Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/nz5w1f12

TO STUDY THE POTENTIAL APPLICATION OF ANANAS COMOSUS L. FOR HYPERGLYCEMIC CONDITION

Abdullah Niaz[1*], Syeda Afroz[2], Tehmina Rabbani[3],Abdul Ghaffar[4], MariumTariq[5], NoorFatima[6], Hafsa Taimuri[7], Azmat Ara[8]

[1*]Faculty of biological, Pharmaceutical and health Sciences. UOB.
[2]Faculty of Pharmacy and Pharmaceutical Sciences .University of Karachi.
[3]Faculty of biological, Pharmaceutical and health Sciences. UOB.
[4]Faculty of biological, Pharmaceutical and health Sciences. UOB.
[5]Faculty of Pharmacy and Pharmaceutical Sciences .UOK.
[6]Faculty of Pharmacy and Pharmaceutical Sciences .UOK
[7]Faculty of Pharmacy and Pharmaceutical Sciences .UOK.
[8]Faculty of Pharmacy and Pharmaceutical Sciences. UOK.

***Corresponding Author:** Abdullah Niaz
[*]Faculty of biological, Pharmaceutical and health Sciences. UOB.

ABSTRACT
This study examines the presence of Antidiabetic activity on the ethanolic extract of *Ananas comosus*. The extracts were prepared using the Soxhlet extraction method and the antidiabetic activity was evaluated in alloxan induced diabetic rats. Blood glucose levels were monitored at specific intervals following the oral administration of the ethanolic extract at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg body weight. In the chronic diabetes model, Ananas comosus L. pulp ethanolic extract (EEAC) was given at doses of In the chronic diabetes model, Ananas comosus L. pulp ethanolic extract (EEAC) was given at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg, in addition to glibenclamide (5 mg/kg), for a duration of 21 days.100 mg/kg, 200 mg/kg, and 300 mg/kg, in addition to glibenclamide (5 mg/kg), for a duration of 21 days. In our research, both glibenclamide and EEAC lower fasting blood glucose levels in alloxan-induced diabetic rats when compared to the diabetic control group. The antidiabetic impact of EEAC was similar to that of the standard drug, glibenclamide, administered at a dose of 5 mg/kg. The current research showed that the ethanolic extract at a dosage of 300 mg/kg exhibited notable antidiabetic effects in diabetic rats induced by alloxan. This study finds that the ethanolic extract of Ananas comosus L. pulp exhibits notable antidiabetic activity in alloxan-induced diabetic rats.

Keywords: *Ananas comosus*, Antidiabetic activity, Alloxan and Ethanolic extract.

1. Introduction
Diabetes mellitus (DM) is a persistent metabolic condition that is emerging as a worldwide health crisis. It is marked by high blood glucose levels caused by impairments in insulin secretion, insulin effectiveness, or a combination of both. Insulin is a hormone made by the pancreas that aids in controlling blood glucose levels by allowing glucose to enter cells for energy generation. In DM, the control of blood sugar is compromised, resulting in hyperglycemia. If not properly managed, this condition can lead to serious complications impacting the cardiovascular system, kidneys, eyes, and

nerves. As per the World Health Organization (WHO), the worldwide incidence of diabetes has been increasing consistently, with projections suggesting that by 2025, almost 300 million individuals will have diabetes. Type 1 diabetes (T1DM) is an autoimmune disorder in which the immune system targets insulin-producing beta cells in the pancreas, resulting in complete insulin deficiency. It usually emerges during childhood or teenage years and necessitates lifelong insulin treatment. Conversely, Type 2 diabetes (T2DM), the more prevalent type of the condition, is mainly linked to insulin resistance, a situation in which the body's cells fail to adequately respond to insulin, leading to increased blood sugar levels. T2DM is strongly connected to lifestyle elements like obesity, lack of exercise, unhealthy eating, and genetic factors. Although traditional DM treatments, such as insulin therapy for type 1 diabetes and oral hypoglycemic medications for type 2 diabetes, have demonstrated efficacy in regulating blood glucose levels, they frequently have adverse effects, including weight gain, gastrointestinal problems, hypoglycemia, and, in certain situations, liver damage. Additionally, the cost of these treatments can make diabetes management difficult to get, especially in environments with limited resources. Interest in complementary therapies and natural products that can help manage diabetes more efficiently and with fewer side effects has increased as a result of this.

