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RESEARCH ARTICLE

Evaluation of *Momordica cochinchinensis* Fruit Methanolic Extract for Potential Medicinal Use

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Abstract

Objectives: This study evaluated the biological and pharmacological activities of *Momordica cochinchinensis* fruit methanolic extract (MCME). **Methods:** The extract was analyzed for its phytochemical constituents and tested for toxicity and antimicrobial properties. The hypoglycemic activity was also evaluated in hyperglycemic mice. **Findings:** The results showed that the extract contained important phytochemical constituents and exhibited antimicrobial activity against *S. aureus* and *E. coli*. The extract was found to contain important phytochemical constituents, including saponins, flavonoids, and phenolic compounds. In a brine shrimp toxicity assay, the extract exhibited toxicity activity, with an LC50 mortality rate of 10 μg/mL after 24 hours. Additionally, in hyperglycemic mice, the extract was found to be more efficient in hypoglycemic activity than the positive control, Metformin, with a decrease in blood glucose levels of 64.8% and 54.2%, respectively, after 24 hours of treatment. **Nove lty:** This study serves as the first unprecedented investigation of the hypoglycemic effect of MCME including novel antibacterial activity against *E. coli*.

Keywords: Gac fruit; phytochemical analysis; toxicity test; antibacterial activity; hypoglycemic activity

1 Introduction

Momordica cochinchinensis (Lour.) Spreng, also known as gac fruit, is a tropical plant belonging to the Cucurbitaceae family. It is widely consumed in Southeast Asia, particularly in the Philippines, for its nutritional and medicinal properties. *M. cochinchinensis* is known to be rich in carotenoids, tocopherols, and essential fatty acids, which have been shown to have potent antioxidant and anti-inflammatory activities [(1)]. Numerous studies have documented the utilization of *M. cochinchinensis* extract [(2–4)] for medicinal applications. However, these investigations have primarily concentrated on the plant's antioxidant, antiproliferative, and anticancer properties, while neglecting to explore its potential hypoglycemic activities and its antibacterial effects against gram-negative bacteria like *E. coli*. Thus, this research aims to fill these gaps in the existing literature.

The phytochemical analysis is an essential step in evaluating plant extracts for their biological activities. Several studies have reported that *M. cochinchinensis* fruit extract

contains important phytochemical constituents [5]. Additionally, to ensure the safe utilization of the extract, the study employed a comprehensive toxicity assay that examined the extract's impact on the toxicity of various harmful compounds [6]. This assessment aims to evaluate the extract's safety and address any concerns regarding potential toxic effects, thus providing valuable insights for its safe usage.

Microbial infections pose a significant global health concern, which worsens by the escalating problem of antimicrobial resistance [7]. Recognizing the urgency to combat this growing issue, this study extends beyond phytochemical and toxicity tests to explore the antimicrobial potential of *M. cochinchinensis* fruit extract. The evaluation focused on two prevalent bacterial pathogens, *Staphylococcus aureus*, known for causing skin and soft tissue infections as well as nosocomial infections, and *Escherichia coli*, a common culprit behind urinary tract and gastrointestinal infections. To determine the extract's efficacy, a disc diffusion method was done. By investigating the antimicrobial properties of *M. cochinchinensis*, this research contributes to the search for alternative solutions to combat microbial infections, in the face of the mounting challenge of antimicrobial resistance.

Furthermore, as diabetes is a metabolic disorder characterized by hyperglycemia, this study sought to assess the hypoglycemic activity of *M. cochinchinensis* in hyperglycemic mice. To establish a benchmark, the extract's effectiveness was compared to that of Metformin, a widely prescribed hypoglycemic drug known for its ability to reduce hepatic glucose production and enhance insulin sensitivity. The evaluation of the extract's hypoglycemic activity was conducted through blood extraction methods in hyperglycemic mice, aiming to shed light on its potential as a natural alternative or complement to existing treatment options for diabetes management.

