Tropical Journal of Pharmaceutical Research January 2024; 23 (1): 91-97
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v23i1.12

Original Research Article

Crude extracts of *Momordica cochinchinensis* (Lour.) Spreng exerts antioxidant and anti-neuroinflammatory properties in LPS-stimulated BV2 microglia

Nootchanat Mairuae[1]*, Benjaporn Buranrat[1], Poonlarp Cheepsunthorn[2]
[1]Faculty of Medicine, Mahasarakham University, 296 Nakhornsawan Road, Talad Sub-district Muang District, Mahasarakham 44000, [2]Department of Anatomy, Faculty of Medicine, Chulalongkorn University, 1873 Rama 4 Rd. Patumwan Bangkok 10300, Thailand

For correspondence:* ***Email:* *nootchanat.m@msu.ac.th; mairuae.n@gmail.com*

Sent for review: 11 October 2023 *Revised accepted: 2 January 2024*

Abstract

Purpose: *To evaluate the antioxidant and anti-neuroinflammatory properties of the leaves, seeds and pulp of Momordica cochinchinensis Spreng (commonly known as Gac) in vitro and in lipopolysaccharide (LPS)-activated BV2 microglial cells.*
Methods: *Lipopolysaccharide was applied to BV2 cells for 24 h in the presence or absence of Gac extracts. Then, levels of reactive oxygen species (ROS) were assayed using CM-H_2DCFDA method, while nitric oxide (NO) level was determined with Griess reagent. The levels of TNF-α and IL-6 were determined using ELISA. Three different assays were used for determination of in vitro antioxidant activities of the extracts, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging; 2,2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) scavenging, and ferric-reducing antioxidant power (FRAP). Total flavonoids content (TFC) and total phenolic content (TPC) were also determined.*
Results: *There were significantly higher levels of ROS, NO, TNF and IL-6 in LPS-treated BV2 cells than in control cells ($p < 0.05$). However, exposure of LPS treated BV2 cells to the leaf, seed and pulp extracts of Gac led to marked decreases in levels of ROS and inflammatory mediators, when compared with untreated cells, with the seed extract producing significantly larger decreases in levels of ROS and inflammatory mediators than the leaf and pulp extracts ($p < 0.05$). The DPPH and ABTS radical scavenging activities and total reducing power activities of the seed extract were superior to those of the leaf and pulp extracts. Moreover, the seed extract showed higher contents of TFC and TPC than the other two extracts.*
Conclusion: *Gac extracts exert antioxidant and anti-neuroinflammatory effects on LPS-activated BV2 cells and in an in vitro model. Therefore, Gac extracts, especially seed extract, may be beneficial in the preparation of innovative antioxidative and anti-neuroinflammatory herbal remedies, as well as dietary supplements.*

Keywords: *Antioxidant, Anti-neuroinflammation, Microglia, Momordica cochinchinensis (Lour.) Spreng, Neuroinflammation*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Neuroinflammation is a central nervous system (CNS) innate immune response aimed at preventing infection and eliminating pathogens, cell waste, and misfolded proteins [1]. Initially, short-term immune cell activation is considered neuroprotective, since it supports tissue restoration and pathogen clearance [2]. However, persistent immune responses may disrupt neurochemical processes, accelerate neuronal mortality, cause deficits in cellular support capacity, and disrupt blood-brain barrier, leading to chronic inflammation [3]. Microglia are brain-resident immune cells which are key contributors to neuroinflammation through production of pro-inflammatory mediators such as IL-1β, TNF-α, IL-6, nitric oxide (NO), and reactive oxygen species (ROS). These factors contribute to neurodegenerative illnesses such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease and multiple sclerosis [4,5].

