Int.J.Curr.Microbiol.App.Sci (2025) *14*(02): 136-143

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 14 Number 2 (2025)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2025.1402.012

Phytochemical analysis, *in vitro* Anti-diabetic and Anti-inflammatory activity of Chloroform extract of *Artocarpus hirsutus* Root

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Keywords

Artocarpus hirsutus, Antidiabetic activity, Anti-inflammatory activity, α-amylase, α-glucosidase, IC50

Article Info

Received:
14 December 2024
Accepted:
29 January 2025
Available Online:
10 February 2025

A B S T R A C T

The incidence and prevalence of diabetes mellitus have risen significantly in recent decades. Our study therefore concentrated on evaluating the phytochemical analysis, anti-inflammatory activity, and antidiabetic activity. Enzyme inhibition has antidiabetic effects in vitro, while protein denaturation produces anti-inflammatory effects. Reducing the synthesis and absorption of glucose in the gastrointestinal tract by inhibiting enzymes that break down carbohydrates, such as α-amylase and α-glucosidase, is one method of treating diabetes. A key approach in blood glucose management is the inhibition of the enzyme's amylase and glucosidase, which are involved in the digestion of carbohydrates. This can considerably reduce the postprandial rise in blood glucose following a mixed carbohydrate diet. According to our testing results, *Artocarpus hirsutus* chloroform extract shows a dose-dependent increase in % inhibitory activity on the enzymes α-glucosidase (IC50 300 µg/ml µg/ml) and α-amylase (IC50 166 µg/ml). As a standard medication, acarbose was used. The Denaturation of proteins was inhibited by chloroform extract. The root extract of *A. hirsutus* (100–500 µg/mL) decreased the denaturation of egg albumin in a dose-dependent manner. The extract's anti-inflammatory qualities, as evaluated in vitro, were comparable to those of the reference drug diclofenac sodium (100 and 200 µg/mL). At 200 µg/mL, the inhibitory actions of diclofenac sodium and chloroform extract were very similar.

Introduction

The chronic endocrine condition known as diabetes mellitus impairs the metabolism of water, proteins, fats, carbs, and electrolytes. It comprises a class of metabolic disorders known as hyperglycemia, in which there is an increase in blood sugar levels due to either insufficient insulin synthesis by the pancreas or insufficient cell response to the insulin that is produced (Sangeetha *et al.,* 2015).

The World Health Organization estimates that 3% of people worldwide suffer from diabetes, and by 2025, the prevalence is predicted to rise (Megha *et al.,* 2013).

There are around 400 traditional plants that have been used to cure diabetes (Upwar *et al.,* 2011). In India, diabetes mellitus is on the rise due to societal influences and shifting lifestyles. For centuries, diabetes was considered "a disease of rich man" in India, but it has since spread to all social classes (Gupta and Misra, 2007). The prevalent medication used to treat inflammatory diseases is non-steroidal anti-inflammatory medicines, which have negative side effects, particularly causing gastrointestinal irritation that can result in the development of gastric ulcers. Inflammation is a significant issue (Sangita *et al.,* 2012). *Artocarpus hirsutus* belongs to the Moraceae family, which has about fifty species. Skin conditions, snake bites, diarrhea, joint discomfort, and ulcers have all been treated with it in the past. Throughout India, they are particularly prevalent in Kerala and Kanyakumari. The fruit is tasty, and the wood is used to manufacture furniture. It is commonly referred to as wild jack. Proteins, carbohydrates, glycosides, alkaloids, flavonoids, phenolic compounds, terpenoids, lactones, tannins, and steroids are all present in *A. hirsutus*. Their physiologic advantages include improved cardiovascular health, anti-aging, anti-carcinogen, anti-inflammatory, and anti-apoptosis (Shervinjose and Vinothini, 2024).