Overall, this study aimed to bridge the gap between the traditional use and actual efficacy of *M. cochinchinensis* fruit extract and explore its potential as a source of natural medicine. The findings of this study have the potential to contribute to the development of new natural products with antimicrobial and hypoglycemic activities, which have not been extensively explored in the scientific community [8]. By encompassing various aspects such as phytochemical analysis, toxicity testing, antimicrobial activity, and hypoglycemic activity, this study provides a comprehensive understanding of the potential of *M. cochinchinensis* fruit methanolic extract.

2 Methodology

2.1 Preparation of Fruit Extracts

Dried fruits of *M. cochinchinensis* were cut into small pieces and put in a blender. 100 g of *M. cochinchinensis* fruit was decocted in a 1-liter methanol solvent (95%) for 3 days. Only the skin and the pulp of the fruit were used. The extract was filtered through a qualitative filter paper with 30–50 μM pore size in a funnel and collected the extract. Finally, the extracts were concentrated on a rotary evaporator at 45°C and 35 rpm (rotation per minute).

2.2 Phytochemical Screening for Plant Secondary Metabolites

The following secondary metabolites screening method was conducted to determine the potency of *M. cochinchinensis* fruit extract for various biochemical tests.

1. **Tests for the presence of alkaloids**

   (a) **Dragendorff's test:** 1ml was taken from the filtrate and was tested by adding 2 to 3 drops of Dragendroff´s reagent. An orange precipitate gives a positive result for the presence of an Alkaloid.
   (b) **Mayer's test:**1 ml was taken from the filtrate and was tested by adding 2 to 3 drops of Mayer´s reagent. A white precipitate gives a positive result for the presence of an Alkaloid.
   (c) **Hager's test:** To 3 ml of filtrate, 1 ml of Hager´s reagent (saturated picric acid solution) was added. The appearance of a yellow precipitate indicates the presence of alkaloids.

2. **Tests for the presence of saponins**

   (a) **Foam Test:** 2 grams of each of the sample extracts was added to 15ml water and warmed in a water bath for 15 minutes. The resulting solution was filtered and left to cool in a room temperature. Then, after cooling the solution it was transferred to a 10ml test tube. This was shaken thoroughly for 10 seconds and the highest persistent (5-10 minutes) honeycomb froth was measured. The honey comb froth higher than 1 cm confirmed the presence of saponins.

(b) **Froth Test**: Each of the plant extracts (0.5 g) was separately shaken with distilled water (10 ml) in a test tube. The formation of frothing, which persists on warming in a water bath for 5 minutes, shows the presence of saponins.

3. **Tests for the presence of flavonoids**

(a) **Lead Acetate Test:** 1 ml of each extract was treated with a few drops of lead acetate solution. The formation of a yellow precipitate indicated the presence of flavonoids.
(b) **Ferric chloride Test:** 1 ml of each extract was added to a few drops of ferric solution. Development of intense green color indicates the presence of flavonoids.

4. **Tests for the presence of phenolic compounds**

(a) **Ferric chloride Test:** To 3 ml of ferric chloride solution was added to the extract. The bluish-black color indicates the presence of phenols.
(b) **Lead acetate Test:** To 3 ml of extract, 3 ml of lead acetate solution was added. The occurrence of a white precipitate indicates the presence of phenols.

2.3 Brine Shrimp Toxicity Bioassay

The brine shrimp hatchery was made from an improvised plastic bottle. The brine shrimp cysts were collected from the College of Aquatic Sciences and Applied Technology of the Mariano Marcos State University, Currimao, Ilocos Norte. The hatchery was filled with artificial seawater which was prepared by 1 tablespoon of table salt, a pinch of baking soda, and 800 ml of tap water. $\frac{1}{2}$ tablespoon of the brine shrimp cyst was added to the hatchery and an aerator was needed for them not to run out of oxygen. The researchers waited 24 hours for them to hatch until ready for the experiment. The researchers used nauplii at the early stage of development. The selection criteria for nauplii included their mobility and absence of any physical deformities or abnormalities.