Several plant-based compounds have produced significant anti-inflammatory effects in animal and *in vitro* models. *Momordica cochinchinensis* Spreng, commonly known as Gac, belongs to the Cucurbitaceae family, and it is native of Southeast Asia [6]. It has been reported that Gac fruit which is rich in carotenoids such as lycopene and β-carotene, in addition to phenolics, flavonoids, and trypsin inhibitors, exerts various beneficial bioactivities [7]. In Southeast Asia, Gac fruit and seeds are utilized in traditional medicine for treating various conditions such as rheumatic pain, hemorrhoids, hemangiomas, wounds, bruises, and muscle spasms [8]. Additionally, the seeds have been associated with anti-cancer, anti-inflammatory, and antioxidant activities [9]. Despite its claimed medical benefits, not much is known on the impact of Gac on neuroinflammation and microglial activation. This study was carried out to investigate the antioxidant and anti-neuroinflammatory activities of the leaves, seeds, and pulp of Gac, both *in vitro* and in LPS-stimulated BV2 microglial cells.

EXPERIMENTAL

Preparation of Gac extracts

The Gac extracts were prepared as previously described [10]. The leaves, seeds and pulp of Gac were separately dried, weighed, chopped and macerated in 95 % (v/v) ethanol. Then, each homogenate was subjected to filtration. The ethanol solvent was evaporated from the crude extract obtained, after which the residue was lyophilized.

Culturing and treatment of murine BV2 cells

Murine BV2 microglial cell line was kindly provided by Dr James R. Connor of Department of Neurosurgery, College of Medicine, Pennsylvania State University; Hershey, PA, USA. The cells were cultured in DMEM supplemented with penicillin/streptomycin and 5 % fetal bovine serum at 37 ˚C in a humidified environment containing 5 % CO_2. All cell culture supplies were purchased from HyClone (Cytiva).

Assessment of cell viability

After subjecting the cells to a 24-h treatment with Gac extracts from the seed, leaf and pulp at doses ranging from 0 to 100 g/mL, in the presence or absence of LPS, the culture medium was aspirated. Subsequently, MTT (0.4 mg/mL) reagent (Millipore Sigma) was added to the culture medium, followed by further incubation at 37˚C in a humidified atmosphere containing 5 % CO_2 for 2 h. Thereafter, the MTT solution was removed and the resultant purple formazan crystals were dissolved in DMSO. The optical density of the formazan solution in each well was measured at 570 nm using a microplate reader (Spectramax 340 PC; Molecular Devices, LLC).

Nitric oxide (NO) assay

Nitric oxide (NO) levels in the cell culture supernatants were quantified utilizing Griess reagent (Thermo Fisher Scientific, Inc.). The culture medium (150 µL) was dispensed into each well of a 96-well plate. Subsequently, 20 µL of Griess reagent was introduced, followed by 130 µL of deionized water. After a 30-min incubation at room temperature, the absorbance of each well was read at 540 nm using a microplate reader (BioTek Instruments, Inc.).

Assay of intracellular ROS

The intracellular levels of ROS were determined using the 2',7' dichlorofluorescein diacetate (DCFH DA) oxidation method. The cells were treated with 10 µM DCFH DA for 20 min at 37 °C in a humidified incubator containing 5 % CO_2. This was followed by 24-h treatment of the cells with LPS (1 µg/mL) in serum-free medium in the presence and absence of different concentrations of Gac extracts. The resultant fluorescence intensity was quantified using a microplate reader (BioTek Instruments, Inc.) at excitation and emission wavelengths of 495 nm and 525 nm, respectively.

Determination of levels of IL-6 and TNF-α

The levels of IL-6 and TNF-α in the culture supernatant were determined using ELISA kits. Absorbance was read at 450 nm in a microplate reader (BioTek Instruments, Inc.), and values obtained were used for extrapolating the concentrations of IL-6 and TNF-α from standard curves for IL-6 and TNF-α, respectively.

Evaluation of FRAP and DPPH and ABTS radical-scavenging activities

The total free radical scavenging potential of the seed, leaf and pulp extracts of Gac were assayed using DPPH, FRAP and ABTS methods, as previously described [11].

Assessment of total phenol and flavonoid contents

The total phenolic and flavonoid contents of the seed, leaf and pulp extracts of Gac were determined in accordance with the procedure described earlier [10].

