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ORIGINAL ARTICLE **OPEN ACCESS**

Immunomodulatory Activity of Extracts of *Bauhinia Racemosa* Lam. Leaves.

Prajakta R. More[1*], Sangeeta S. Tanavade[1], Nutan M. Dudhal[2], Vidya D. Atole[3], Snehal S. More[4]
[*1]Department of Pharmaceutical Chemistry, Appasaheb Birnale College of Pharmacy, Sangli (MS)-416416
[2]Department of Pharmaceutical Chemistry, College of Pharmacy, Akluj
[3]Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Science and Research for Girls, Swami Chincholi, India
[4]Shri Ganpati Institute of Pharmaceutical Sciences and Research, Tembhurni
*Corresponding author email: moreprajacta6655@gmail.com

ABSTRACT
Bauhinia Racemosa Lam. is a rare medicinal species belonging to the Caesalpiniaceae family and utilized historically for a variety of medical purposes. The study was designed to investigate the immunomodulatory activity of aqueous and ethanolic extracts of leaves of Bauhinia Racemosa Lam. in wistar rats. The aqueous and ethanolic extracts were administered orally at a dose of 500 mg/kg/day of body weight in Wistar rats. The immunostimulatory activities on specific and non-specific immunity were studied by haemagglutination antibody (HA), delayed type hypersensitivity and carbon clearance test (CCT), using sheep red blood cells (SRBC) as the antigen. Distilled water served as a control in all the tests and cyclophosphamide (30 mg/kg), an induction agent, was used for disease control. Levamisole 50 mg/kg was used as a standard. Humoral antibody response was found to be increased significantly (P < 0.001) in haemagglutination antibody titre. Mean footpad thickness was found to be increased significantly (P < 0.0001) in delayed type hypersensitivity and Phagocytic index was found to be increased significantly (P < 0.0001) in carbon clearance test (CCT). The present study concludes that Bauhinia racemosa Lam. leaves showed significant immunomodulatory activity in both extracts.
Keywords: *Bauhinia Racemosa Lam., Caesalpiniaceae, Delayed type hypersensitivity, Antibody, Immunomodulatory activity.*

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INTRODUCTION

The immune system is involved in defending our bodies from particular diseases and microorganisms. A variety of external and endogenous factors may have an impact on the immune system's effectiveness and functionality. Certain substances, referred to as immunomodulators, have the ability to have pharmacological or biological impacts on the immune system. Finding parts of the host response that can be strengthened or inhibited in order to supplement or improve a desired immune response is the fundamental concept behind immunomodulation. [1]

Immunomodulators can be either synthetic medications or natural herbs. When the host defence mechanism needs to be activated in the presence of a compromised immune response, immunomodulation using herbal drugs can offer an alternative to conventional chemotherapy for a variety of diseases due to the severe side effects associated with synthetic drugs. Herbal medications are more readily available, less expensive, and less strong than synthetic prescription immunomodulators. They also have a lower risk of having negative effects. In order to provide a fresh method for the treatment of infectious diseases, it is necessary to look for plants that have immunomodulatory action. [2]

The small Caesalpiniaceae tree *Bauhinia racemosa* (Lam.), a rare medicinal species of flowering shrub, has religious significance. Between three and sixteen feet (10 to three metres) tall, it is a small, crooked tree with drooping limbs. White racemes with terminal or axillary blooms. March through June is when plants bloom. Fruit is an oblong, tightly packed, dark green pod that is frequently twisted. The bark is black and strong. The leaves have complete margins, are orbicular, bifolate, and alternate distichously. *Bauhinia racemosa* (Lam.) belongs to the Caesalpiniaceae Family. The name "Sittacha" (Tamil) is most frequently used to describe it and is regularly seen in India, Ceylon, China, and Timor. Pharmacological experiments on the plant reveal that the ethanol extract of *Bauhinia racemosa* (Lam.) leaves has antibacterial activity in addition to analgesic, antipyretic, antiinflammatory, and antispasmodic properties. In addition to these

compounds, the plant's leaves were also used to extract a minimum of five flavonols (including kaempferol and quercetin) and two coumarins (scopoletin and scopolin). The heartwood of *Bauhinia racemosa* (Lam.) could be separated from stilbene (resveratrol). [3,4]

Since COVID -19; Need of immunomodulator have been essential. Different herbal plants may be the source of immunomodulatory action, according to recent investigations and study. However, there are no reports on the experimental pharmacological research on the leaves of *Bauhinia Racemosa* Lam. In rural Maharashtra, Ayurvedic practitioners have employed the leaves of *Bauhinia Racemosa* Lam. to boost immunity and fight a variety of ailments. The present study was undertaken to measure the immunomodulatory activity of the aqueous and ethanolic extracts derived from the leaves of *Bauhinia Racemosa* Lam.