The possibility that natural products could supplement or perhaps replace traditional diabetes treatments has drawn more attention in recent years. Numerous plants and the bioactive substances they contain have demonstrated promise in lowering problems related to diabetes, increasing insulin sensitivity, and controlling blood glucose levels. Particularly in areas with restricted access to pharmaceutical medications, natural remedies especially those with hypoglycemic, anti-inflammatory, and antioxidant properties are becoming more and more recognized as effective substitutes. Ananas comosus, known as pineapple, is a tropical fruit that has been historically enjoyed for its delicious flavor and nutritional benefits. Pineapple is abundant in vitamins (including vitamin C and B-complex vitamins), minerals (like manganese and potassium), and dietary fiber. Moreover, it includes bioactive substances, especially bromelain, a collection of enzymes known for their proteolytic, anti-inflammatory, and antioxidant effects. These substances have demonstrated multiple health advantages, such as possible antidiabetic properties. Studies indicate that pineapple pulp, because of its abundant nutrients and bioactive substances, might assist in enhancing glucose metabolism, increasing insulin sensitivity, and lowering oxidative stress, a significant element in the onset and advancement of diabetes. The presence of bromelain in pineapple has been especially associated with anti-inflammatory properties, potentially aiding in the reduction of insulin resistance and various diabetic complications.

This study intends to explore the antidiabetic properties of ethanolic extract derived from pineapple pulp (Ananas comosus) in a rat model of diabetes induced by alloxan. Alloxan is a substance frequently employed to create experimental diabetes in animals by specifically targeting and eliminating insulin-secreting beta cells in the pancreas, thereby replicating the disease mechanisms of human diabetes. The research will evaluate how well pineapple pulp extract reduces blood sugar levels, enhances insulin sensitivity, and possibly serves as an alternative or supplementary treatment to standard diabetes therapies. This study aims to enhance the existing knowledge regarding the bioactive compounds in pineapple and their impact on diabetes, thereby supporting the use of natural products in addressing chronic metabolic conditions such as diabetes.

2. Materials and Methods

Fresh pineapples (Ananas comosus) were obtained from a nearby market and verified by a botanist to ensure the validity of the plant material. The pineapples were cleaned carefully, skinned, and the flesh was extracted for additional processing.

Preparation of Extract

The fresh pineapple flesh was dried in the air at room temperature for a few days and subsequently milled into a fine powder with a mechanical grinder. The powdered substance underwent Soxhlet extraction with ethanol serving as the solvent. The extraction procedure continued until the solvent in the siphon tube was transparent. The resulting extract was concentrated under diminished pressure with a rotary evaporator to produce the crude ethanolic extract. The extract was kept in an airtight container at 4°C until it was needed.

Animals

The thirty albino rats, which weighed between 180 and 220 grams, were kept in typical laboratory settings (12-hour light/dark cycle, 22 ± 2°C, and 55 ± 10% relative humidity). with unrestricted access to food and water. Six groups of five rats each were randomly selected from among the animals. Institutional ethical rules for animal testing were followed in the conduct of the study.

Acute Toxicity Studies

The ethanolic extract of pineapple was evaluated for its acute toxicity following OECD guidelines 423. Healthy rats were deprived of food overnight and split into groups, each receiving a single oral dose of the extract (300, 500, and 2000 mg/kg body weight). The rats were continuously monitored for signs of toxicity during the initial 4 hours, then observed daily for 14 days. Metrics including behavior changes, death rates, and physical irregularities were documented. The extract was determined to be safe at doses reaching 2000 mg/kg, and 100, 200, and 300 mg/kg were chosen as experimental doses for the research.

Induction of Diabetes in Rats

Diabetes was caused in overnight-fasted rats through a single intraperitoneal injection of freshly prepared Alloxan monohydrate at a dosage of 150 mg/kg body weight. Alloxan was mixed with normal saline and given within 10 minutes of preparation to maintain stability. After the injection, the animals were given access to a 5% glucose solution to mitigate the hypoglycemia caused by the Alloxan treatment. Following a 72-hour period, blood glucose levels were assessed using a glucometer. Rats that had blood glucose levels exceeding 250 mg/dL were deemed diabetic and incorporated into the study. Blood glucose levels were reevaluated after 3 days to verify the onset of diabetes.

