



## RESEARCH ARTICLE

# *In-vitro* study on the antidiabetic property of *Pisonia grandis*

J. Mohan, R. Arun Kumar

## Abstract

The prevalence of diabetes seems to be growing, and many people report unfavorable reactions to currently available antidiabetic drugs. The use of ethnomedicinal plants has the potential to provide cost-effective and safe alternatives to conventional diabetic treatments. This *in-vitro* investigation aims to investigate the antidiabetic properties of *Pisonia grandis*. The extract's cytotoxic potential on HepG2 cells was evaluated using the MTT assay. Additionally, it investigated its impact on glucose metabolism in HepG2 cells and its ability to inhibit enzymes like alpha-amylase and alpha-glucosidase. Both doses of plant extract caused less than 50% cell death. The extract was more effective than berberine in enhancing glucose uptake by "HepG2 liver cells". When compared to the positive controls, the crude extract did not affect alpha-amylase, protein glycation, collagenase, alpha-glucosidase, DPP-IV, or lipase. *P. grandis* exhibits hypoglycaemic effects that may operate independently of insulin, suggesting its potential as an antidiabetic agent.

**Keywords:** Diabetes, Herbal, HepG2, Pharmacology, *Pisonia grandis*.

## Introduction

Diabetes mellitus is a metabolic condition in which the body is unable to properly use the hormone insulin, leading to persistently high blood sugar levels. With the rising global prevalence of diabetes, there is an urgent need to explore new therapeutic options to effectively manage this condition. Natural products derived from medicinal plants have long been recognized as potential sources of novel antidiabetic compounds. Among these plants, *Pisonia grandis*, commonly known as the grand devil's-claws tree, has gained attention for its traditional use in various folk medicines (Yadav *et al.*, 2014; Rosenzweig *et al.*, 2018; Wu *et al.*, 2014).

WHO reports that in most underdeveloped countries, 80% of the population relies on traditional medicine derived from plant components such as leaves, stems, bark, and flowers. Bioactive compounds found in plants are known

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as phytochemicals. There was a considerable decrease in calcium bioavailability due to the plant's high total oxalate, tannin, and dietary fiber content (Yadav *et al.*, 2014). As a result of the potential lack of relevance to human needs, their production by plants is considered a secondary metabolic activity. These "green factories" have yielded a wealth of active phytochemicals, which have been extracted and characterized to generate a variety of medications with outstanding activity profiles.

*P. grandis* is a tropical tree native to several regions, including Asia, Africa, and the Pacific Islands. It has been traditionally employed in herbal remedies for various ailments, including diabetes. The plant's therapeutic potential has led to increased scientific interest, prompting researchers to explore its bioactive compounds and their effects on diabetes-related mechanisms (Modak *et al.*, 2007).

Phytochemicals are made up of both main and secondary components; primary constituents include proteins, chlorophyll, and simple sugars, while secondary contents include total terpenoids, flavonoids, gums, phenolic compounds, tannins, glycosides, alkaloids, and essential oils. *In-vitro*, propagation of *P. alba* is only one of several successful actions performed with this plant (Pugazhendy *et al.*, 2017). The flowering plants of the genus *Pisonia* belong to the Nyctaginaceae family, sometimes known as the "4 O'clock" family. Some members of this genus are called "catch bird trees," "bird catcher trees," and "birdlime trees" due to the widespread belief that the sticky seeds of these trees snare little birds (Pramanick *et al.*, 2015; Nie *et al.*, 2020).

Plants may benefit from the guano that seabirds that take refuge in this tree produce. It serves as a model of ectomycorrhizal fungal interaction in autotrophic plants and is a symbiotic host to several mycobionts (Poongothai *et al.*, 2023). Another investigation on symbiotic associations confirms the plant's usefulness as a nitrogen source due to its composition. Both the Australasian and Polynesian *Pisonia grandis* and the tropical Indo-Pacific *P. grandis* are included in the taxonomy of plants in India (Narayanan *et al.*, 1987; Gopal *et al.*, 2013). There have been claims that the plant may help with a wide variety of health issues, including diabetes, wounds, bacteria, cancer, inflammation, anxiety, fever, and even hepatitis (Chang *et al.*, 2004). The leaves of the plant have yielded pinitol and allantoin, two compounds with potential medical applications that have been isolated and described (Kannaiyan *et al.*, 2022). This research aims to evaluate the antidiabetic property of the plant *P. grandis*.