Four test tubes were used and labeled 1-4. 1000 μg was introduced to the first test tube then 9 ml of distilled water was added. 1 ml of the plant extract from the first test tube was pipetted into the second test tube and mixed well. This represents 100 μg of the plant extract. Next, 1 ml from the second test tube was removed and placed into the third test tube, and swirled. From the third test tube, 1 ml was transferred to the last test tube and again swirled. This represents 1 μg of the plant extract.

A clean and dry exposure from the wells was obtained. The extract dilutions of the *M. cochinchinensis* fruit extract were poured within each well. With a wide-mouth dropper or pipette, 10 brine shrimps were transferred into each well plate containing the extract. The actual number of brine shrimp was recorded. The numbers of the alive nauplii were counted in each well plate after 24 hours of exposure.

To determine the concentration killing 50% of the larvae (LC_{50}) Abbott's formula was used:

$$\%\, death = \frac{Death\ in\ Test\ Well - Death\ in\ Control\ Well}{Death\ in\ Control} x100$$

2.4 Antibacterial Test

- **Test Microorganism:** The following microorganisms- *Staphylococcus aureus* and *Escherichia coli* were chosen based on their clinical and pharmacological importance and were used for evaluating antimicrobial activity. The bacterial strains were obtained from the Microbiology Laboratory, Department of Biological Sciences, College of Arts & Sciences, Mariano Marcos State University, City of Batac, Ilocos Norte.
- **Agar Well Cut Diffusion Method:** The agar well-cut diffusion method was used for antibacterial activity. Agar plates are inoculated with a standardized inoculum of the test microorganism. 0.5 ml of *M. cochinchinensis* methanolic fruit extract was impregnated into a hole that was bored 4 mm in diameter. Ninety-five percent (95%) methanol solution was used as a negative control and Cephalexin was used as a positive control for both *S. aureus* and *E. coli* strains. The Petri dishes are incubated under suitable conditions. Generally, an antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism, and then the diameters of inhibition growth zones are measured. The assay was repeated twice. The zones of growth inhibition around the disks were measured after 24 hours of incubation Antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the fruit extract.

2.5 Hypoglycemic Activity

The use of animals in the study and the experimental protocol complied with the guidelines set by the Institutional Animal Care and Use Committee.

Twelve (12) female white mice (*Mus musculus* L.) were used in the study. The source of animals was at the Laboratory Animal Care Facility of Mariano Marcos State University.

2.6 Experimental Protocol

The study utilized a total of twelve (12) female white mice. Four (4) treatments were used:

T1 = untreated
T2= negative control (distilled water)
T3= *M. cochinchinensis* methanolic fruit extract (MCME)
T4= positive control (Metformin)

Each treatment consisted of three (3) mice. This sample size per treatment is the minimum number of replicates required to allow statistical analysis of the measured blood sugar level at different exposure times.

- **Quarantine and/or acclimatization or conditioning process:** The mice were acclimatized to standard normal conditions at the Laboratory Animal Care Facility – MMSU for two (2) weeks. The standard normal condition was the following: 22 – 25° Celsius room temperature, 55 – 75% relative humidity, 10 – 15 cycles of air changes, and 12 hours of light/dark cycle. Mice were fed with standard chow pellets *ad libitum* and given free access to distilled water.

2.6.1 ***Animal Care Procedures***

- Caging (dimensions and material, number of animals per cage, type of litter used): Plastic cages with metal screen cover with dimensions of 18 x 12 x 7 inches (length x width x depth) were used to house the mice. Beddings made with sterilized rice hulls with a 1.5 – 2.0 inches thickness were used. Each cage house four (4) mice to provide enough space for the movement of the animals.
- Disinfection and Cage Cleaning Method: Cages were cleaned at least twice a week. The increments were removed and the cages were washed with soap and water. Disinfection using isopropyl alcohol was done also.
- Room Temperature, Humidity, Ventilation, and Lighting: Room Temperature: 22 – 25° Celsius, Humidity: 55 – 75%, Ventilation: 10 – 15 cycles of air changes, Lighting: 12 hours light/dark cycle,
- Animal Diet/Feeding/Watering Method: Standard chow pellets, 40 – 50 grams, were fed per head per day. Sippers were installed in each cage to provide free access to distilled water.

