution, calcium chloride, concentrated HCl, Folin and Ciocalteu's phenol reagent, Indomethacin, α-carrageenan (Sigma-Aldrich, USA), olive oil, polysorbate 80 (SRL, India) and sterile normal saline solution were used in animal testing. All the solvents were of analytical grade.

Preparation of plant extracts

Approximately 260 g coarse powder of the air-dried flowers was subjected to successive solvent extraction method using solvents of

increasing polarity from petroleum ether, chloroform and followed by methanol in a Soxhlet extraction unit. The plant material was suspended in the main chamber of Soxhlet extractor which was then placed onto a flask containing the extraction solvent. The flask was heated by the heating mantle and the solvent evaporated into the condenser and moved down the extraction chamber containing the sample. The Soxhlet extraction process was continuously run for more than 18 h. Subsequently, each extract was filtered at the end of the hot extraction process to remove impurities. The succeeding drying process of petroleum ether, chloroform, and methanol filtrate was conducted using rotary evaporator at 60 °C (699 mbar), 62 °C (500 mbar) and 65 °C (360 mbar), respectively. The concentrated filtrate yield was collected and kept in a desiccator containing calcium chloride to remove the moisture. The yield of each extracts was collected in airtight containers and stored at 4 °C to prevent the growth of microorganisms.

Preliminary phytochemical analysis of the extracts

Phytochemical analysis of *Moringa oleifera* extracts was performed to identify the presence of various phytoconstituents alkaloids, phytosterols, triterpenes, tannins, phenolic compounds, carbohydrates, proteins and free amino acids, fats and oils, flavonoids and saponin glycosides [11].

Preliminary identification of phytochemical compounds was subjected to the following qualitative tests for the identification of various active constituents which exert pharmacological effects.

Effect of extracts of *Moringa oleifera* on inflamed mice paw

Twenty-five healthy Swiss albino mice were selected for the *in vivo* study. All mice were equally divided and kept in five separate cages during habituation period. Prior to the study, each mice from each cage was randomly assigned to a handled control group, a standard reference group and three flower extract groups, with a total of five mice in each group as stated in table 1. Three different solvent extracts were selected to proceed to this study due to their reported potent anti-inflammatory phytochemicals in the previous study. It is a single dose study.

Table 1: Grouping in different experimental designs

Groups (n=5)	Experimental designs groups
1	Negative control receiving normal saline
2	Animal treated with petroleum ether extract
3	Animal treated with chloroform extract
4	Animal treated with methanolic extract
5	Animal treated with Standard reference (Indomethacin)

The extracts given were freshly prepared based on the weight of mice. Before oral administration, petroleum ether and chloroform extracts were suspended in 10 ml of a 4:2:1 emulsion of olive oil:

distilled water: acacia gum, while methanol extract was dissolved in 10 ml of distilled water, given that 1 ml containing 0.125 mg dose. Dosage description is given in table 2.

Table 2: Dosage description of experimental designs

Groups	Description
1.	Normal mice under treatment of carrageenan+10 ml/kg b. w normal saline
2.	Inflamed paw mice under treatment of carrageenan+500 mg/kg b.w. petroleum ether extract orally
3.	Inflamed paw mice under treatment of carrageenan+500 mg/kg b.w. chloroform extract orally
4.	Inflamed paw mice under treatment of carrageenan+500 mg/kg b.w. methanolic extract orally
5.	Inflamed paw mice under treatment of carrageenan+10 mg/kg b.w. Indomethacin (standard reference) orally

Inductions of hind paw edema on experimental mice by α-carrageenan

The anti-inflammatory activity on the different extracts of *Moringa oleifera* was determined through the α-carrageenan induced left hind paw edema method in albino mice. 0.05 ml of 1% w/v carrageenan suspension was reconstituted with normal saline (0.9% NaCl) to give a homogenous solution which then be injected into the sub plantar tissue of the left hind paw of each mice to induce acute

inflammation. Paw thickness was measured in mm of the dorsa-plantar axis at the metatarsal level before injecting carrageenan and every h post-carrageenan injection for 5 h using a digital caliper. An h prior to edema induction, Indomethacin is used as the reference anti-inflammatory agent and it was suspended in 4% Tween 80 and administered at a dose of 10 mg/kg by oral gavage, and each test extracts were orally administered at 500 mg/kg body weight [12]. The negative control group was given an equal volume of carrageenan suspension in saline solution. The degree of swelling

was evaluated by the delta volume (a-b), where (a) and (b) are the mean volume of the left hind paw after and before the carrageenan treatment, respectively [13].