The root of *Artocarpus hirsutus* containing antioxidant and anti-cancer properties and root of ethanolic extract contain Ergosterol peroxide, alpha-amyrin which is responsible for prevention and treatment of cancer (Vinothini and Shervinjose, 2024). Numerous investigations have been done on different Artocarpus species, and they also have additional pharmacological qualities like anti-inflammatory, antifungal, immunomodulatory, anticholinergic, chelating, cosmetic, ACE, anthelmintic, protease, inhibition of melanin biosynthesis, and wound-healing effects (Hari *et al.,* 2014). Thus, objective of our study is to investigate the root of *Artocarpus hirsutus* for its *invitro* antidiabetic and anti-inflammatory properties.

Materials and Methods

Plant Material

The root of *Artocarpus hirsutus* were collected from nithiravilai, Kanyakumari district in October and authenticated by Dr. D. Stephen Ph.D., Lecturer, Department of Botany, The American College, Madurai 625020.

Drugs and chemicals

The following were purchased from SD Fine Pvt. Ltd., Mumbai: potato starch, trichloroacetic acid, and Folin-Ciocalteau reagents; 3,5-dinitrosalicylic acid, Tris buffer, linoleic acid, and ammonium molybdate; and α-amylase and α-glucosidase enzymes from Hi-Media Pvt. Ltd., Mumbai. Every other chemical utilized in the research was of analytical quality and purchased commercially.

Preparation of Extract

After shade-drying at room temperature, the plant roots were ground into a coarse powder using a dry grinder. Chloroform (250 mL) was used to extract 50 g of this coarse powder, which was then placed in a Soxhlet apparatus. The extraction process was repeated until the solvent in the siphon tube turned colorless. A rotary evaporator set at 60°C was used to evaporate the chloroform extract.

Qualitative phytochemical analysis

The chloroform extract was tested for the presence of various active phytoconstituents namely flavonoids, steroids, tannins, phenolic compounds, diterpenes, triterpenes, alkaloids, and saponins (Harborne, 1998).

In vitro methods employed in antidiabetic studies

Inhibition of alpha-amylase enzyme

500 µl of test samples and reference drug (100–1000 µg/ml) were combined with 500 µl of 0.20 mM phosphate buffer (pH 6.9) that contained a solution of α-amylase (0.5 mg/ml). The mixture was then incubated for 10 minutes at 25°C. Each tube was then filled with 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9). After that, the reaction mixtures were incubated for ten minutes at 25°C. A 1.0 ml solution of 3, 5 dinitrosalicylic acid colorant was used to halt the process. After five minutes of incubation in a boiling water bath, the test tubes were allowed to cool to room temperature.

After adding 10 milliliters of distilled water to the reaction mixture, it was diluted, and the absorbance at 540 nm was measured. 100% enzyme activity is represented by the control, which was carried out similarly by substituting a vehicle for the extract (Thalapaneni *et al.,* 2008; Heidari *et al.,* 2005).

Inhibition of alpha glucosidases enzyme

The inhibitory action was assessed by incubating a starch substrate solution (2 percent w/v maltose or sucrose) in 1 milliliter with 0.2 M Tris buffer pH 8.0 and different root extract concentrations for 5 minutes at 37°C. To start the process, 1 milliliter of α-glucosidase enzyme (1U/ml) was added, and it was then incubated for 10 minutes at 37°C. The reaction was then stopped by heating the reaction mixture in a boiling water bath for two minutes. The amount of liberated glucose is determined using the glucose peroxidase technique (Andrade-Cetto *et al.,* 2008; Matsuura *et al.,* 2002; Tietz, 1999).

Calculation of 50% Inhibitory Concentration (IC50)

The percentage scavenging activities at five distinct extract concentrations were used to determine the concentration of the root extracts needed to scavenge 50% of the radicals (IC50). The calculation of percentage inhibition (I%) was calculated by

I % = (Ac-As)/Ac X 100

where As is the sample's absorbance and Ac is the control's absorbance (Shai *et al.,* 2010).