MATERIAL AND METHODS

Plant material

From Tembhurni (Solapur, Maharashtra), the *Bauhinia racemosa* Lam. plant's leaves were harvested in October 2022. The Kasturbai Walchand College in Sangli's Department of Botany carried out the plant material's authentication. To eliminate dirt and debris, the plant leaves were thoroughly cleaned. The foliage of the plant was air dried in the shade. Once the leaves had dried completely, they were ground into a coarse powder using a mixer grinder.

Extraction: [5]

The extraction was carried out using a variety of techniques.

1. Maceration
2. Soxhlet extraction

1. Maceration: Maceration was used to create an aqueous extract. A 500ml (1:9) solution of chloroform and water was made in one beaker. A small amount of dried powder, about 25gm, was added, blended, and wrapped in foil paper. Up to 7 days, this solution was mixed three times each day. After filtering, the marc was then compressed, and the filtrate was then collected. It was kept cold in the fridge. The first aqueous extraction was this one. Then, the identical process was done for aqueous extractions no. 2 and no. 3. **[6]**

2. Soxhlet extraction: Organic solvents like pet ether, chloroform, and ethanol were used for the extraction process. The dried leaf powder of *Bauhinia racemosa* Lam. was extracted using pet ether and continuous Soxhlet extraction for around 30 g. Up till the solvent lost its colour, the extraction was continued. The pet ether extract was filtered, and the powder in the extraction device was taken out of the extractor, dried, and utilised for extraction with chloroform and ethanol. These pet ether, chloroform, and ethanol extracts were kept in separate bottles and marked. **[7-8]**

Both aqueous and ethanolic extracts was further used for pharmacological screening.

Pharmacological screening

Immunomodulatory activity

Animals

Male wistar rats weighing 200-250 gm were obtained from Crystal Biological Solutions, Pune. All rats were fed with pellet diet, and water ad. libitum. rats were maintained at 22 ± 3°C with 55± 5% relative humidity, and kept under 12-hrs light and dark cycles. The animals were allowed to adapt to laboratory conditions prior to experimentation. All experiments were conducted during the light period of 12 hours of the day/night cycle. All the experiments were permitted and conducted as per the guidelines of Institutional Animal Ethical Committee (Approval no. CRY/2223/121). **[9-10]**

Figure 1: Wistar rats

Study design

Six animals in each group were used for this study, Extracts were dosed as per standard protocol and animals were observed for signs and symptoms along with weekly Body weight.

Collection of Sheep RBC

Sheep RBC (SRBC) were collected in Alsever's solution, washed three times in phosphate buffered saline (PBS) and adjusted to a concentration of 0.5×109 cells/ml.

Alsever's solution (Dextrose - 2 g, Trisodium citrate dehydrate - 0.8 g,Citric acid monohydrate - 0.055 g, Sodium chloride - 2.1 g, Distilled water - 100 ml).

Phosphate buffered saline (Sodium chloride – 8 g, Potassium chloride - 0.2 g, Disodium hydrogen phosphate - 1.15 g, Potassium dihydrogen phosphate - 0.2 g, Magnesium chloride - 0.1 g, Calcium chloride - 0.1 g, Distilled water - 100 ml).

Evaluation of Immunomodulatory Activity

Treatment Protocol

An experimental laboratory-based study was done following standard methods and procedures. Five experimental groups (I, II, III, IV, V) each comprising of six animals were used. Group I received vehicle. Groups II received Cyclophosphamide 30mg/kg i.p. on 18th, 19th and 20th day. Group III received Levamisole 50 mg/kg p.o of an immunostimulatory drug. Groups IV received (aqueous extract) and V (ethanolic extract) were dosed daily with extract 500mg/kg, respectively, using an intragastric tube. Delayed-type hypersensitivity reaction (DTH), Carbon Clearance test (CCT) and hemagglutination antibody titer were determined using standard methods and procedures. **[11-13]**

Hemagglutination Antibody (HA) Titre

Day 0- Rats were immunized by injecting 0.1 ml of SRBCs suspension containing 0.5 × 109 cells intraperitoneally.