The anti-inflammatory activity was calculated as percentage inhibition of edema in the mice treated with reference drug and extract under test in comparison to the negative control group. The percentage (%) of edema inhibition was calculated using the formula:

$$\% \text{ inhibition} = (T_c - T_t) / T_c \times 100$$

Where: T_c is the paw thickness of mice of the control group and T_t is the paw thickness of mice given drug or test extract at the corresponding time.

Oral gavage administration in mice

Gavaging is a common method procedure in scientific experiments using laboratory animals and typically is achieved in conscious animals by using intragastric gavage technique. The dose volume for gavage administration in an animal should not exceed 10 ml/kg and metal gavage needle was recommended to be used in mice since it is not easily been bitten [14].

Intraplantar injection

Experimentally induced paw edema was assessed by intraplantar injection which was the combination of an intradermal and subcutaneous injection. In this procedure, drug-induced inflammation was performed at the ventral hind paw in accordance with the guideline stated by IACUC. The paw thickness of the mice was measured by using digital Vernier calipers, before and after injection of extracts.

Statistical analysis method

All numerical results were expressed as mean±standard deviation (SD) and analyzed using statistical package for the social sciences (SPSS) IBM SPSS Statistic. One-way analysis of variance (ANOVA) was conducted for statistical comparison of study whereby p<0.05 being the criterion for statistical significance. The significant treatment means were further subjected to Tukey's post hoc test.

RESULTS

Soxhlet extraction of *Moringa oleifera* flowers

In this study, the three solvents employed in the extraction of *Moringa oleifera* flowers were petroleum ether, chloroform and methanol ranging from low polarity to high polarity respectively. The percentage yield of extracts obtained is methanolic extract 19.95% w/w, petroleum ether extract 4.35% w/w and chloroform extract 3.17% w/w.

Preliminary phytochemical analysis of the extracts

The results of qualitative analysis depicted that flower extracts of *Moringa oleifera* as shown in table 3, contains potent anti-inflammatory phytoconstituents including alkaloids, phytosterols, triterpenes, phenolic compounds, tannins, flavonoids, carbohydrates, proteins, oil fats and saponin glycosides. The methanolic extract showed the presence of all phytochemical constituents in abundance except for saponin glycoside. Chloroform extract demonstrated higher concentration of phytochemical content compared to petroleum ether extract but both crude extracts were devoid of tannins and proteins. However, chloroform extract was the only solvent amongst all showing positive result in saponin glycoside.

Table 3: Phytochemical analysis of different extracts of *Moringa oleifera*

Constituent of detection	Types of test	Results		
		Petroleum ether	Chloroform	Methanol
Alkaloids	Mayer's reagent	+	++	++
	Hager's reagent	+	++	++
Phytosterol	Salkowski reaction	+	++	+++
	Liebermann-Buchard reaction	+	++	+++
	Liebermann's reaction	+	++	+++
Triterpenes	Salkowski reaction	+	++	+++
	Liebermann-Buchard reaction	+	++	+++
	Tschugajen test	+	++	++
Phenolic compounds	Folin Ciocalteu Micro test	+	+	++
Tannins	5% Ferric Chloride solution test	-	-	+
Carbohydrates	Molisch test	+	+	++
	Barfoed's test (monosaccharides)	-	-	++
	Benedict's test (reducing sugar)	-	-	+
Proteins and free amino acids	Biuret test	-	-	+
Fats and oils	Sudan red III test	Red globules present	Red globules present	Red globules present
	Solubility test	insoluble	insoluble	soluble
Flavonoids	Alkaline reagent test	-	-	+
Glycosides	Foam test	-	+	-

Note:- not detected, +: present in low concentration, ++: present in moderate concentration, +++: present in high concentration

Initial paw thickness of normal albino mice

The initial paw thickness of each group had been analyzed as shown in table 4 where there is no significant difference in the

initial paw thickness between the mice in control group and experimental group. This procedure is to further prove on all albino mice has no major difference among the groups in the paw thickness initially.