2.6.2 ***Experimental or Animal Manipulation Methods***

- General Description of Animal Manipulation Methods in the Study: Mice were purchased from Laboratory Animal Care and Facility and acclimatized for two (2) weeks under standard normal conditions. The mice were grouped into four (4) treatments, each group consisting of three mice. All groups were administered with glucose solution for the induction of diabetes with a concentration of 200 mg/kg. Extracts were orally administered to three (3) groups with the same dosage. Prior to blood extraction, the mice were restricted using an improvised restrictor. Blood was extracted through tail snipping after one (1) hour, two (2) two, three (3) hours of exposure, and 24-hour post–treatment to determine the blood sugar level. The snipped tails were dabbed to stop the blood flow.
- Dosing Method (Drugs, extracts, etc. used, frequency, volume, route of administration, method of restraint, and expected outcome or effects): To induce hyperglycemia, all mice were subjected to a 12-hour fasting period while having unrestricted access to water. Hyperglycemia was then induced orally in all mice using a glucose solution with a concentration of 200 mg/kg. Blood sugar levels were measured 48 hours after administration, and only mice with glucose levels of 120 mg/dL were included in the study. A single dose of the following treatment was administered orally using oral gavage in each group:

Group 1 = diabetic untreated mice
Group 2 = negative control (distilled water)(10 mL/kg body weight)
Group 3 = MCME (10 mL/kg body weight)
Group 4= Positive control (Metformin) (100 mg/kg body weight)

An improvised restrictor was used to restrain the mice during blood extraction through tail snipping. It is expected that groups 3 and 4 will have lower blood sugar levels after 1, 2, 3, and 24 hours of treatment.

Based on the toxicity tests and similar studies conducted using the plant extracts, it has been proven that the non-toxic single dosage of MCME is 100 mg/kg. Therefore, there is no need to subject the plant extracts to toxicity tests.

- Specimen or biological agent (blood, urine, etc.) collection method (including frequency, volume, route, and method of restraint): A drop of 0.05 mL of blood was extracted through tail snipping to determine if the mice hyperglycemic (>120 mg/dL) after glucose induction. Three blood collection of 0.05 mL was done for each of the mice. Blood was collected and tested for the blood sugar level using a glucometer after 1 hour, two hours, three hours, and 24 hours of treatment administration. For the induction of hyperglycemia, the test animals were restrained using the one-handed mouse restraint. For the administration of the different treatments, a restrainer was used.
- Animal Examination procedures and frequency of examinations (including restraining method): Hyperglycemia was induced in mice through oral gavage. Blood sugar level was measured after induction of hyperglycemia, I hour, 2 hours, 3 hours, and 24 hours of extract administration by extracting blood through tail snipping. Restrainer was used during the blood extraction procedure.

2.6.3 ***Cervical dislocation***

Was used for euthanasia and was disposed of according to the guidelines given by the IACUC. This procedure was done under the supervision of the Animal Laboratory Personnel.

2.7 Statistical Analysis

The data measured was expressed as mean and was analyzed using Post Hoc Test- Tukey Test for pairwise comparison between and among all the treatments against positive controls (for antibacterial activity and hypoglycemic activity). P values less than or equal to 0.05 was considered indicative of significance. The statistical test was conducted using SSPS Version 20.0.

3 Results and Discussion

3.1 Qualitative Phytochemical Screening

The results of the qualitative phytochemical screening of MCME (*M. cochinchinensis* methanolic extract) are shown in Table 1. The pH of the sample was determined to be basic, with a value above 7.0.