Day 0- day 21- Vehicle, Test drug and standard drug were given orally upto 21 Days. Cyclophosphamide was given 50mg/kg on day 18th, 19th, and 20th. Blood was collected in micro-centrifuge tubes by retro-orbital puncture. Half Blood was stored and used for Lipid peroxidation (LPO) estimation, Super oxide dismutase (SOD) estimation, Catalase (CAT) estimation and Glutathione (GSH) estimation will be done as per standard protocol. Blood was centrifuged and serum obtained of each group were used for hemagglutination assay. Two-fold serial dilutions of serum made in 25 microliters of normal saline in microtitration plates + 25 microliter of 1% suspension of SRBCs in saline. After mixing, plates were incubated at 37 °C for 2 h & examined for hemagglutination Antibody levels were determined by taking the reciprocal of the highest dilution of the test serum agglutination as the antibody titer.

Delayed- type Hypersensitivity (DTH) Response

Day 0- Foot thickness was measured and rats were challenged by injection of 0.5 × 109 cells SRBCs in right hind foot pad. Foot thickness was measured after 24 hrs. and 48 Hrs. Day 0 -Day 21- Vehicle, extracts and standard herbal drug were given orally. Day 18,19,20- Cyclophosphamide 30mg/kg i.p on 18th, 19th & 20th day. Right hind footpad thickness was measured with Vernier caliper on 21st day (prior to injection), 22ndand 23rdday of the study. Difference between prior and post injection footpad thickness was reported as DTH response. **[14-15]**

Carbon Clearance Assay (Phagocytic Response)

For 21 days, the rats were treated with extracts and standard drug. On 21stday, rats are treated by an intravenous injection of 0.2 ml/animal Indian ink dispersion (pre-warmed at 37 °C) through tail vein. 0, 5, 10 & 15 min, Blood is collected in micro-centrifuge tubes by retro-orbital puncture. 25µl of blood samples are added to 2 ml of 0.1% Na2CO3 solution. Absorbance is measured at 660 nm using UV visible spectrophotometer. Rate of carbon clearance (phagocytic index, K) is calculated k (LogeOD1 – LogeOD2)/15. **[16-17]**

Statistical Analysis

Data is expressed as Mean ± S.E.M. was analysed for significance of variance by one-way ANOVA followed by student T test using Graph Pad prism Software. **[18]**

RESULTS AND DISCUSSION

Haemagglutination antibody (HA) Titre:

Table 1: Effect of leaves of *Bauhinia Racemosa* Lam. on Haemagglutination antibody Titre

Group	HA Titre
Control	7.33 ±1.63**
(Change) Disease Control	3.00 ± 1.10
Standard	26.67 ± 8.26**
Experimental-1 (Aqueous extract)	17.33 ± 7.87 *
Experimental- 2 (Ethanoilic extract)	24.00 ± 8.76 **

Results are expressed as mean ± S.E.M (n=6), one-way ANNOVA followed by Student T test. *P <0.05, **P<0.001, ***P < 0.001 ****P < 0.0001 When compared with disease control group.

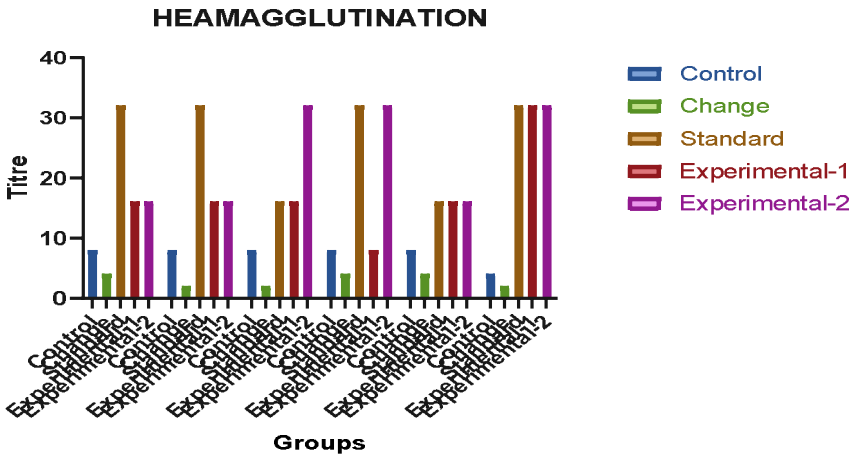

Figure 1: The effect of leaves of *Bauhinia racemosa* Lam. on humoral immune response (HA Titre).