Evaluation of anti-inflammatory activity

Inhibition of Albumin Denaturation

The anti-inflammatory properties of the *A. hirsutus* root extract were assessed using the slightly modified protein denaturation method outlined by Padmanabhan. The standard medication included the potent nonsteroidal anti-inflammatory medicine diclofenac sodium.

A reaction mixture comprising 2 mL of standard diclofenac sodium (100 and 200 µg/mL) or *Artocarpus hirsutus* root extract (100-500 µg/mL) at varying concentrations, along with 2.8 mL of phosphate buffered saline (pH 6.4), was combined with 2 mL of egg albumin (from fresh hen's egg) and incubated at (27 ± 1) °C for 15 min.

The reaction mixture was maintained in a water bath at 70 °C for 10 minutes to induce denaturation. After cooling, double-distilled water was used as a blank to measure the absorbance at 660 nm. Three duplicates of each experiment were conducted, and the average was calculated (Padmanabhan *et al.,* 2012). The following formula, the % inhibition of protein denaturation was determined using the following formula:

% inhibition= At -Ac /Ac ×100

Where, At =absorbance of test sample; Ab=absorbance of control

Results and Discussion

Phytochemical screening

The phytoconstituents found in chloroform extract include phenolic compounds, alkaloids, steroids, carbohydrates, saponins, flavonoids, and terpenoids. (table:1) The percentage yield was found to be 2.5 % w/w.

Evaluation of in vitro α-amylase inhibitory activity using *A. hirsutus* Root extract

The percent of inhibitory action against the α-amylase enzyme increased dose depended manner. The percentage inhibition was 31.72 ± 0.36 at a concentration of 100 µg/ml of extract and 76.12 ± 0.26 at 1000 µg/ml. According to the extract, the IC50 value was 300 µg/ml. Acarbose, a standard drug, with an IC50 value of 380 µg/ml.

Every determination was made in triplicate, and the mean ± SEM is used to express the results. The concentration of an inhibitor required for inhibiting 50% of its action under the test conditions is known as the IC50 value.

Evaluation of in vitro α-glucosidase inhibitory activity using *A. hirsutus* Root extract

The chloroform extract of *A. hirsutus* demonstrated a notable inhibitory effect on the enzyme α-glucosidase. The percentage inhibition increased concentration-dependent manner at doses of 100–1000 µg/ml of *A. hirsutus* extract.

The percentage inhibition ranged from 93.62 ± 0.20 for the highest concentration to 42.66 ± 0.33 for the lowest concentration, which was 100 µg/ml. The α-glucosidase inhibitory activity of positive control acarbose produced percentages of 42.66 ± 0.33 for 100 µg/ml and 93.62 ± 0.20 for 1000 µg/ml, whereas the dose needed for 50%

inhibition (IC50) was determined to be 166 µg/ml. It was discovered that the conventional medication acarbose had an IC50 value of 220 µg/ml against α-glucosidase.

Every determination was made in triplicate, and the mean ± SEM is used to express the results. The concentration of an inhibitor required for inhibiting 50% of its action under the test conditions is known as the IC50 value.

Inhibition of Albumin Denaturation

The given table shows how varying the dose of chloroform extract inhibited protein denaturation. *A. hirsutus* root extract (100–500 µg/mL) reduced egg albumin denaturation in a dose-dependent manner. When tested in vitro, the anti-inflammatory properties of the extract were similar to those of the reference medication diclofenac sodium (100 and 200 µg/mL). The chloroform extract and diclofenac sodium had almost similar inhibitory activities at a concentration of 200 µg/mL.