### Material And Methods

The *P. grandis* leaves were gathered from a dense forest area in India. The leaves were dried in an oven at 40°C to a consistent weight before being further processed in an electric blender to create a uniform powder (Waring Products Division, Torrington, USA). In 1000 mL of distilled water were used to extract 60 gm of the plant's powder. Whatman filter paper and a Buchner funnel were then used to filter the extract. A yield of 9 grams was obtained from the extract after it was frozen at -40°C and 48 hours dry in a freeze dryer. Before conducting different bioactivity tests, the dried extract was reconstituted in DMSO after being first held at -4°C. The extracts of leaf, stem, and bark, the remainder of the sample from each extract, were sent for phytochemical evaluation to check for the existence of sterols, flavonoids, carbohydrates, tannins, triterpenoids, and glycosides. For the phytochemical analysis, we used the TLC method (Mir *et al.*, 2013).

### Cell Reagents, Lines, and Media Kits

The "HepG2 liver cells" were all purchased from India. The "Eagle's Minimum Essential Medium (EMEM) and MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide)" were supplied by Themofischer in India. Both the "fetal calf serum (FCS) and the phosphate-buffered saline (PBS)" were generously donated by "Lonza Biologics". Analytical grade Merck Chemicals and Sigma reagents were utilized for the rest of the experiments.

### Maintenance of Cell Cultures

In a humidified environment containing 5% CO<sub>2</sub>, all cell cultures were maintained at 37°C. On average, new batches of Roswell Park Memorial Institute 1640 medium with 10% of FCS to HepG2 cells were supplied every two to three days. Cells from the 3T3-L1 mouse were grown in fetal bovine serum-supplemented DMEM. After 90% confluence was attained for each cell line, they were all subcultures.

### Cytotoxicity Assay

The cytotoxicity experiment was conducted using a slightly modified version of the procedure reported by Mosmann *et al.*, (1983). In 100 µL of "HepG2 liver cells" were seeded into 96-well plates at a density of eight thousand cells per well. The cells were allowed to adhere overnight, and the next day, 100 µL extract of the plant was added to the well at various concentrations (50, 100, and 200 µg per mL). Following 48 hours of 37°C incubation, the medium was aspirated, and 100 µL of "Eagle's Minimum Essential Medium media" with 10% FCS and 0.5 mg per mL MTT was added (dissolve 50 mL of full culture media with 25 g of MTT). Three hours of incubation were performed at 37°C. The purple formazan MTT crystals generated in the cells were then dissolved with DMSO (200 mL per well) after the media had been aspirated. The absorbance was calculated using a microplate reader and done at 540 nm (Figure 1). The proportion of the medium-only control, which was set at zero, was used to measure the plant extract's cytotoxicity and IC<sub>50</sub> values were calculated.

$$\% \text{Cell death} = 1 - (\text{Absorbance of test well} / \text{Average of the untreated}) \times 100$$

### HepG2 C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> Utilization Experimental Method

The rate of glucose intake in "HepG2 cells" was evaluated by using the method explained by van de Venter *et al.*, (2008). The "HepG2 cells" were counted, then separated by incubation in 0.25% trypsin in PO<sub>4</sub><sup>3-</sup>-buffered saline for the night. The following day, they were suspended in fresh medium and inoculated at a density of 6000 cells per well in a 96-well culture plate. They were then incubated for three days at 37°C in a humidified incubator containing 5% CO<sub>2</sub>. Two more rows with no cells were also added. On the third day after seeding, 10 mL extract of the plant at concentrations of 25 and 100 mg per mL was administered to each well without changing the medium. The utilized culture media was removed after 48 hours of incubation and replaced with a 25 µL incubation buffer, which was then incubated for an additional 3 hours at 37°C. The positive controls were metformin (0.1 µg per mL) and berberine (18 µg per mL), whereas the incubation buffer alone from the untreated group was used as the negative control. To evaluate the amount of glucose in the medium, 200 µL of the glucose oxidase reagent was added to each well after 10 µL of the incubation media had been withdrawn from each well and transferred onto a fresh 96-well plate. The absorbance at 492 nm was measured using a "Multiscan microtitre plate reader" from Lab Systems after 15 minutes of incubation at 37°C. The difference between the wells with and without cells was used to determine glucose utilization (Figure 2). The amount of consumed glucose was calculated using the untreated controls. Using the MTT test, cell viability in the representative well was evaluated.