Table 1. Phytochemical constituents of *Momordica cochinchinensis*

Phytochemical Constituents	Presence (+), Absence (-)
Alkaloids	-
Saponins	+
Flavonoids	+
Phenolic Compounds	+
pH	basic

The results of this qualitative phytochemical screening indicate that MCME contains several potentially bioactive compounds, including saponins, flavonoids, and phenolic compounds. Saponins are known to have a range of biological activities, including anti-inflammatory, antitumor, and antiviral effects[9]. Flavonoids and phenolic compounds are also well-known for their antioxidant and anti-inflammatory properties and have been shown to have potential therapeutic applications in the treatment of various diseases[10]. Interestingly, alkaloids were not detected in the sample. Alkaloids are a large and diverse group of natural compounds that are commonly found in plants and have a wide range of pharmacological effects[11].

Moreover, parallel to these results were also observed in a study conducted by Marnpae, M. et al.[12] where the qualitative phytochemical screening of *M. cochinchinensis* revealed the presence of several potentially bioactive compounds, including saponins, flavonoids, and phenolic compounds. While these results are promising, further results will be explored for the pharmacological potential of this plant and to identify any potential therapeutic applications.

3.2 Brine Shrimp Toxicity Bioassay

The mean percentage of mortality of brine shrimps after 24 hours of exposure to different concentrations of MCME is presented in the Table 2. The control group, which was not exposed to MCME, showed a mean mortality rate of 76.67%. The mortality rates for the treatment groups exposed to different concentrations of MCME were 23.33% for 1000 μg/mL, 36.67% for 100 μg/mL, 50% for 10 μg/mL, and 70% for 1 μg/mL.

Table 2. The Mean Percentage of the Mortality of Brine Shrimps after 24 Hours

	Concentrations				
	Control	**1000 μg/mL**	**100 μg/mL**	**10 μg/mL**	**1 μg/mL**
MCME	76.67	23.33	36.67	50	70

Values are in percent (%) mortality

Out of the four concentrations tested, only 10 μg/mL exhibited a mortality rate equivalent to the LC50, indicating that any concentration higher than this is potentially lethal. In addition to assessing efficacy, it is crucial to consider the safety of herbal medicines, particularly given the limited knowledge available about many traditional remedies. The findings align with a previous study by Clemen-Pascual, L et al. that investigated the use of different parts of the Cucurbitaceae family and also showed a mortality rate of 10 μg/mL [13].

3.3 Antibacterial Activity

The results of this study demonstrated the antibacterial activity of MCME against *S. aureus* and *E. coli* are presented in Table 3. The mean zone of inhibition produced by the extract was found to be 20.55±0.05 mm against *S. aureus* and 10.75±0.22 mm against *E. coli*. These results suggest that MCME possesses moderate activity against *S. aureus*, but only weak activity against *E. coli*.

In comparison, the positive control, cephalexin, exhibited a mean zone of inhibition of 30.5±0.47 mm against *S. aureus* and 1717±0.47 mm against *E. coli*. These results indicate that cephalexin was more effective in inhibiting the growth of both bacteria when compared to MCME.

The negative control, 95% methanol, did not exhibit any significant antibacterial activity against either *S. aureus* or *E. coli*, indicating that the observed activity of MCME was not due to any residual solvent effects.

Table 3. Mean Zone of Inhibition in Diameters (mm) of the Treatments against *S. aureus* and *E. coli* after 24 hours of Incubation

Treatments	***S. aureus***	***E. coli***
	Mean Zone of Inhibition in Diameters (mm)	**Mean Zone of Inhibition in Diameters (mm)**
Positive Control (Cephalexin)	30.5± 0.47	17± 0.47
Negative Control (95% Methanol)	1.5± 0.14*	0.55± 0.03*
M. cochinchinensis fruit extract	20.55± 0.05	10.75± 0.22

* The mean difference is significant at the 0.05 level. Means of all the treatments were compared with the positive control group (Cephalexin).