Insulin deficiency significantly disrupts water and electrolyte homeostasis and has an impact on the metabolism of fat, proteins, and carbohydrates (Frier and Fisher, 2006). For the medical community, managing diabetes without adverse effects remains a problem (Rhabaso Lhoret and Chiasson, 2004). More recent developments in the understanding of intestinal enzyme activity (α-amylase and α-glucosidase are both crucial for the digestion of carbohydrates and the absorption of glucose) have resulted in the creation of additional pharmaceutical medicines. More so than fasting blood glucose, a high postprandial blood glucose response is linked to the risk of cardiovascular illnesses and micro- and macrovascular problems in diabetes. The intestinal lumen and brush border membrane contain α-glucosidase enzymes, which are essential for the digestion of carbohydrates because they break down starch and oligosaccharides into monosaccharides before they can be absorbed. It was suggested that inhibiting the activity of these digestive enzymes would postpone the breakdown of oligosaccharides and starch, which would reduce glucose absorption and, as a result, lower the spike of postprandial blood glucose levels (Puls *et al.,* 1977).

An inhibitor of alpha-glucosidase slows down the absorption and digestion of carbohydrates. Acarbose and miglitol decrease the absorption of starch and disaccharides by acting as competitive inhibitors of α-glucosidases. It is therefore possible to lower postprandial (PP) blood glucose levels in patients with diabetes mellitus by blocking the absorption of carbohydrates after meals. When α-amylase and α-glucosidases were inhibited, the elevated postprandial (PP) blood glucose peaks in diabetics were decreased (Davis and Granner, 2001; Conforti *et al.,* 2005). The prevention and treatment of type 2 diabetes can be improved by an "anti-inflammatory" diet and exercise routine.

Type 2 diabetes develops as a result of internal inflammation in the body. Although it can be a consequence of diabetes, chronic inflammation is a risk factor for both type 1 and type 2 diabetes. Researchers found that individuals with type 2 diabetes had increased amounts of inflammation in their systems decades ago. Persons with type 2 diabetes frequently have higher levels of cytokines, which are inflammatory molecules, than persons without the disease.

Table.1 Result of Phytochemical analysis root of Artocarpus hirsutus chloroform extract

Constituents	Chloroform extract
Carbohydrate	Present
Alkaloids	Present
Glycosides	Absent
Saponins	Present
Protein and Aminoacids	Absent
Aminoacids	Absent
Phenolic compound	Present
Flavanoids	Present
Steroids	present
Terpenoids	Present

Table.2 α-amylase inhibition by root of *Artocarpus hirsutus* chloroform extract

Sample	Concentrationn (µg/ml)	% inhibition	IC 50 (µg/ml)
Chloroform extract	100 200 400 800 1000	31.72 ± 0.36 45.22 ± 0.22 56.60 ± 0.14 64.89 ± 0.28 76.12 ± 0.26	300 µg/ml
Acarbose	100 200 400 800 1000	30.55 ± 0.14 41.34 ± 0.37 53.22 ± 0.66 69.22 ± 0.27 74.57 ± 0.24	380 µg/ml

Table.3 α-glucosidase inhibition by root of *Artocarpus hirsutus* Chloroform extract

Sample	Concentrationn (µg/ml)	% inhibition	IC 50 (µg/ml)
Chloroform extract	100 200 400 800 1000	42.66 ± 0.33 54.12 ± 0.36 66.26 ± 0.24 76.29 ± 0.62 93.62 ± 0.20	166 µg/ml
Acarbose	100 200 400 800 1000	38.24 ± 0.22 48.44 ± 0.32 60.26 ± 0.26 74.22 ± 0.93 90.77 ± 0.21	220 µg/ml

Table.4 In-vitro anti-inflammatory effect of *A. hirsutus* root extract

Extract	Concentration (µg/ml)	Inhibition of protein of denaturation (%)
Chloroform extract	100 200 500	70.24 ± 1.22 94.36 ± 2.66 110.20 ± 1.24
Diclofenac sodium	100 200	82.57 ± 1.22 120.26 ± 2.87

Figure.1 Inhibition α-amylase by root of *Artocarpus hirsutus* chloroform extract