### Alpha-amylase Inhibition Assay

Odeyemi *et al.*, (2015) protocol was followed for the alpha-amylase test. In brief, 15  $\mu$ L of plant extract diluted in phosphate buffer (50 to 200  $\mu$ g per mL) was added to 5 mL of pig pancreatic solution containing an enzyme on a 96-well plate. After 10 minutes at 37°C, 20  $\mu$ L of starch solution were added to initiate the reaction, which was followed by 30 minutes of incubation. Ten mL (1-mL) of 1M HCl and 75 mL of iodine reagent were added to each well to halt the reaction. Acarbose (64 g/mL) was used as a positive control, and phosphate buffer (pH 6.9) was used as a blank. There was no starch control or enzyme control used in this experiment. The proportion of inhibitory action was estimated using the following method after measuring absorbance at 580 nm.

$$\% \text{Inhibition} = \frac{(1 - \text{Absorbance of the untreated (Control)})}{\text{Absorbance of the test well}} \times 100$$

### $\alpha$ -Glucosidase Inhibition Assay

The " $\alpha$ -glucosidase inhibition" experiment was performed using a modified version of the protocol published by Sancheti *et al.*, (2010). In brief, a 96-well plate was filled with 20  $\mu$ L of a 50 g/mL alpha-glucosidase solution and 5  $\mu$ L of the plant extract (at conc. of 50, 100, and 200  $\mu$ g/mL). Then, 60  $\mu$ L of 67 millimoles  $\text{KH}_2\text{PO}_4$  buffer (pH 6.8) was added thereafter. Incubation at 37°C continued for a further 20 minutes with the addition of 10 L of a 10 mM -nitrophenyl- -D-glucoside solution after the first 5 minutes. The absorbance at 405 nm was measured after incubation by adding 25  $\mu$ L of a 100 mM sodium carbonate solution. Five  $\mu$ L of deionized water were used in place of the plant extract and 20  $\mu$ L of the enzyme to make a blank and a sample blank, respectively. The concentration of epigallocatechin gallate utilized in the experiment was 10  $\mu$ g/mL. The formula for determining the percentage of inhibition is as follows:

$$\% \text{Inhibition} = \frac{(1 - \text{Absorbance of the test well})}{\text{Absorbance of the untreated (control)}} \times 100$$

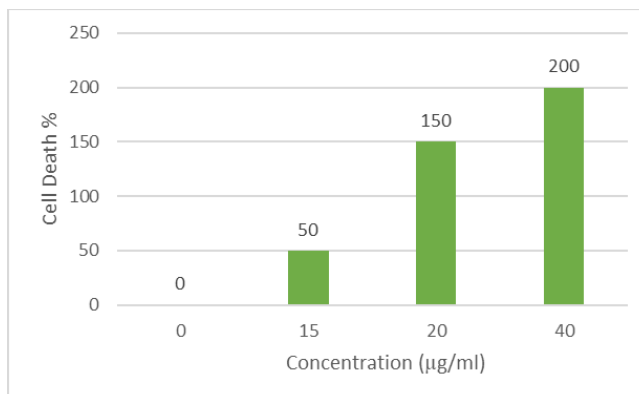
### Statistical Analysis

Statistical analysis was performed using SPSS version 25, and significance was determined using a one-way analysis of variance and a student's t-test (two-tailed). Each treatment's replicated values were compared to the controls' replicated values. The significance level was between  $p < 0.001$  and  $p < 0.05$ .

## Results

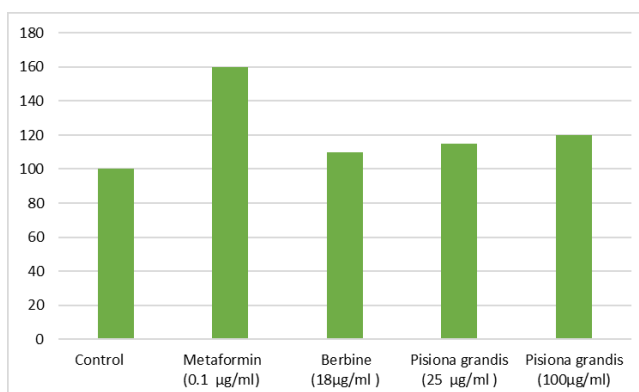
### Cytotoxicity

The MTT test was used to determine the cytotoxic activity of *P. grandis*'s extract against the "HepG2 liver cell" line *in-vitro*. The cytotoxicity data showed a dose-dependently low degree of toxicity of *P. grandis*'s extract towards HepG2 cells. The maximum dose of the extract (200  $\mu$ g per mL) resulted in less than 50% cell mortality, while the  $\text{IC}_{50}$  value was determined to be 250 g/mL (a concentration that may result in 50% cell death).