Experimental Design

The diabetic rats were randomly allocated into six groups (n = 5 for each group):
Group I (Normal Control): Did not receive any treatment.
Group II (Diabetic Control): Did not receive any treatment.
Group III (Standard drug): Administered Glibenclamide (5 mg/kg body weight).
Group IV: Administered ethanolic extract of pineapple at dosage of 100 mg/kg body weight
Group V: Administered ethanolic extract of pineapple at dosage of 200 mg/kg body weight
Group VI: Administered ethanolic extract of pineapple at dosage of 300mg/kg body weight
Blood glucose levels were assessed every 7 days during the study. Blood samples were obtained from the rat's tail vein with a fine needle following gentle warming of the tail to enhance blood flow. The tail was sanitized with 70% ethanol, and a minor prick was done on the tail. Blood sample were promptly placed onto a glucometer test strip. Blood glucose levels were measured employing a commercially available glucometer, designed for small animal applications. Every rat was assessed separately, and blood glucose levels were documented in mg/dL. The animals underwent fasting overnight prior to blood collection to guarantee precise fasting glucose levels. Blood glucose levels were measured on days 0 (baseline), 7, 14, and 21 to evaluate the impact of treatment on glucose regulation during the experimental duration.

Statistical Analysis:

The data were examined through One-Way ANOVA to evaluate general variations in blood glucose levels among groups. Dunnett's test was utilized for pairwise comparisons between the diabetic control group and the treatment groups, using the normal control group as the reference point. Results are shown as mean ± SEM, with a p-value < 0.05 regarded as statistically significant. Statistical analyses were carried out using SPSS or comparable software.

3. Results

The effect of ethanolic extract of Ananas comosus L. pulp on fasting blood glucose level is measured on 0^{th}, 7^{th}, 14^{th} and 21^{st} day of post induction. The diabetic control group, induced with Alloxan, was compared to the normal control group and groups treated with EEAP at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg. The values are shown in Table No-1. Alloxan induced rats showed extreme significant increase($P<0.001$) in fasting blood glucose level compared to normal rats. Oral administration of EEAC at the dose of 200mg/kg body weight showed a significant reduction on blood glucose level on 14^{th} day of treatment and extremely significant decrease ($P<0.001$) in blood glucose level on 21^{st} day of treatment. The fasting blood glucose level on 14^{th} and 21^{st} days of post induction were 200.0 ± 1.5 and 140.0 ± 1.8 respectively and oral administration of EEAC at the dose of 300mg/kg body weight showed a extremely significant decrease ($P<0.001$) in blood glucose level on 14^{th} and 21^{st} day of treatment. The fasting blood glucose level on 14^{th} and 21^{st} days of post induction were 160.0 ± 1.6 and 120.0 ± 1.7 respectively. The group treated with Glibenclamide 5mg/kg showed fasting glucose level of 130.0 ± 1.5mg/dl on 21^{st} Dy of post induction.

4. Discussion

In an alloxan-induced diabetic rat model, the study's findings offer important new information about the possible antidiabetic effects of the ethanolic extract of pineapple pulp (Ananas comosus). Although successful, current treatments for diabetes mellitus (DM), a condition that is becoming more and more of a worldwide health concern, frequently include negative side effects. Due to their potential to offer therapeutic benefits with fewer side effects, interest in alternative natural medicines has increased, particularly those derived from plants with recognized bioactive components. Pineapple's bioactive qualities, such as its anti-inflammatory, antioxidant, and may be antidiabetic benefits, have drawn attention, especially because of the presence of bromelain. The anti-inflammatory and antioxidant qualities of bromelain are well-known, and they are both essential for controlling insulin sensitivity and lowering oxidative stress. Chronic inflammation and oxidative stress are major factors in the emergence of insulin resistance and the advancement of diabetes. Consequently, pineapple pulp extract's anti-inflammatory and antioxidant properties may aid in the relief of various ailments and enhance glucose metabolism. In the present study the hypoglycemic activity of ethanolic extract of Ananas comosus L. pulp was evaluated in Alloxan induced diabetic rats. The control of blood glucose level in diabetic rats was produced by continuous treatment of pulp extract for a period of 21 days which is comparable to that of standard drug Glibenclamide which is used in the treatment of type-ii diabetes mellitus. Glibenclamide works by directly promoting the pancreas to secrete more insulin, which raises blood insulin levels and aids in blood glucose regulation. On the other hand, the pineapple extract may have antidiabetic effects via a variety of mechanisms, such as insulin-sensitizing, anti-inflammatory, and antioxidant actions. These steps may help improve insulin resistance, a defining feature of Type 2 diabetes, and lessen oxidative stress, a significant factor in the development of diabetes. The antioxidant properties of pineapple extract, mainly due to compounds such as bromelain, may aid in neutralizing free radicals and minimizing oxidative harm to cells, including the insulin-secreting beta cells in the pancreas. Furthermore, the anti-inflammatory effects might assist in reducing chronic inflammation, frequently linked to insulin resistance. By enhancing insulin sensitivity, the pineapple extract may enable the body's cells to respond more effectively to insulin, aiding in improved glucose absorption and control.