The findings of this study suggest that MCME fruit extract may have the potential as a natural antibacterial agent against *S. aureus*. This is consistent with previous studies that have reported the antimicrobial activity of other parts of MCME against a wide range of bacteria, including *S. aureus*, and *E. coli*.

The antibacterial activity of MCME may be attributed to its phytochemical content, which includes flavonoids, phenolics, and triterpenoids. These compounds have been reported to exhibit antimicrobial activity through various mechanisms, including disruption of cell membranes, inhibition of bacterial enzymes, and inhibition of bacterial growth. The results have been consistent with the works of Alkhafaji, Q., et al., and Puksee, P., et al. It was found that the seeds *M. cochinchinensis* exhibited antimicrobial properties amounting to 12.5 mg/ml and 8.170±0.290 mm, respectively [14,15].

However, the weak activity of MCME against *E. coli* suggests that the extract may have a limited spectrum of activity against Gram-negative bacteria. This is consistent with previous reports that have shown that Gram-negative bacteria are more resistant to natural antimicrobial agents due to the presence of an outer membrane that acts as a barrier to the penetration of antibacterial compounds [16,17].

3.4 Hypoglycemic Activity

The use of animals in the study and the experimental protocol was in compliance with the guidelines set by the Institutional Animal Care and Use Committee. The whole experiment was done in the Laboratory Animal Care Facility of Mariano Marcos State University. Hyperglycemia was induced in mice through oral gavage. Blood sugar level was measured after induction of hyperglycemia, 1 hour, 2 hours, 3 hours, and 24 hours of extract administration.

Administration of concentrated glucose (200 mg/kg) resulted in to increase in sugar levels thereby inducing hyperglycemia in animals, especially in the MCME which had the highest blood glucose level. As can be seen in Figure 1, an obvious decrease in blood glucose level was observed after 1 hour among all treatments after 1 hour except for the negative control (distilled water) and the untreated mice. It can be noticed that positive control is statistically comparable to the MCME and to the other treatment except for the untreated mice. The simultaneous decrease in blood glucose levels was observed after 2 and 3 hours of observation except for the untreated and the negative control as expected. The positive control (metformin) and the MCME have always been comparable throughout the experiment until the 24-hour observation wherein the peak of the hypoglycemic activity of both treatments was observed. Surprisingly, the MCME even exceeded the hypoglycemic activity of the Metformin on the last hour of observation having a 96.33 mg/dL difference from the initial observation.

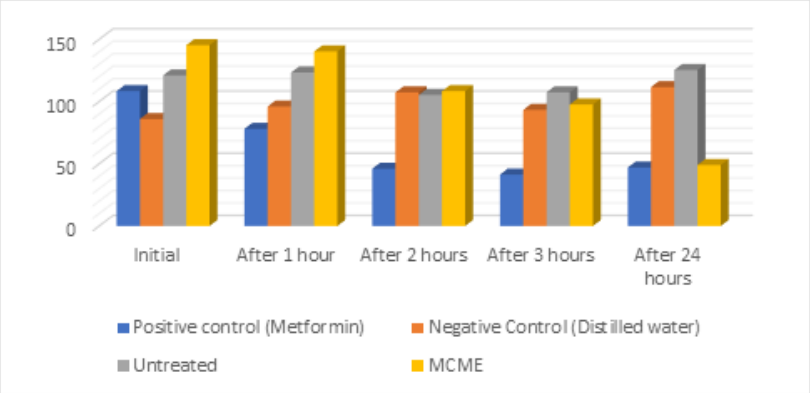

Fig 1. A Bar Graph Representation of the Blood Glucose Levels (mg/dL) after 1, 2, 3, and 24 hour(s) of treatment

The presence of flavonoids, phenolic compounds, and other constituents in the fruit of the plant may be responsible for its hypoglycemic effect, as not all phytochemical constituents were tested. These constituents may act together or independently to lower blood sugar levels. It is hypothesized that flavonoids play a significant role in the hypoglycemic effect of the plant fruit extract, as they are polyphenolic and known to have hypoglycemic effects [18–20].