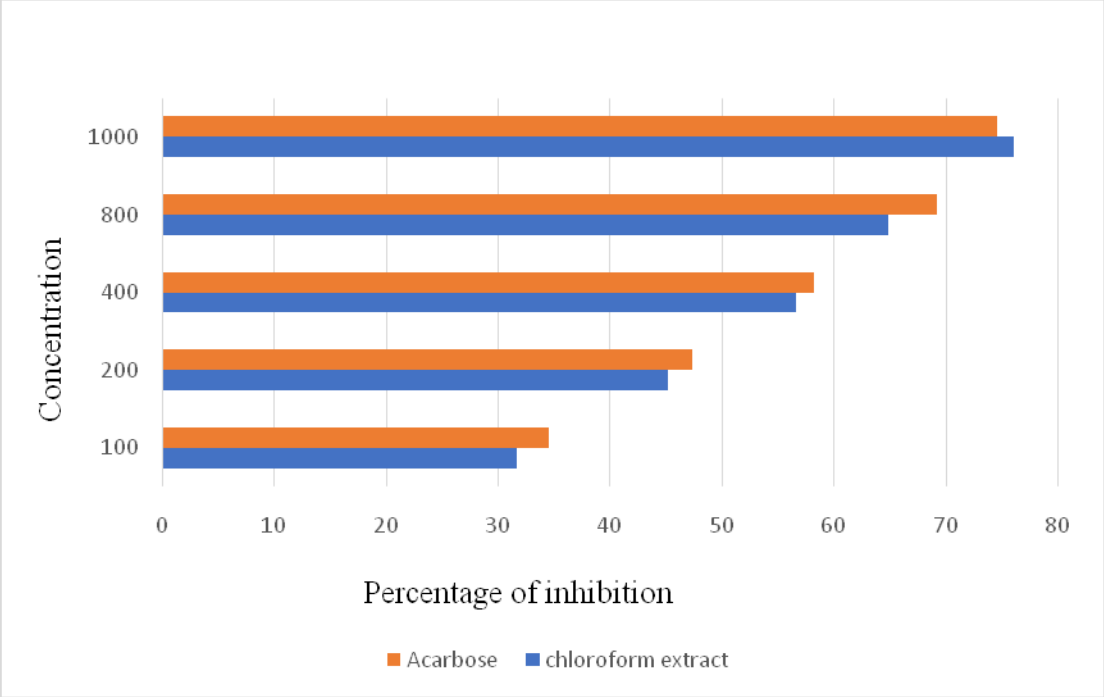
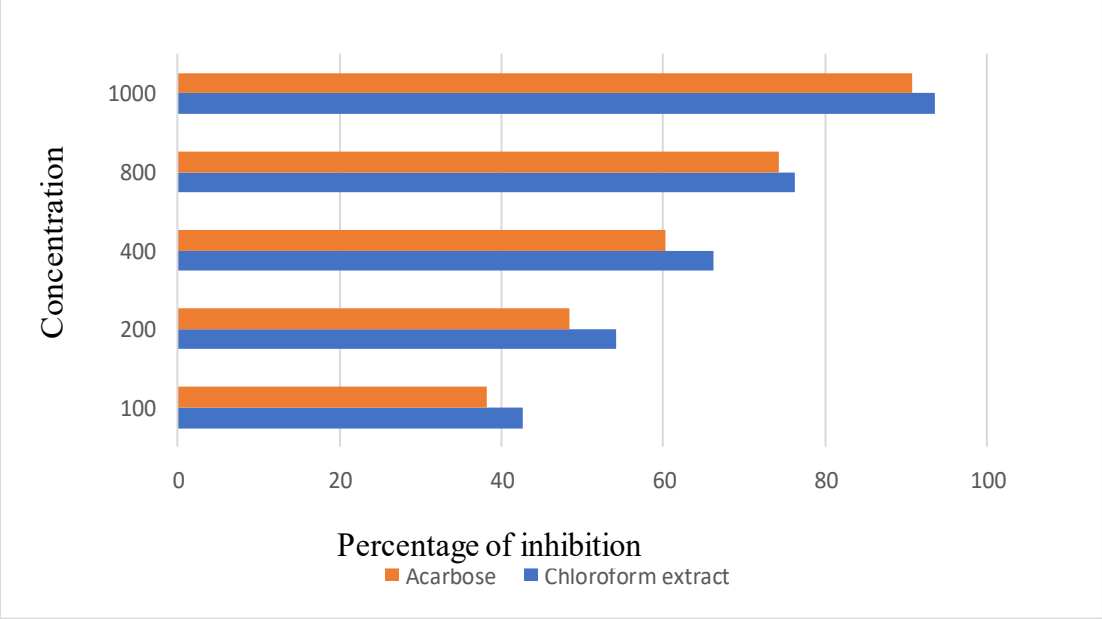