As compare to disease control group all the animals showed significant result. The HA titer was used to assess humoral immune response. Administration of both the tested groups and standard produced a significant increase in HA titer as evident from hemagglutination after incubation of the serum with SRBCs. The antibody titre for the control and disase control group was found to be 7.33 ±1.63 and 3.00 ± 1.10 respectively. Aqueous and ethanolic extracts the antibody titre is 17.33 ± 7.87 and 24.00 ± 8.76 respectively. Ethanolic extract was found to be effective than Aqueous extract when compared with the disease control group showed a possible immunostimulant effect.

Delayed-Type Hypersensitivity Response

Table 2: Effect of leaves of *Bauhinia Racemosa* Lam. on Delayed-Type Hypersensitivity Response.

Group	Rats paw volume in mm (48 hrs)
Control	0.01 ± 0.00****
(Change) Disease Control	2.31 ± 0.11
Standard	1.23 ± 0.03****
Experimental-1 (Aqueous extract)	1.30 ± 0.01****
Experimental-19 ethanolic extract)	1.38 ± 0.03****

Results are expressed as mean ± S.E.M (n=6), One-way ANNOVA followed by student T test. *P <0.05, **P<0.001, ***P < 0.001 ****P < 0.0001 When compared with disease control group.

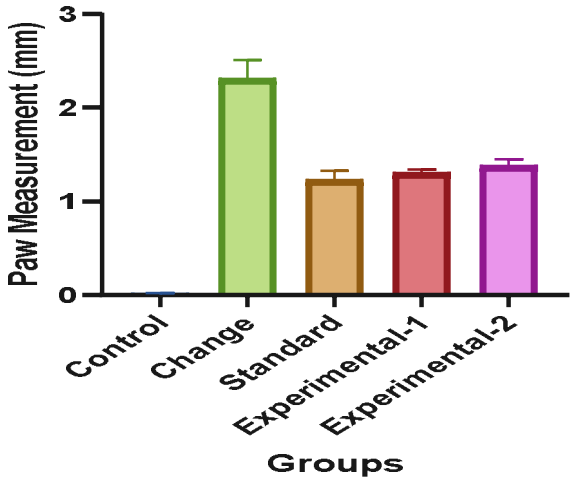

Figure 2: The effect of leaves of *Bauhinia racemosa* Lam. on Delayed Type Hypersensitivity (DTH).

Effect of *Bauhinia Racemosa* Lam. leaves extracts on cell-mediated immune response by DTH-induced footpad oedema is measured by footpad thickness in the hind paw. The mean difference for the control and disease group was found to be 0.01 ± 0.00 and 2.31 ± 0.11 respectively. Also mean diference of aqueous

and ethanolic extracts shows 1.30 ± 0.01 and 1.38 ± 0.03 respectively. The mean difference of ethanolic extract is greater than that of aqueous extract. when compared with the disease control groups, showed a possible immunostimulant effect.

Carbon Clearance Assay (CCT)

Table 3: Effect of leaves of *Bauhinia Racemosa* Lam. on Phagocytic response (Carbon Clearance Assay)

Group	Phagocytic index
Control	0.003845 ± 0.000681***
(Change) Disease Control	0.000731 ± 0.000329
Standard	0.011734 ± 0.00231****
Experimental-1 (Aqueous extract)	0.005900 ± 0.00050****
Experimental- 2 (Ethanoilic extract)	0.007176 ± 0.00121****

Results are expressed as mean ± S.E.M (n=6), one-way ANNOVA followed by Student T test. *P <0.05, **P<0.001, ***P < 0.001 ****P < 0.0001 When compared with disease control group.