Table 4: Initial paw thickness of different experimental groups on normal albino mice

Group no	Description	Initial paw thickness (mm)
1	Negative control	1.78±0.08
2	Petroleum ether extract	1.82±0.12
3	Chloroform extract	1.72±0.04
4	Methanol extract	1.73±0.04
5	Indomethacin (standard)	1.76±0.11

Note: Data represented as mean±SD, n=5, *All mean values are not a significant difference; p>0.05.

Evaluation of different extracts of *Moringa oleifera* flower on mice paw edema induced by carrageenan

As shown in table 5, the mean paw thickness and edema inhibition percentage were reported comparable to that of the negative control. Experimental results showed that three different solvent extracts of the flower were found to possess a prominent anti-inflammatory action, shown significantly by suppressed hind paw edema in mice induced by intra plantar injection of carrageenan

compared to the control group. A significant ($p < 0.05$) increase of left hind paw thickness after the drug injection was noticed in the negative control mice group as time increases (table 5). It showed the highest paw thickness at the fifth h with $4.72 \text{ mm} \pm 0.07$. Whereas the Indomethacin treated group showed the highest percentage of edema inhibition amongst all experimental groups with 38.60% at the fifth h post-carrageenan induction. It exhibited a significant inhibition of 29.02% against the edema after the third h of carrageenan injection.

Table 5: Effects of different extracts of *Moringa oleifera* and Indomethacin on mice paws' thickness and edema inhibition percentage at studied time intervals

Group	Parameter	Time Interval				
		1 st h	2 nd h	3 rd h	4 th h	5 th h
1	thick.±SD	3.30±0.07	4.29±0.07	4.68±0.06	4.71±0.06	4.72±0.07
	Inh. %	0.00% c,d,e	0.00% b,c,d,e	0.00% b,c,d,e	0.00% b,c,d,e	0.00% b,c,d,e
2	thick.±SD	3.19±0.04	3.69±0.15	4.19±0.06	4.46±0.10	4.37±0.09
	Inh. %	3.27% c,d,e	14.08% a,d,e	10.55% a,c,d,e	5.22% a,c,d,e	7.33% a,c,d,e
3	thick.±SD	2.84±0.07	3.48±0.07	3.90±0.08	4.00±0.08	3.82±0.09
	Inh. %	14.00% a,b,d,e	18.93% a,d,e	16.71% a,b,d,e	15.03% a,b,d,e	19.15% a,b,d,e
4	thick.±SD	2.66±0.04	3.22±0.14	3.35±0.10	3.37±0.07	2.95±0.05
	Inh. %	19.33% a,b,c,e	24.99% a,b,c	28.33% a,b,c	28.49% a,b,c	37.59% a,b,c
5	thick.±SD	2.43±0.09	3.14±0.19	3.32±0.16	3.22±0.17	2.89±0.17
	Inh. %	26.48% a,b,c,d	26.71% a,b,c	29.02% a,b,c	31.55% a,b,c	38.60% a,b,c

Note: thick. = swelling thickness (mm); SD= standard deviation; Inh. = inhibition, Differences between inhibition percentage were analyzed using ANOVA by Tukey's HSD and regarded as significant at $p < 0.05$, n= 5, ^aSignificant when compared to Group 1, negative control group; $p < 0.05$, ^bSignificant when compared to Group 2, petroleum ether extract group; $p < 0.05$, ^cSignificant when compared to Group 3, chloroform extract group; $p < 0.05$, ^dSignificant when compared to Group 4, methanol extract group; $p < 0.05$, ^eSignificant when compared to Group 5, indomethacin (reference) group; $p < 0.05$

The anti-inflammatory activity displayed by the methanol extract, compared with the negative control group was greater than petroleum ether and chloroform extract, although both extracts have demonstrated anti-inflammatory activity. The peak percent inhibition of 37.59% was developed at the fifth h of methanolic extracts. Meanwhile, chloroform extract and petroleum ether extract showed 19.15% and 7.33% inhibition respectively at the same time interval. Both chloroform and petroleum ether extract showed a significant difference in the percentage of inhibition of edema with methanolic extract throughout the study.