Figure.2 Inhibition α-glucosidase by root of *Artocarpus hirsutus* Chloroform extract



In vitro, *Artocarpus hirsutus* effectively inhibits the α-amylase enzyme, according to our findings. Unknown underlies the mechanisms that plant protein inhibitors use to inhibit α-amylase enzymes. However, there are indications that the plant protein flavanols may result in structural conformational changes. In vitro, the results indicate that *Artocarpus hirsutus* root chloroform extract effectively inhibits the α-glucosidase and α-amylase enzymes. The chloroform extract and diclofenac sodium had almost similar inhibitory activities at a concentration

of 200 µg/mL. The anti-inflammatory activity of the extracts is increased when the concentration increases.

Acknowledgements

We want to pay all my Respect and emotions to my beloved parents who have been with me as a pillar of love, support and care in all my difficulties and happiness which is the source of strength throughout our life. without whose blessings this task would not have been accomplished.

Author Contributions

S. Shervinjose: Investigation, formal analysis, writing—original draft. R. Roshinee: Validation, methodology, writing—reviewing. G. M. Vishal Bharathi:—Formal analysis, writing—review and editing. J. Jenisha: Investigation, writing—reviewing. S. Aishwarya Laxmi: Resources, investigation writing—reviewing. V. Vinslet: Validation, formal analysis, writing—reviewing. M. J. Nishani: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript.

Data Availability

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

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Andrade-Cetto A, Becerra-Jimenez J, Cardenas-Vazquez R. Journal of Ethnopharmacology 2008; 116: 27-32. https://doi.org/10.1016/j.jep.2007.10.031

Conforti F, Statti G, Loizzo MR, Sacchetti G, Poli F, Menichini F. Biological & Pharmaceutical Bulletin 2005; 28 (6): 1098-1102. https://doi.org/10.1248/bpb.28.1098

Davis SN, Granner DK. Insulin, oral hypoglycemic agents and the pharmacology of endocrine pancreas. In: Brunton LL, Lazo JS, Parker KL (Ed.), Goodman and Gilman's: The pharmacological basis of therapeutics, 11th ed. (McGraw-Hill Medical Publication Division: New York 2001) 1706-1707.

Frier BM, Fisher M. Diabetes mellitus. In: Boon NA, Colledge NR, Walker BR, Hunter JAA, (Ed.), Davidson's principle and practice of medicine, 20th ed.(Churchill Livingstone Elsevier: Ediburgh 2006) 805-845.

Gupta R, Misra A. British Journal of Diabetes and Vascular Disease 2007; 7: 12-16. http://dx.doi.org/10.1177/14746514070070010301

Harborne J B, Phytochemical methods: A guide to modern techniques of plant analysis, (Chapman and Hall, London), 1998.

Hari, A., Revikumar, K.G. and Divya, D., 2014. Artocarpus: A review of its phytochemistry and pharmacology. *Journal of Pharma Search*, *9*(1), pp.7-12.