Statistical analysis

Results are presented as mean ± standard error of the mean (SEM). Differences among multiple groups were analyzed using one-way ANOVA, followed by Bonferroni's post-hoc tests. A significance level of $p < 0.05$ was considered as indicative of statistical significance.

RESULTS

Antioxidant properties of the seed, leaf and pulp extracts of Gac

Table I presents the total phenolic content (TPC) and total flavonoid content (TFC) of the dried extracts obtained from Gac seeds, leaves and pulp. The TPC values of the seed, leaf and pulp extracts were 1.568 ± 0.077, 1.334 ± 0.052 and 0.237 ± 0.003 mg GE/g, respectively, while the TFC of the seed, leaf and pulp extracts were 143.225 ± 0.471, 89.525 ± 0.517 and 54.499 ± 0.435 mg QE/g, respectively. The seeds exhibited the highest antioxidant activity, followed by the leaves and pulp. These findings suggest a positive correlation between the antioxidant activities and TPC/TFC.

In addition, the present study evaluated the antioxidant potential of seeds, leaves and pulp of Gac using DPPH, ABTS and FRAP assays. The results are also shown in Table I. The extracts of seeds, leaves and pulp of Gac had IC_{50} values of 0.0595 ± 0.0153, 0.0641 ± 0.0008, and 0.0804 ± 0.0011 mg/mL, respectively, with respect to DPPH radical scavenging activities, while the corresponding ABTS values were 0.0060 ± 0.0005, 0.0157 ± 0.0003 and 0.0193 ± 0.0022 mg/mL, respectively. The FRAP values for the seed, leaf and pulp extracts were 409.738 ± 0.820, 382.295 ± 0.319, and 199.570 ± 0.804 mg TE/g Ext, respectively. The results obtained in the DPPH, ABTS and FRAP assays indicate that Gac seeds had the highest antioxidant activities, while the pulp had the lowest antioxidant activities.

Effects of seed, leaf and pulp extracts of Gac on BV2 cell viability in the presence of LPS

Before determination of the potential anti-neuroinflammatory and antioxidant effects of the extracts in BV2 cells, their effects on the viability of BV2 cells were measured. The MTT assay results showed that the seed, leaf and pulp extracts of Gac at concentrations up to 50 g/mL did not have a cytotoxic effect on BV2 microglia when compared with the control (untreated) cells after 24 h of treatment (Figures 1 A - C).

Table I: TFC and TPC of Gac extracts and their corresponding antioxidant activities

Sample	DPPH	ABTS	FRAP	TPC	TFC
	IC_{50} (mg/mL)	IC_{50} (mg/mL)	mg TE/g Ext	mg GE/g Ext	mg QE/g Ext
Pulp	0.0804±0.0011	0.0193±0.0022	199.570±0.804	0.237±0.003	54.499±0.435
Leaves	0.0641±0.0008	0.0157±0.0003	382.295±0.319	1.334±0.052	89.525±0.517
Seeds	0.0595±0.0153	0.0060±0.0005	409.738±0.820	1.568±0.077	143.225±0.471
Trolox	0.0162±0.0012	0.0086±0.0006	-	-	-
Ascorbic acid	0.0036±0.0002	0.0019±0.0000	-	-	-

Note: The IC_{50} values were determined by calculating 50 % inhibitions of DPPH and ABTS radicals. IC_{50} = half maximum inhibitory concentration; ABTS = 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid); DPPH = 2,2'-diphenyl-1-picrylhydrazyl; FRAP = ferric-reducing antioxidant power; TPC = total phenolic content; TFC = total flavonoid content

Therefore, Gac extracts at concentrations of 25 and 50 g/mL were selected for subsequent antioxidant and anti-neuroinflammatory studies.