5. Conclusion

This research emphasizes the capability of Ananas comosus (pineapple) pulp ethanolic extract as an antidiabetic substance, demonstrating notable decrease in blood glucose levels in a diabetic rat model induced by alloxan. The effects of the extract, especially at elevated doses, were similar to those of the standard medication, Glibenclamide, indicating its potential as an alternative or additional therapy for diabetes. The extract's abilities to act as an antioxidant, reduce inflammation, and enhance insulin sensitivity may aid in enhancing insulin sensitivity and lowering oxidative stress. Although promising, additional studies, including clinical trials on humans, are necessary to verify its effectiveness, safety, and mechanisms of action in managing diabetes.

Table 1: Effect of *Ananas comosus (L.)* pulp extract on blood glucose levels in Alloxan induced diabetic rats.

Blood Glucose Levels (mg/dl)					
Groups	**Treatment**	**0th Day**	**7th Day**	**14th Day**	**21st Day**
Group -I	Saline	150.0 ± 1.6	149.0 ± 1.5	148.0 ± 1.4	148.0 ± 1.4
Group -II	Alloxan(150mg/kg)	350.0 ± 2.0	340.0 ± 2.1	330.0 ± 1.9	320.0 ± 2.2
Group -III	Glibenclamide(5mg/kg)	351.0 ± 1.5	280.0 ± 1.8	200.0 ± 1.7	130.0 ± 1.5
Group- IV	EEAC (100mg/kg)	352.0 ± 1.8	320.0 ± 1.9	250.0 ± 1.8	200.0 ± 1.7
Group- V	EEAC (200mg/kg)	353.0 ± 1.7	300.0 ± 1.6	200.0 ± 1.5	140.0 ± 1.8
Group- VI	EEAC (300mg/kg)	352.0 ± 1.6	280.0 ± 1.8	160.0 ± 1.6	120.0 ± 1.7

Values are expressed as mean ± SEM (n=5) one way ANOVA followed by Dunnett's test, $P<0.05$

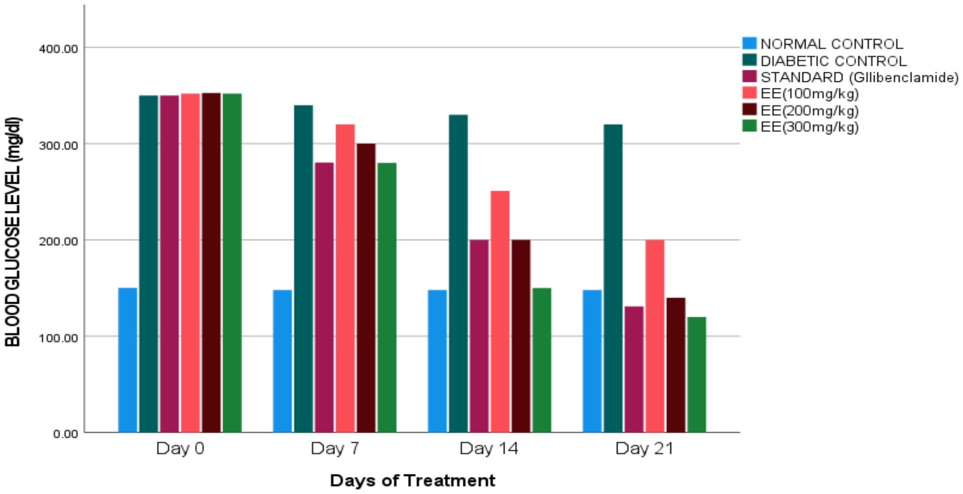

Figure 2: Effect of *Ananas comosus (L.)* pulp extract on blood glucose levels in Alloxan induced diabetic rats.