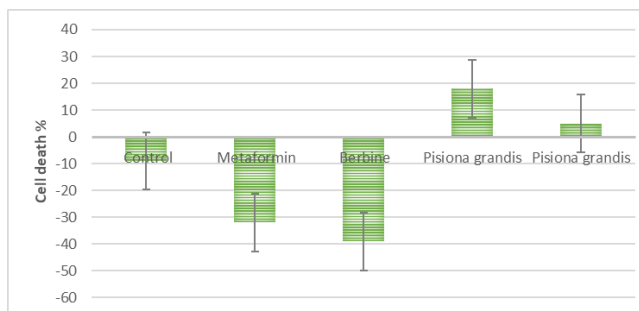
**Figure 1:** Effect of *P. grandis*'s aqueous extract on HepG2 liver cells' MTT cytotoxicity.

Data are shown as a percentage of control  $\pm$ SD (n = 4). \*denotes a significant increase in comparison to the untreated control ( $p < 0.05$ ).



**Figure 2:** Glucose utilization by "HepG2 hepatocytes" was affected by *P. grandis* extract.

In different concentrations of the plant extract, either present or absent, cells were treated for 48 hours. Information presented as mean  $\pm$  SD (n = 4).  $P < 0.05$  denotes a significant increase in comparison to the untreated control. A substantial increase ( $p < 0.05$ ) has been observed when compared to the positive control (berberine). No appreciable improvement in comparison to metformin (a positive control) was seen.

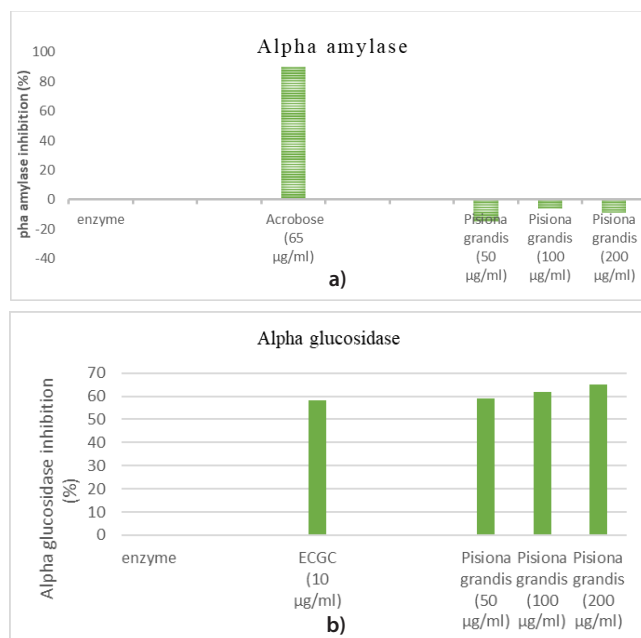


**Figure 3:** HepG2 cell toxicity of *P. grandis* extract during glucose uptake test.

Data are presented as the mean  $\pm$  standard deviation (n = 4). \*denotes a significant increase in comparison to the untreated control ( $p < 0.05$ ).

### Glucose Utilization in "HepG2 cells"

The uptake of glucose by HepG2 cells was measured after they were exposed to plant extract at 25 and 100  $\mu$ g per mL of concentrations (Figure 3). The crude extract of *P. grandis* enhanced glucose absorption in HepG2 cells in



**Figure 4:** The impact of *P. grandis* aqueous extract on a) alpha-glucosidase and b) alpha-amylase activity.

Epigallocatechin gallate, or EGCG. information presented as mean  $\pm$  SD ( $n = 4$ ). \*denotes a substantial increase in comparison to the enzyme-only control ( $p < 0.05$ ). In contrast to the active controls (acarbose and epigallocatechin gallate), no significant improvement was seen.

a concentration-dependent manner as compared to the untreated control and berberine, yet it was less efficient than metformin. When comparing the effects on glucose uptake at 100  $\mu\text{g}$  per mL, the crude extract of *P. grandis*'s was less effective than berberine but more effective than metformin.

With less than 10% cell mortality at both dosages tested, the toxicity experiment showed that *P. grandis*'s extract was not harmful to "HepG2 cells". However, neither berberine nor metformin significantly harmed the cells; instead, they promoted cell growth.