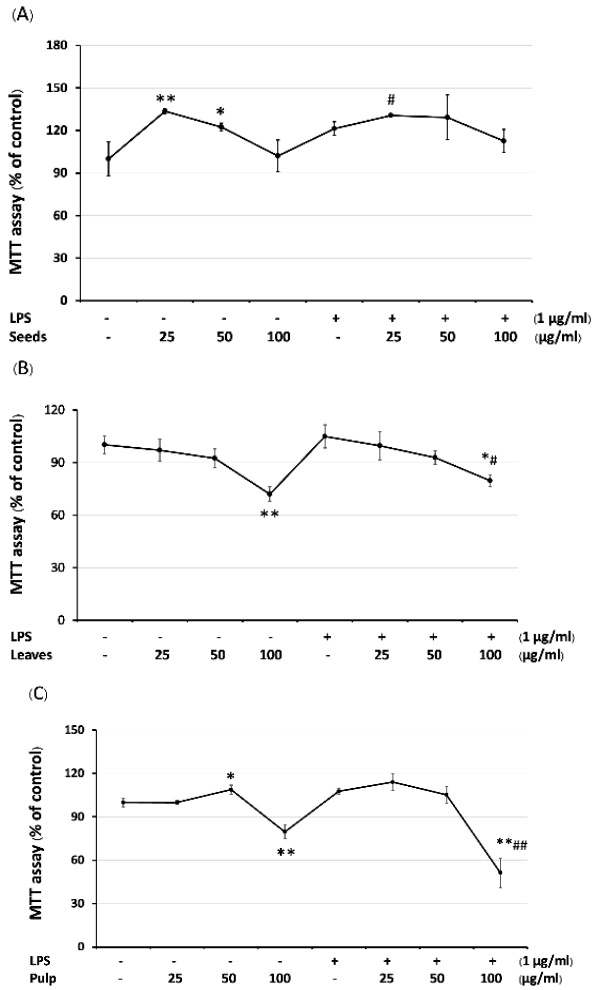

Figure 1: Effect of seed (a), leaf (b) and pulp (c) extracts of Gac on the viability of BV2 cells, as determined using MTT assay. *P < 0.05, vs. control group; $^{\#}p$ < 0.05, vs. LPS group

Gac reduced the production of intracellular ROS in LPS-stimulated BV2 microglia

The intracellular levels of ROS were determined in order to evaluate the antioxidant properties of the Gac extracts in LPS-stimulated BV2 cells. Compared with control cells, the level of ROS in LPS-treated cells was significantly increased. However, compared with BV2 cells treated with LPS only, the ROS levels was markedly reduced in a concentration-dependent manner in the presence of Gac extracts (p < 0.01; Figure 2). However, when applied at the same concentrations, the seed extract resulted in lower ROS production, when compared with the leaf and pulp extracts.

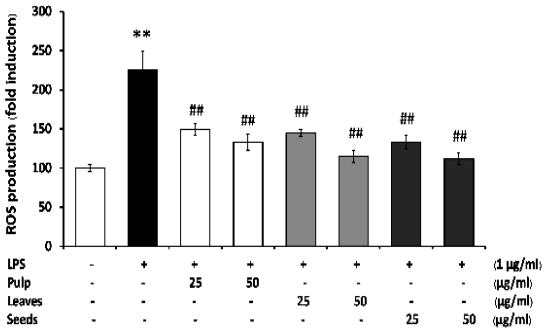

Figure 2: Effect of Gac extracts on ROS levels in BV2 cells in the presence of LPS. **P < 0.01, vs. control group; $^{\#\#}p$ < 0.01 vs. LPS-treated group

Gac extract decreased NO production in LPS-stimulated BV2 microglia

To investigate the anti-inflammatory properties of Gac extracts in LPS-stimulated microglial cells, NO production was indirectly measured by determining the nitrite content of BV2 cells after 24 h of treatment. Compared with untreated control cells, LPS treatment significantly raised the NO level in the culture medium of BV2 microglial cells, as shown in Figure 3. However, the level of NO in the groups treated with extracts from the seeds, leaves and pulp were dose-dependently much lower than that of the LPS group. Furthermore, the effect produced by the Gac seed extract was comparable to that of the leaf extract, but significantly better than that of the pulp extract at the same concentration.