Methanolic extract displayed edema inhibition of 19.33% to 37.59% where the inhibitory effect of the methanolic flower

extract (24.99%) was closely related to that of Indomethacin (26.71%) after the second h of edema induction. The data between both groups showed no significant difference ($p > 0.05$) after the second h. In this regard, methanolic extract of *Moringa oleifera* at 500 mg/kg reduced the carrageenan-induced edema to a similar extent as the potent anti-inflammatory drug indomethacin, in a time-dependent manner.

The degree of swelling was expressed as an increase in the paw thickness at various time intervals in mm as shown in table 6. Data was tabulated as the mean of swelling degree (mm)±standard deviation.

Table 6: Degree of swelling at different time interval

Group	Degree of swelling				
	1 st h	2 nd h	3 rd h	4 th h	5 th h
1	1.52±0.07	2.51±0.07	2.90±0.06	2.93±0.06	2.94±0.07
2	1.37±0.08	1.87±0.14	2.37±0.09	2.64±0.11	2.55±0.10
3	1.12±0.05	1.76±0.05	2.18±0.06	2.28±0.06	2.10±0.07
4	0.93±0.04	1.49±0.09	1.62±0.25	1.64±0.05	1.22±0.05
5	0.67±0.10	1.38±0.15	1.56±0.14	1.46±0.14	1.13±0.14

According to the data shown in table 6, the negative control group had the highest degree of swelling throughout the experiment. The degree of swelling was decreased in extracts and the drug-treated group as time increased. A decrease trend demonstrated in the methanolic extract, chloroform extract and petroleum ether extract treated group at the fourth h of edema induction, indicating the anti-inflammatory response of *Moringa oleifera* flowers. Indomethacin showed a diminished response in swelling degree after the third h of edema induction.

DISCUSSION

To the best knowledge, no other published data on using the flowers of *Moringa oleifera* in assessing the anti-inflammatory effect, other than those used in assessing antimicrobial and antihypertensive activity. The findings in this study agreed with earlier studies which also found that, not all phytochemicals were present in all plant

parts and that those present differed depending on the types of the extracting solvent used [15]. Thereafter, the study stated that flavonoids, tannins and alkaloids were potentially inhibiting both the lipoxygenase and cyclooxygenase pathways of arachidonic acid metabolism resulting notable anti-inflammation action. Besides, a study by Fawole *et al* asserted that some presented alkaloids such as isoquinoline, indole and diterpene were known to have good anti-inflammatory activity [16]. Tannins and saponins have shown potential anti-inflammatory effects. Moreover, phytochemicals like phytosterols and triterpenes were attributed to the inhibition of activities related to lipoxygenase and cyclooxygenase in the pathways. An Immunoblotting study was conducted to evaluate the expression of inflammatory mediators in macrophages treated with the 80% hydroethanolic *M. oleifera* flower extract at concentrations 100 and 200 5g/ml [17]. Another study was proven the anti-inflammatory activity at varying concentrations to inhibit nitric

oxide production. There was an increase before a gradual decrease in nitrite concentration [18].

CONCLUSION

In a nutshell, the current study gives evidence for the promising therapeutic benefits of *Moringa oleifera* in treating inflammation. The edema is sensitive to both the extracts and Indomethacin mainly in second phase. *Moringa oleifera* is as effective as the clinically proven drugs in showing anti-edematous effect. From the phytochemical result obtained, it is believed that *Moringa oleifera* flowers potentially beneficial for the management of inflammation-related consequences such as analgesic and antipyretic. The results provided experimental evidence for its traditional use in treating various diseases associated with inflammation. However, further investigations in the areas of plant-drug interactions, mutagenic and teratogenic effects, and dose-dependent and time-dependent manner adverse effect of *Moringa oleifera* on the human study are advocated to verify the long-term use and its effectiveness in chronic and acute inflammation. Overall, natural phytochemicals are still considered as potent candidates for drug discovery and are playing a crucial role in drug development programmes.

ACKNOWLEDGEMENT

Appalaraju wishes to express his thanks and gratitude to MAHSA University for providing financial support to conduct the research.