Heidari R, Zareae S, Heidarizadeh M. Pakistan Journal of Nutrition 2005; 4 (2): 101-105. https://doi.org/10.3923/pjn.2005.101.105

Matsuura H, Asakawa C, Kurimoto M, Mizutani J. Bioscience Biotechnology and Biochemistry 2002; 66(7): 1576-1578. https://doi.org/10.1271/bbb.66.1576

Megha G, Chaudhari, Bhoomi B, Joshi, Kinnari N. Mistry, Brijesh Dabhi and Lal S. In vitro antimicrobial activity of stem bark of Bauhinia purpurea. Int Sci Press, 2013; (India),4:29-35.

Padmanabhan P, Jangle SN. Evaluation of in-vitro anti-inflammatory activity of herbal preparation, a combination of four herbal plants. Int J App Basic Med Sci 2012; 2(1): 109-116.

Puls W, Keup U, Krause HP, Thomas G, Hoffmeister F. Naturwissenschaften 1977; 64 :536 –537 (1977). https://doi.org/10.1007/BF00483562

Rhabaso Lhoret, R., and Chiasson, J.L.- Glucosidase inhibitors. In: Defronzo R.A, Ferrannini E, Keen H, Zimmet P. (Eds.), International Textbook of Diabetes Mellitus, Vol. 1, third ed. John Wiley and Sons Ltd., UK, 2004 pp. 901-914.

Sangeetha MS, Priyanga S, Hemmalakshmi S, Devaki K. In vivo antidiabetic potential of Cyclea peltata in

streptozotocin-induced- diabetic rats. Asian J Pharm Cli Res, 2015; 8:103-108.

Sangita C, Priyanka C, Protapaditya D, Sanjib B.Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pac J Trop Biomed, 2012; S178-S180. https://doi.org/10.1016/S2221-1691(12)60154-3

Shai LJ, Masoko P, Mokgotho MP, Magano SR, Mogale MA, Boaduo N, Eloff JN. South African Journal of Botany 2010; 76: 465–470. https://doi.org/10.1016/j.sajb.2010.03.002

Shervinjose, S. and Vinothini, K.V.World journal of pharmaceutical research.2024:Volume 13, Issue 6, 224-233.

Thalapaneni NR, Chidambaram KA, Ellappan T, Sabapati ML, Mandal SC. Inhibition of Carbohydrate Digestive Enzymes by *Talinum portulacifolium* (Forssk) Leaf Extract. Journal of Complementary and Integrative Medicine 2008; 5(1): 1-10. http://dx.doi.org/10.2202/1553-3840.1120

Tietz, N.W., 1999. In: Burtis, C.A., Ashwood, E.R. (Eds.), Tietz Textbook of Clinical Chemistry, third ed. Saunders W.B., pp. 750–778.

Upwar N, Patel R, Waseem N, Mahobia NK. Hypoglycemic effect of methanolic extract of *Berberis aristata* DC stem on normal and streptozotocin induced diabetic rats. Int J Pharm Pharm Sci, 2011; 3:222- 224.

Vinothini, K.V. and Shervinjose, S., evaluation of anti-oxidant and anti-cancer activity of root extracts of *Artocarpus hirsutus* by in-vitro methods. World journal of pharmaceutical research. 2024:Volume 13, Issue 18, 615-636. https://doi.org/10.20959/wjpr202418-33845

How to cite this article:

Shervinjose, S., R. Roshinee, G. M. Vishal Bharathi, J. Jenisha, S. Aishwarya Laxmi, V. Vinslet and Nishani, M. J. 2025. Phytochemical analysis, *in vitro* Anti-diabetic and Anti-inflammatory activity of Chloroform extract of *Artocarpus hirsutus* Root. *Int.J.Curr.Microbiol.App.Sci.* 14(02): 136-143.
doi: https://doi.org/10.20546/ijcmas.2025.1402.012