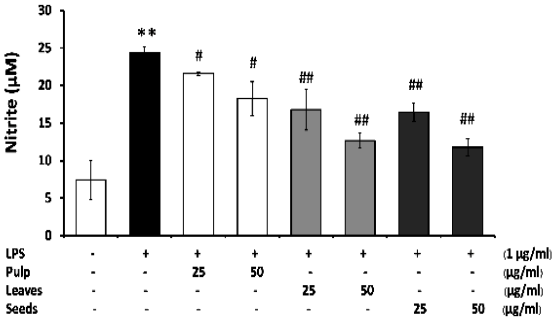

Figure 3: Effect of Gac extract on NO levels. **P < 0.01, vs. control group; $^{\#}p$ < 0.05, vs. LPS-treated group

Gac attenuated TNF-α levels in LPS-stimulated BV2 microglia

The TNF-α level in the culture medium of BV2 cells was measured using ELISA in order to further assess the anti-inflammatory effects of Gac extract in LPS-stimulated BV2 microglial

cells. As presented in Figure 4, BV 2 cells treated with LPS had significantly higher levels of TNF-α in the culture medium than control group. However, the levels of TNF-α were significantly and concentration-dependently reduced when the cells were co-treated with LPS and Gac extract, relative to cells treated with LPS alone ($p < 0.01$). At the same concentrations, the seed extract resulted in lower TNF-α production than the leaf and pulp extracts.

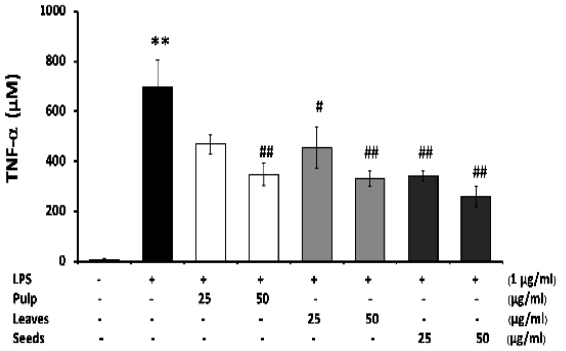

Figure 4: Effect of Gac extracts on TNF-α levels. $^{**}P < 0.01$, vs. control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, vs. LPS-treated group

Gac decreased IL-6 levels in LPS-stimulated BV2 microglia

The levels of IL-6 were measured in LPS-activated BV2 microglial as an additional indicator of the anti-inflammatory potential of Gac extracts. As presented in Figure 5, BV 2 cells treated with LPS had significantly higher levels of IL-6 in the culture medium than control cells. However, the IL-6 levels were significantly reduced in a concentration-dependent manner when the cells were treated with LPS and the Gac extract, when compared to when the cells were exposed to LPS only ($p < 0.01$). The seed extract produced higher decreases in IL-6 levels than the leaf and pulp extracts.

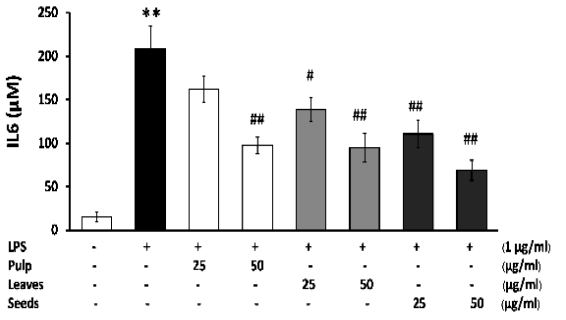

Figure 5: Effect of Gac extracts on IL-6 levels. $^{**}P < 0.01$, vs. control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, vs. LPS-treated group

DISCUSSION

Previous studies indicate that neurodegenerative illnesses involve microglia-mediated neuroinflammation and oxidative stress which accelerate disease progression and neuronal death [12]. The identification of drugs for mitigation of microglia-induced oxidative stress and neuroinflammation is a crucial goal in developing disease-modifying therapeutics for neurodegenerative disorders. Interest in naturally-occurring phytochemicals with antioxidant and neuroprotective properties has grown, due to concerns about side effects associated with conventional medicines, in addition to the ready accessibility of natural products in edible forms [13]. Medicinal plants are historically effective and perceived as safe, with minimal side effects, and are often preferred to conventional medicines.