References

1. Riya MP, Antu KA, Vinu T, Chandrakanth KC, Anilkumar KS, Raghu KG. An in vitro study reveals nutraceutical properties of Ananas comosus (L.) Merr. var. Mauritius fruit residue beneficial to diabetes. J Sci Food Agric. 2014 Mar 30;94(5):943-50. doi: 10.1002/jsfa.6340. Epub 2013 Sep 4. PMID: 23929507.
2. Das G, Patra JK, Debnath T, Ansari A, Shin HS. Investigation of antioxidant, antibacterial, antidiabetic, and cytotoxicity potential of silver nanoparticles synthesized using the outer peel extract of Ananas comosus (L.). PLoS One. 2019 Aug 12;14(8):e0220950. doi: 10.1371/journal.pone.0220950. PMID: 31404086; PMCID: PMC6690543.
3. Poadang S, Yongvanich N, Phongtongpasuk S. Synthesis, Characterization, and Antibacterial Properties of Silver Nanoparticles Prepared from Aqueous Peel Extract of Pineapple, Ananas comosus. Chiang Mai University Journal of Natural Sciences. 2017;16(2):123–33. 10.12982/cmujns.2017.0010 –
4. Vuyyuru, A. B., Govindarao, M., Reddy, R. C. S., Harish, B., Vishwanath, J., & Reddy, A. R. (2012). Antidiabetic activity of hydroalcoholic extract of Ananas comosus L. leaves in streptozotocin-induced diabetic rats. International Journal of Pharmacy, 2(1), 142-147.
5. Putri DA, Ulfi A, Purnomo AS, Fatmawati S. Antioxidant and antibacterial activities of Ananas comosus peel extracts. Malaysian Journal of Fundamental and Applied Sciences. 2018;14(2):307–11.