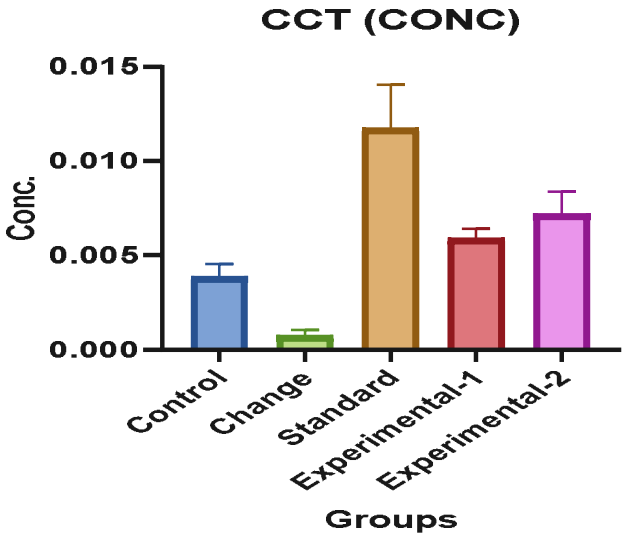

Figure 3: The effect of leaves of *Bauhinia racemosa* Lam. on Phagocytic response (Carbon Clearance Assay)

The rate of elimination of carbon particles from the blood stream is typically used to estimate the phagocytic activity of the reticuloendothelial system. The phagocytic index for the control and disease control group was found to be 0.003845 ± 0.000681 and 0.000731 ± 0.000329 respectively. experimental-2 Ethanolic extract showed highest phagocytic index (0.007176 ± 0.001214) than experimental-1 Aqueous extract (0.005900 ± 0.000508). when compare with disease control group showed possible immunostimulant effect.

CONCLUSION

Leaves of *Bauhinia racemosa* Lam. showed significant Immunomodulatory activity at both the extracts. In all the immunomodulatory model's significant activity was observed. Ethanolic extract was found to have more significance when compared with disease control group.

REFERENCES

1. Agarwal, S.S. and V. K. Singh. (1999). Immunomodulators: A review of studies on Indian medicinal plants and synthetic peptides Part I: Medicinal Plants; P1NSA 65, 179-204.
2. Srikumar, R., Jeya, P.N., Manikandan, S., Sathya, et al. (2006). Effect of Triphala on oxidative stress and on cell mediated immune response against noise stress in rats. Mol. Cell Biochem. 283, 67-74.
3. https://en.wikipedia.org/wiki/*Bauhinia racemosa*
4. R.S. Kumar, T. Sivakumar, R.S. Sunderam, et al. (2005). Antioxidant and antimicrobial activities of *Bauhinia racemosa* L. stem bark. Brazilian journal of medical and biological research. 38(7); 1015-1024.
5. Khadbadi SS, Deore SL, Baviskar BA. (2013). Experimental pharmacognosy. 2nd ed; Nirali prakashan, p. 1.2, 1.3, 2.4, 2.5.3.1-3.9.
6. Mazumder PM, Pattnayak S, Parvani H, et al. (2012). Evaluation of immunomodulatory activity of Glycyrhiza glabra L roots in combination with zing. Asian Pac J Trop Biomed ;1:15-S20.