AUTHORS CONTRIBUTION

Appala raju and Lim Li Chee involved in the phytochemical analysis of *Moringa olifera* flower extract. Nagaraja and Nahlah contributed in anti-inflammatory activity of different extracts in mice paw edema.

CONFLICT OF INTERESTS

The author declares that they have no conflict of interest.

REFERENCES

1. Padayachee B, Baijnath H. An overview of the medicinal importance of *Moringaceae*. *Med Plant Res* 2012;6:39-41.
2. Lim TK, Edible plant and non-medicinal plants: fruits, New York, USA, Springer Science Business Media 2012;2:453-80.
3. Marcu MG. *Miracle tree*, Toronto, Canada, KOS Health Publishing 2005.
4. Chukwuebuka E. *Moringa oleifera* "The Mother's Best Friend". *Int J Nutr Food Sci* 2015;4:624-30.
5. Navie S, Csurhes S. Weed risk assessment: *Moringa oleifera*. Department of Agriculture and Fisheries Biosecurity Queensland, State of Queensland; 2016.
6. Sachan A, Meena AK, Kaur R, Pal B, Singh B. *Moringa oleifera*: a review. *J Pharm Res* 2010;3:840-2.
7. Fuglie LJ. *The miracle tree: the multiple attributes of mooring*, Dakar, Senegal, Church World Service; 2001. p. 172.
8. Babu NP, Pandikumar P, Ignacimuthu S. Anti-inflammatory activity of *Albizia lebbek* Benth. An ethnomedicinal plant, in acute and chronic animal models of inflammation. *J Ethnopharmacol* 2009;125:356-60.
9. Mueller T. Neuroinflammation in Huntington's disease. *J Neural Transm* 2010;117:1001-8.
10. Acarin L, Peluffo H, Gonzalez B, Castellano B. Expression of inducible nitric oxide synthase and cyclooxygenase-2 after excitotoxic damage to the immature rat brain. *J Neurosci Res* 2010;68:745-54.
11. Khandelwal KA. *Practical Pharmacognosy: Techniques and Experiments*, Pune, India, Nirali Prekashan; 2008.
12. Hafeez A, Jain U, Sajwan P, Srivastava S, Thakur A. Evaluation of carrageenan-induced anti-inflammatory activity of ethanolic extract of the bark of *Ficus virens* Linn. in swiss albino mice. *J Phytopharmacol* 2013;2:39-43.
13. Rakesh S, Singh VJ. Anti-inflammatory activity of *Moringa oleifera* leaf and pod extracts against carrageenan induced paw edema in albino mice. *J Pharm Sci Innovation* 2011;1:22-4.
14. Brown AP, Dinger N, Levine BS. Stress produced by gavage administration in the rat. *J Am Assoc Lab Anim Sci* 2000;39:17-21.
15. Brett JW, Stephen KP, Shixin D, Afa KP. Antimicrobial activity of an iridoid rich extract from *Morinda citrifolia* fruit. *Curr Res J Biol Sci* 2012;4:52-4.
16. Fawole OA, Ndhlala AR, Amoo SO, Finnie JF, Van-Staden J. Anti-inflammatory and phytochemical properties of twelve medicinal plants used for treating gastrointestinal ailments in South Africa. *J Ethnopharmacol* 2009;123:237-43.
17. Woan ST, Palanisamy A, Govindarajan K, Sharida F. *Moringa oleifera* flower extract suppresses the activation of inflammatory mediators in lipopolysaccharide-stimulated RAW 264.7 macrophages via NF- κ B pathway. *Mediators of Inflammation* 2015:1-11. <http://dx.doi.org/10.1155/2015/720171>
18. Julia PC, Yanping X, Hong C, Min-Hsiung P, Chi-Tang H, Rodolfo J, et al. Determination of flavonoids by LC/MS and anti-inflammatory activity in *Moringa oleifera*. *J Functional Foods* 2013;5:1892-9.

How to cite this article

- Venkatasthya Sai Appala Raju Velaga, Nagaraja Suryadevara, Lim Li Chee, Nahlah Elkudssiah Ismail. Phytochemical analysis and immuno-modulatory effect of *Moringa oleifera* flowers. *Int J Pharm Pharm Sci* 2017;9(6):24-28.