#### Alpha-Amylase Inhibition Assay

The findings revealed that at all concentrations examined, extract from *B. elliptica* had no significant effect on alpha-amylase but did have a modest significant effect on alpha-glucosidase (Figure 4).

#### Alpha-glucosidase Inhibition Assay

Alpha-glucosidase activity was reduced by 32.6% when the extract was used at the maximum concentration (200 g/mL) tested. In contrast, the positive controls acarbose and EGCG were much more effective than the extract and untreated control in inhibiting the activities of alpha-amylase and alpha-glucosidase, respectively, by percentages of 94.7 and 57.5%, respectively, in the relevant experiments (Figure 4).

#### Discussion

The use of herbal medications to manage diabetes is rising. Several traditional herbs may be used to manage diabetes (Kasole *et al.*, 2019; Governa *et al.*, 2018), but their effectiveness

has to be supported by research. In the current investigation, an indigenous herb with a reputation for having antidiabetic properties was evaluated in comparison to the widely used antidiabetic medicine acarbose. The antidiabetic properties of an ethanol extract of *P. grandis* plant leaves have been described, although the fractionates of this extract have not been studied extensively. Crude extracts often include a complicated assemblage of several metabolites (Vimalavalli *et al.*, 2015; Thakur *et al.*, 2017; Megala *et al.*, 2019). Using the TLC technique, we found that the presence of phytochemicals such as flavonoids, phenols, flavonols, and terpenoids inhibited glucose release and enhanced glucose absorption by the liver. Therefore, the existence of the polyphenols previously mentioned in the extract is responsible for the glucose absorption seen in HepG2 cells for this extract in our investigation. In contrast Narayanan *et al.* in 1987 reports, the molecules pinitol and allantoin are both antidiabetics. In diabetic rats, pinitol has been shown to exhibit acute and chronic insulin-like anti-hyperglycemic effects. It can also help transport glucose into the muscle and enhance glycogen storage. Pinitol was initially discovered as an antidiabetic drug (Narayanan *et al.*, 1987). In the 2016 research by Lalitha *et al.*, *P. grandis* leaf ethanol extract column fractions were tested for their ability to suppress the activity of beta-amylase, a marker of diabetes. The effectiveness of inhibition was 67% when acarbose (250 g/mL) was employed as a standard. The study's findings reveal that all *P. grandis* fractions have amylase inhibition capacity, which is connected with antidiabetic potential. With a concentration of 250 g/mL, pinitol showed the strongest inhibition of  $\alpha$ -amylase, followed by the liquid-liquid extracted PGL aq.1 fraction from *P. grandis* leaves (20 g/mL), and the column fractionate PGL1% (10 g/mL). Moderate inhibition of  $\alpha$ -amylase was likewise seen for all other fractionates (at concentrations of 20 g/mL) (Lalitha *et al.*, 2016).

In the current investigation, the cytotoxicity effect demonstrated that even at the maximum dosage of 200 g/mL, less than 50% cell death was obtained for "HepG2 cells" treated with the extract. Since *P. grandis*'s extract showed a low degree of toxicity, the aqueous extract of *P. grandis*'s might be harmless to its consumers. To determine how *P. grandis*'s extract could help diabetics, researchers here used a succession of biochemical and cell-based experiments.

This research examined the effects of berberine and *P. grandis*'s extract on glucose absorption in HepG2 and found that the latter significantly boosted glucose uptake. This study indicates that the crude extract of *P. grandis* has a comparable effect to metformin in stimulating glucose uptake in the liver. Metformin is an oral hypoglycemic medication that is classified as a biguanide. It lowers blood sugar levels by stimulating AMP-activated protein kinase in the liver. Possible pharmacological effects include reduced hepatic glucose and fat synthesis and improved insulin

sensitivity in the liver. Based on these observations, it is possible to postulate that *P. grandis*'s mode of action involves stimulating GLUT 2, which allows for the translocation of glucose into the cell through the activation of the insulin signaling cascade. Abnormal lipid accumulation outside of adipose tissue causes physiological dysfunction (lipotoxicity), notably in skeletal muscle, the liver, and the islets of the pancreas.