4 Conclusion

This study represents the first attempt to investigate the hypoglycemic effect of *Momordica cochinchinensis* fruit methanolic extract (MCME) through a comprehensive evaluation of its biological and pharmacological activities. The findings bridge the gap between the traditional use of the plant in folkloric medicine and its actual efficacy. The phytochemical analysis revealed the presence of significant constituents, including saponins, flavonoids, and phenolic compounds in the extract. Moreover, it demonstrated antimicrobial properties, including the first-time observation of antibacterial activity against *E. coli* (about 10.75 mm). Importantly, the extract had more efficient hypoglycemic activity compared to the positive control, Metformin, in tests conducted on hyperglycemic mice (about 49.82% change and 13.72% change in blood glucose levels, respectively). These promising results suggest that MCME holds potential as a natural remedy for various health conditions although further research is necessary to determine its safety and effectiveness for human use.

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References

1) Othman HS, Rahman NAA, Nizam NIM. Preliminary Morphological and Phytochemical Evaluation of Momordica cochinchinensis Spreng. *Acta Chemica Malaysia*. 2020;4(1):1–8. Available from: https://doi.org/10.2478/acmy-2020-0001.
2) Anunthawan T, Phosri S, Woraratphoka J, Somwatcharajit R, Terdsaksri P, Prangphet P. Effects of Momordica cochinchinensis Aril Extract on Sterilized Low-Fat Milk, Antioxidant and Antiproliferative Activities. *International Journal of Food Science*. 2022;2022:1–9. Available from: https://doi.org/10.1155/2022/7934454.