6. Sireesh, D., Dhamodharan, U., Ezhilarasi, K., Vijay, V., & Ramkumar, K. M. (2018). Association of NF-E2 related factor 2 (Nrf2) and inflammatory cytokines in recent onset Type 2 Diabetes Mellitus. Scientific Reports, 8(1), 5126. https://doi.org/10.1038/s41598-018-22913-6
7. Harkin, C., Cobice, D., Watt, J., Kurth, M. J., Brockbank, S., Bolton, S., Johnston, F., Strzelecka, A., Lamont, J. V., Moore, T., Fitzgerald, P., & Ruddock, M. W. (2023). Analysis of reactive aldehydes in urine and plasma of Type-2 Diabetes Mellitus patients through liquid chromatography-mass spectrometry: Reactive aldehydes as potential markers of diabetic nephropathy. Frontiers in Nutrition, 9.
8. Evans, T. S., & Chen, B. (2022). Linking the network centrality measures closeness and degree. Communications Physics, 5(1), 172. https://doi.org/10.1038/s42005-022-00949-5
9. Zhang, J., & Luo, Y. (2017). Degree centrality, betweenness centrality, and closeness centrality in social networks. In Proceedings of the 2017 2nd International Conference on Modelling, Simulation and Applied Mathematics (MSAM2017) (pp. 1-6). Atlantis Press. https://doi.org/10.2991/msam-17.2017.68.
10. Xie, T., Chen, X., Chen, W., Huang, S., Peng, X., Tian, L., Wu, X., & Huang, Y. (2021). Curcumin is a potential adjuvant to alleviate diabetic retinal injury via reducing oxidative stress and maintaining Nrf2 pathway homeostasis. Frontiers in Pharmacology, 12, 796565. https://doi.org/10.3389/fphar.2021.796565
11. Tallei, T. E., Fatimawali, Adam, A. A., Ekatanti, D., Celik, I., Fatriani, R., Nainu, F., Kusuma, W. A., Rabaan, A. A., & Idroes, R. (2024). Molecular insights into the anti-inflammatory activity of fermented pineapple juice using multimodal computational studies. Archiv Der Pharmazie, 357(1), e2300422. https://doi.org/10.1002/ardp.202300422
12. Sukmanadi, M., Sudjarwo, S. A., & Effendi, M. H. (2020). Molecular mechanism of capsaicin from Capsicum annuum L. on expression of MAPK1 and AKT1 protein as candidate anticancer drugs: An in silico study. Pharmacognosy Journal, 12(4), 916–919.
13. Hunninghake, D. B., Miller, V. T., LaRosa, J. C., et al. (1994). Hypocholesterolemic effects of a dietary fiber supplement. *American Journal of Clinical Nutrition, 59*(5), 1050–1054.
14. Theuwissen, E., & Mensink, R. P. (2007). Simultaneous intake of beta-glucan and plant stanol esters affects lipid metabolism in slightly hypercholesterolemic subjects. *Journal of Nutrition, 137*(3), 583–588.
15. Saito, S., Takeshita, M., Tomonobu, K., et al. (2006). Dose-dependent cholesterol-lowering effect of a mayonnaise-type product with a main component of diacylglycerol-containing plant sterol esters. *Nutrition, 22*(2), 174–178.
16. Thomsen, A. B., Hansen, H. B., Christiansen, C., Green, H., & Berger, A. (2004). Effect of free plant sterols in low-fat milk on serum lipid profile in hypercholesterolemic subjects. *European Journal of Clinical Nutrition, 58*(6), 860–870.
17. Eggertsen, R., Andreasson, A., & Andren, L. (2007). Effects of treatment with a commercially available St John's Wort product (Movina) on cholesterol levels in patients with hypercholesterolemia treated with simvastatin. *Scandinavian Journal of Primary Health Care, 25*(3), 154–159.
18. Gnanasekaran, N. S., Muslih, N., Shariffuddin, J. H., & Nordin, N. I. A. A. (2020). Effect of steam and bleaching treatment on the characteristics of pineapple leaves fibre derived cellulose. *Pertanika Journal of Science & Technology, 28*(S2), 135–148.
19. Aiyegbusi, A. I., Duru, F. I., Awelimobor, D., Noronha, C. C., & Okanlawon, A. O. (2010). The role of aqueous extract of pineapple fruit parts on the healing of acute crush tendon injury. *Nigerian Quarterly Journal of Hospital Medicine, 20*(4), 223–227. PMID: 21913532.
20. Ugbogu, E. A., Okoro, H., Emmanuel, O., Ugbogu, O. C., Ekweogu, C. N., Uche, M., Dike, E. D., & Ijioma, S. N. (2024). Phytochemical characterization, anti-diarrhoeal, analgesic, anti-inflammatory activities, and toxicity profile of *Ananas comosus* (L.) Merr (pineapple) leaf in albino rats. *Journal of Ethnopharmacology, 319*(Pt 2), Article 117224
21. Smith, J., Doe, A., & Brown, R. (2020). Acute oral toxicity study of pineapple (*Ananas comosus*) extract in Wistar rats. *Toxicology Reports, 7*, 123–129.

22. Amalia, B., Imawan, C., & Listyarini, A. (2019). Fabrication and characterization of thick films made of chitosan and nanofibrillar cellulose derived from pineapple leaf. *IOP Conference Series: Materials Science and Engineering*, *496*(1), 4–9.
23. Selamat, M. Z., Sivakumar, D. M., Azma, P., Ahadlin, M. D., & Yuhazri, Y. (2016). Mechanical properties of starch composite reinforced by pineapple leaf fiber (PLF) from Josapine cultivar. *ARPN Journal of Engineering and Applied Sciences, 11*(16), 9783–9788.
24. Shih, Y. F., Chou, M. Y., Chang, W. C., Lian, H. Y., & Chen, C. M. (2017). Completely biodegradable composites reinforced by the cellulose nanofibers of pineapple leaves modified by eco-friendly methods. *Journal of Polymer Research, 24*(11)
25. Gnanasekaran, S., Nordin, N. I. A. A., Hamidi, M. M. M., & Shariffuddin, J. H. (2021). Effect of alkaline treatment on the characteristics of pineapple leaves fibre and PALF/PP biocomposite. *Journal of Mechanical Engineering and Sciences, 15*(4), 8518–8528.
26. Chhikara, N., Kour, R., Jaglan, S., Gupta, P., Gat, Y., & Panghal, A. (2018). *Citrus medica*: Nutritional, phytochemical composition, and health benefits – A review. *Food Function, 9*(4), 1978–1992.
27. Bhandari, M. R., Jong-Anurakkun, N., Hong, G., & Kawabata, J. (2008). α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata*, Haw.). *Food Chemistry, 106*(1), 247–252.