This study investigated and compared the antioxidant potential of three parts of Gac (leaves, seeds, and pulp) using DPPH, ABTS, and FRAP assays. The Gac seed extract showed the highest antioxidant activities, while the Gac pulp extract exhibited the lowest antioxidant activity in DPPH, ABTS and FRAP assays. This study also evaluated the TPC and TFC of the Gac extracts. The results revealed that the seeds had the highest levels of TPC and TFC, followed by the leaves, while the pulp had the lowest TPC and TFC. Phenolic acids exert antioxidant properties, and they have potential to function as primary antioxidants. The results obtained in this study indicate a strong association between antioxidant activity and TPC of the Gac extracts. Flavonoids are free radical scavengers which are important for human health. The ethanol extracts of Gac demonstrated potent antioxidant potential due to their rich contents of total phenolics and flavonoids, with the seed extract having the highest phenolic content, followed by the leaves and pulp. The study observed good agreement amongst TPC, TFC, DPPH scavenging activity, reducing power, and chelating activity.

Excessive reactive oxygen species (ROS) damage biomacromolecules in living cells, leading to ageing and ailments such as Alzheimer's disease, Parkinson's disease, atherosclerosis, diabetes, and cancer [14]. In this study, LPS-stimulated BV2 cells showed a six-fold increase in ROS production, but this was effectively and concentration-dependently reduced by the Gac extracts. Interestingly, the seed extract exhibited lower ROS production than the leaf and pulp extract at the same concentrations. This is consistent with previous findings in a different cell line [15]. The present

study has revealed, for the first time, the potential of Gac extracts to suppress ROS production in BV2 microglial cells, the resident immune cells in the CNS. Bioactive compounds like β-carotene, lycopene, phenolic compounds, and flavonoids in the extracts are known to reduce LPS-induced ROS generation [16,17]. This accounts for the observed effects of the extracts on ROS levels in this study. The extracts also suppressed neuroinflammation by reducing the levels of NO, TNF-α, and IL-6 in LPS-activated BV2 microglia. The extracts, at concentrations of 25 and 50 µg/mL, significantly decreased NO production in LPS-exposed BV2 microglial cells in a concentration-dependent manner, with the seed extract resulting in the lowest NO production. Elevated NO levels within the central nervous system contribute to neurodegenerative diseases. Therefore, this study has demonstrated that the Gac extracts have potential to mitigate excess NO-induced neuroinflammation. Exposure of the BV2 cells to LPS led to increased release of TNF-α and IL-6, while Gac extract treatment significantly and dose-dependently reduced the levels of these inflammatory factors. Again, the seed extract resulted in the lowest production levels of TNF-α and IL-6 amongst the Gac extracts. These findings are consistent with earlier reports which showed that ethanol extract of Gac decreased IL-6 levels in LPS-stimulated murine and human macrophages [18]. Therefore, Gac extracts are potential sources of anti-inflammatory compounds that may be useful for targeting pro-inflammatory cytokines linked to neurotoxicity.

CONCLUSION

The current study has established the antioxidant properties of the ethanol extracts of Gac *in vitro* and in LPS-activated BV2 microglial cells. The ethanolic extracts exhibit anti-neuroinflammatory effect in LPS-activated BV2 microglial cells. The seed extract produces stronger antioxidant activity and higher levels of the well-known antioxidants TPC and TFC than the leaf and pulp extracts. Moreover, the seed extract has the strongest anti-neuroinflammatory activity, followed by the leaf extract and the pulp extract. Therefore, Gac extracts may be useful in the production of innovative anti-oxidative and anti-neuroinflammatory herbal medications, as well as food supplements.