3) Abdulqader A, Faisal A, Ismail A, Esa NM. Antioxidant compounds and capacities of Gac (Momordica cochinchinensis Spreng) fruits. *Asian Pacific Journal of Tropical Biomedicine*. 2019;9(4):158–167. Available from: https://journals.lww.com/aptb/Fulltext/2019/09040/Antioxidant_compounds_and_capacities_of_Gac.4.aspx.
4) Wimalasiri D, Dekiwadia C, Fong SY, Piva TJ, Huynh T. Anticancer activity of Momordica cochinchinensis (red gac) aril and the impact of varietal diversity. *BMC Complementary Medicine and Therapies*. 2020;20(1):1–15. Available from: https://doi.org/10.1186/s12906-020-03122-z.
5) Ali A, Ali F, Ismail A, Esa NM. Antioxidant compounds and capacities of Gac (Momordica cochinchinensis Spreng) fruits. *Asian Pacific Journal of Tropical Biomedicine*. 2019;9(4):158–167. Available from: https://journals.lww.com/aptb/Fulltext/2019/09040/Antioxidant_compounds_and_capacities_of_Gac.4.aspx.
6) Nerdy N, Lestari P, Sinaga JP, Ginting S, Zebua NF, Mierza V, et al. Brine Shrimp (Artemia salina Leach.) Lethality Test of Ethanolic Extract from Green Betel (Piper betle Linn.) and Red Betel (Piper crocatum Ruiz and Pav.) through the Soxhletation Method for Cytotoxicity Test. *Open Access Macedonian Journal of Medical Sciences*. 2021;9(A):407–412. Available from: https://doi.org/10.3889/oamjms.2021.6171.
7) Sharma K, Kaur R, Kumar S, Saini RK, Sharma S, Pawde SV, et al. Saponins: A concise review on food related aspects, applications and health implications. *Food Chemistry Advances*. 2023;2:1–9. Available from: https://doi.org/10.1016/j.focha.2023.100191.
8) Mutha RE, Tatiya AU, Surana SJ. Flavonoids as natural phenolic compounds and their role in therapeutics: An overview. *Future Journal of Pharmaceutical Sciences*. 2021;7(1):1–13. Available from: https://doi.org/10.1186/s43094-020-00161-8.
9) Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, et al. Important Flavonoids and Their Role as a Therapeutic Agent. *Molecules*. 2020;25(22):1–39. Available from: https://doi.org/10.3390/molecules25225243.
10) Serwecińska L. Antimicrobials and Antibiotic-Resistant Bacteria: A Risk to the Environment and to Public Health. *Water*. 2020;12(12):1–17. Available from: https://doi.org/10.3390/w12123313.
11) Banday MZ, Sameer AS, Nissar S. Pathophysiology of diabetes: An overview. *Avicenna Journal of Medicine*. 2020;10(4):174–188. Available from: https://www.thieme-connect.de/products/ejournals/abstract/10.4103/ajm.ajm_53_20.
12) Marnpae M, Chusak C, Balmori V, Kamonsuwan K, Dahlan W, Nhujak T, et al. Probiotic Gac fruit beverage fermented with Lactobacillus paracasei: Physiochemical properties, phytochemicals, antioxidant activities, functional properties, and volatile flavor compounds. *LWT - Food Science and Technology*. 2022;169:1–9. Available from: https://doi.org/10.1016/j.lwt.2022.113986.
13) Clemen-Pascual LM, Macahig RAS, Rojas NRL. Comparative toxicity, phytochemistry, and use of 53 Philippine medicinal plants. *Toxicology Reports*. 2022;9:22–35. Available from: https://doi.org/10.1016/j.toxrep.2021.12.002.
14) Alkhafaji Q, Mohammed IH, Saadedin S, Al-Awadei S, Abedulhussein TM, Al-Jussani G. Antimicrobial Activity of Momordica cochinchinensis Seeds and Seeds Aril Extract. *Jordan Journal of Earth and Environmental Sciences*. 2019;10(4):252–255. Available from: https://www.researchgate.net/publication/339163271_Antimicrobial_Activity_of_Momordica_cochinchinensis_Seeds_and_Seeds_Aril_Extract.
15) Puksee P, Fakkham S, Sangvichien S, Chinakran T, Arnmanee P. The Study on Phytochemical and Antimicrobial Activities of Momordica cochinchinensis (Lour.) Spreng. Seeds Extract. *Princess of Naradhiwas University Journal*. 2023;15(1):1–17. Available from: https://li01.tci-thaijo.org/index.php/pnujr/article/view/254265.
16) Breijyeh Z, Jubeh B, Karaman R. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. *Molecules*. 2020;25(6):1–23. Available from: https://doi.org/10.3390/molecules25061340.
17) Helmy YA, Taha-Abdelaziz K, Hawwas HAEHAE, Ghosh S, Alkafaas SS, Moawad MMM, et al. Antimicrobial Resistance and Recent Alternatives to Antibiotics for the Control of Bacterial Pathogens with an Emphasis on Foodborne Pathogens. *Antibiotics*. 2023;12(2):1–52. Available from: https://doi.org/10.3390/antibiotics12020274.
18) Yodsaoue O, Chansakaow S, Sirirugsa P, Rojsitthisak P. Flavonoids and phenols, the potential anti-diabetic compounds from Bauhinia strychnifolia Craib. stem. *Molecules*. 2022;27(8):1–16. Available from: https://doi.org/10.3390/molecules27082393.
19) Van LV, Pham EC, Nguyen CV, Duong NTN, Thi TVL, Truong TN. In vitro and in vivo antidiabetic activity, isolation of flavonoids, and in silico molecular docking of stem extract of Merremia tridentata (L.). *Biomedicine & Pharmacotherapy*. 2022;146:1–17. Available from: https://doi.org/10.1016/j.biopha.2021.112611.
20) Shamsudin NF, Ahmed QU, Mahmood S, Shah SAA, Sarian MN, Khattak MMAK, et al. Flavonoids as Antidiabetic and Anti-Inflammatory Agents: A Review on Structural Activity Relationship-Based Studies and Meta-Analysis. *International Journal of Molecular Sciences*. 2022;23(20):1–35. Available from: https://doi.org/10.3390/ijms232012605.