7. Manjeshwar SB, Ramakrishna JP, Harshith PB,et al. (2011). Chemistry and medicinal properties of the Bakul (*Mimusops elengi* Linn): A review. Food Res Int;44:1823-9.
8. Aher VD, Wahi VA. (2010). Pharmacological study of *Tinospora Cordifolia* as an immunomodulator. Int J Curr Pharm Res ; 2:52-54.
9. Patel S, Banji D, Banji OJ, Patel MM, Shah KK. Scrutinizing the role of aqueous extract of Trapa bispinosa as an immunomodulator in experimental animals. Int J Res Pharm Sci 2010; 1:13-9.
10. Balchandran P, Panchanathan S. (1998). Immunomodulatory activity of *Semecarpus anacardium* Linn. Nut milk extract in aflatoxin B1-induced hepatocellular carcinoma in rats. J.Ethnopharmacol, 4, 507–510.
11. Agarwal, S.S. and V. K. Singh. (1999). Immunomodulators: A review of studies on Indian medicinal plants and synthetic peptides Part I: Medicinal Plants; P1NSA 65, 179-204.
12. Srikumar, R., Jeya, P.N., Manikandan, S., Sathya, et al. Effect of Triphala on oxidative stress and on cell mediated immune response against noise stress in rats. Mol. Cell Biochem. (2006). 283, 67-74.
13. R.S. Kumar, T. Sivakumar, R.S. Sunderam, et al. Antioxidant and antimicrobial activities of *Bauhinia racemosa* L. stem bark. Brazilian journal of medical and biological research. 2005; 38(7); 1015-1024.
14. Khadbadi SS, Deore SL, Baviskar BA. Experimental pharmacognocy. 2nd ed; Nirali prakashan, 2013. p. 1.2, 1.3, 2.4, 2.5.3.1-3.9.
15. Mazumder PM, Pattnayak S, Parvani H, et al. (2012). Evaluation of immunomodulatory activity of Glycyrhiza glabra L roots in combination with zing. Asian Pac J Trop Biomed ;1:15-S20.
16. Manjeshwar SB, Ramakrishna JP, Harshith PB,et al. (2011). Chemistry and medicinal properties of the Bakul (*Mimusops elengi* Linn): A review. Food Res Int;44:1823-9.
17. Aher VD, Wahi VA. (2010). Pharmacological study of *Tinospora Cordifolia* as an immunomodulator. Int J Curr Pharm Res ; 2:52-54.
18. Patel S, Banji D, Banji OJ, Patel MM, Shah KK. (2010). Scrutinizing the role of aqueous extract of *Trapa bispinosa* as an immunomodulator in experimental animals. Int J Res Pharm Sci; 1:13-9.
19. Balchandran P, Panchanathan S. (1998). Immunomodulatory activity of Semecarpus anacardium Linn. Nut milk extract in aflatoxin B1-induced hepatocellular carcinoma in rats. J.Ethnopharmacol, 4, 507–510.
20. Galatage ST, Trivedi R, Bhagwat DA. (2010). Characterization of camptothecin by analytical methods and determination of anticancer potential against prostate cancer. Future Journal of Pharmaceutical science,202;7(104):1-9. doi.org/10.1186/s 43094- 021- 00236-0.
21. Galatage ST, Hebalkar AS,Killedar SG (2020).Design and Characterization of camptothecin gel for the treatment of epidermoid carcinoma.Future journal of pharmaceutical sciences.2020,6:50;1-11.
22. Galatage, S. T., Trivedi, R., & Bhagwat, D. A., Oral self-emulsifying nanoemulsion systems for enhancing dissolution, bioavailability and anticancer effects of camptothecin. *Journal of Drug Delivery Science and Technology*, *78* (2022) 103929.
23. Galatage ST, Manjappa AS, Bhagwat DA, Trivedi R, Salawi A, Sabei FY, Alsalhi A. Oral self- nanoemulsifying drug delivery systems for enhancing bioavailability and anticancer potential of fosfestrol: In vitro and In vivo characterization. Eur J Pharm Biopharm. 2023;17:S0939-6411(23)00273-4. doi: 10.1016/j.ejpb.2023.10.013.
24. Galatage, S.T., Hebalkar, A.S., Gote, R.V. Design and characterization of camptothecin gel for treatment of epidermoid carcinoma. Futur J Pharm Sci,2020; 6, 50. https://doi.org/10.1186/s43094-020-00066-6.
25. Galatage ST, Manjappa AS, Kumbhar PS, Salawi A, Sabei FY, Siddiqui AM, Patil RV, Akole VS, Powar RD, Kagale MN. Synthesis of silver nanoparticles using *Emilia sonchifolia* plant for treatment of bloodstream diseases caused by Escherichia coli. Ann Pharm Fr. 2022 Dec 15:S0003-4509(22)00179-1. doi: 10.1016/j.pharma.2022.12.007.
26. Kodoli, R.S., Galatage, S.T., Killedar, S.G. et al. Hepatoprotective activity of Phyllanthus niruri Linn. endophytes. Futur J Pharm Sci 7, 97 (2021). https://doi.org/10.1186/s43094-021-00243-1.
27. Gudise V, Chowdhury B, Manjappa AS.(2019). *In vitro* free radical scavenging and antidiabetic activity of aqueous and ethanolic leaf extracts: a comparative evaluation of *Argyreia pierreana* and *Matelea denticulata*. Future Journal of Pharmaceutical Sciences. ;5(1):1-1.

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