DECLARATIONS

Acknowledgements

The present project was supported financially by Thailand Science Research and Innovation and the Faculty of Medicine, Mahasarakham University.

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Yang QQ, Zhou JW. Neuroinflammation in the central nervous system: Symphony of glial cells. Glia 2019; 67: 1017-1035.*
2. *Jankovic J, Tan EK. Parkinson's disease: etiopathogenesis and treatment. J Neurol Neurosurg Psychiatry 2020; 9: 795-808.*
3. *Chen Z, Trapp BD. Microglia and neuroprotection. J Neurochem 2016; 136: 10-17.*
4. *Hickman S, Izzy S, Sen P, Morsett L, Khoury JE. Microglia in neurodegeneration. Nat Neurosci 2018; 21: 1359-1369.*
5. *Muzio L, Viotti A, Martino G. Microglia in neuroinflammation and neurodegeneration: From understanding to therapy. Front Neurosci 2021; 24: 742065.*

6. Wimalasiri D, Piva T, Urban TS, Huynh T. Morphological and genetic diversity of Momordica cochinchinenesis (Cucurbitaceae) in Vietnam and Thailand. Genet. Resour Crop Evol 2016; 63: 19–33.
7. Kubola J, Siriamornpun S. Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (Momordica cochinchinensis Spreng). Food Chem 2011; 127: 1138–1145.
8. Tran XT, Parks SE, Roach PD, Golding JB, Nguyen MH. Effects of maturity on physicochemical properties of Gac fruit (Momordica cochinchinensis Spreng.). Food Sci Nutri 2016; 4: 305-312.
9. Kha TC, Nguyen MH, Roach PD, Parks SE, Stathopoulos C. Gac fruit: Nutrient and phytochemical composition, and options for processing. Food Rev Int 2012; 29: 92–106.
10. Mairuae N, Buranrat B, Yannasithinon S, Cheepsunthorn P. Antioxidant and anti-inflammatory effects of Aegle marmelos fruit and Moringa oleifera leaf extracts on lipopolysaccharide stimulated BV2 microglial cells. Trop J Pharm Res 2023; 22(6): 1247-1254.
11. Darunee TP, Sumalee A, Konsue A. Phytochemical screening and antioxidant activities of the seedling extracts from Inca peanut, Plukenetia volubilis, Puangpronpitag. Pharmacog J 2021; 1: 52-58.
12. Simpson DSA, Oliver PL. ROS generation in microglia: understanding oxidative stress and inflammation in neurodegenerative disease. Antioxidants (Basel) 2020; 9: 743.
13. Winiarska-Mieczan A, Wójcik EB, Kwiecień M, Grela ER, Szwajgier D, Wiatkowska K, Kiczorowska KB. The role of dietary antioxidants in the pathogenesis of neurodegenerative diseases and their impact on cerebral oxidoreductive balance. Nutrients 2020; 12(2): 43.
14. Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: An overview. Methods Enzymol 1990; 186: 1-85.
15. Kemika P, Pornpan S, Siriporn T, Gac fruit extract suppresses oxidative stress in LPS-induced RAW264.7. Cells J Nutri Assoc Thai 2021; 56: 37-51.
16. Bai SK, Lee SJ, Na HJ, Ha KS, Han JA, Lee H. Beta-carotene inhibits inflammatory gene expression in lipopolysaccharide-stimulated macrophages by suppressing redox-based NF-kappa-B activation. Exp Mol Med 2005; 37: 323-334.
17. Zou J, Feng D, Ling WH, Duan RD. Lycopene suppresses proinflammatory response in lipopolysaccharide-stimulated macrophages by inhibiting ROS-induced trafficking of TLR4 to lipid raft-like domains. J Nutr Biochem 2013; 24: 1117-1122.
18. Jung K, Chin YW, Yoon KD, Chae HS, Kim CY, Yoo H, Anti-inflammatory properties of a triterpenoidal glycoside from Momordica cochinchinensis in LPS-stimulated macrophages. Immunopharmacol Immunotoxicol 2013; 35: 8–14.