The metabolism of glucose in pancreatic  $\beta$  cells is crucial for the release of insulin in response to glucose. Increased insulin release has been linked to enhanced glucose metabolism, which in turn increases ATP synthesis in pancreatic cells. The mechanisms of action of the many antidiabetic medicines now in use to treat or control diabetes are well understood. These blocks the activity of a variety of enzymes, including lipase, alpha-amylase, dipeptidyl peptidase IV, and alpha-glucosidase. Based on our findings, there was no significant difference in the activities of lipase, alpha-amylase, dipeptidyl peptidase-IV, or glucosidase between the extract and their corresponding positive controls. The extract was shown to suppress alpha-amylase activity, although only slightly. This effect is unlikely to have any physiological significance. This shows that *P. grandis*'s antidiabetic mechanism does not include the inhibition of the enzymes. Similarly, according to research by Narkhede *et al.*, (2011), at a concentration of 250 g/mL, the conventional medication acarbose was 67% effective against the  $\alpha$ -amylase enzyme. Andrade-Cetto *et al.*, (2008) found that the  $IC_{50}$  value of acarbose is 128 g/mL, which is comparable to the% inhibition (67%) achieved at 250 g/mL. Acarbose works by suppressing the function of pancreatic amylase, which causes a delay in the digestion of carbohydrates. Evidence like this suggests that enzyme inhibitors generated from food may be used to successfully reduce glucose levels in *P. grandis*.

## Conclusion

The hypoglycaemic effects of *P. grandis* are achieved through multiple mechanisms, including independent action from insulin and promotion of beta-cell and pancreatic health. The activation of the mitogen-activated protein kinase (MAPK) and p38 mitogen-activated protein kinase (P13K) pathways, along with the translocation of glucose transporters, contributes to its antidiabetic properties. The presence of phytochemicals in the extract, known for their antidiabetic action, may account for these observed effects. Overall, this study provides evidence supporting the traditional use of *P. grandis* for diabetes treatment. Furthermore, the results suggest the potential of combining this plant with other antidiabetic treatments that interact with the cytochrome P450 isoform, as well as the promising use of *P. grandis* extracts in the development of new antidiabetic pharmaceuticals.

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## References

- Andrade-Cetto, A., Becerra-Jiménez, J., & Cárdenas-Vázquez, R. (2008). Alfa-glucosidase-inhibiting activity of some Mexican plants used in the treatment of type 2 diabetes. *Journal of Ethnopharmacology*, 116(1), 27–32. <https://doi.org/10.1016/j.jep.2007.10.031>
- Chang, A. M., Smith, M. J., Bloem, C. J., Galecki, A. T., & Halter, J. B. (2004). Effect of lowering postprandial hyperglycemia on insulin secretion in older people with impaired glucose tolerance. *American Journal of Physiology. Endocrinology and Metabolism*, 287(5), E906–E911. <https://doi.org/10.1152/ajpendo.00156.2004>
- Gopal, P., Sripathi, S. K., & Indumathi, P. (2013). GC-MS Analysis of Non-Polar Fractions of Leaves, Stems and Roots of *Pisonia grandis* R. Br. *International Journal of Phytomedicine*, 5(4), 460.
- Governa, P., Bains, G., Borgonetti, V., Cettolin, G., Giachetti, D., Magnano, A. R., ... & Biagi, M. (2018). Phytotherapy in the management of diabetes: a review. *Molecules*, 23(1), 105.
- Kannaiyan, S., Easwaramoorthi, D., Kannan, K., Gopal, A., Lakshmiopathy, R., Mohammedsahle Katubi, K., Almuaiikel, N. S., & Rodriguez Rico, I. L. (2022). *Pisonia Alba* assisted synthesis of nanosilver for wound healing activity. *Bioinorganic Chemistry and Applications*, 2022, 1775198. <https://doi.org/10.1155/2022/1775198>
- Kasole, R., Martin, H. D., & Kimiywe, J. (2019). Traditional medicine and its role in the management of diabetes mellitus: "patients' and herbalists' perspectives". *Evidence-Based Complementary and Alternative Medicine*, 2019.
- Lalitha, P., & Sripathi, S. K. (2016). *In-vitro* anti diabetic efficacy (potential) of leaf ethanol extract fractionates of *Pisonia grandis* R. Br. *Pharmacophore*, 7(6), 625–630.
- Megala, S. (2019). Pharmacognostical, Phytochemical Studies and Evaluation of Anti-Arthritic Activity of Leaves of *Momordica Charantia* Linn (Doctoral dissertation, College of Pharmacy, Madras Medical College, Chennai).
- Mir, M. A., Sawhney, S. S., & Jassal, M. M. (2013). Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. *Wudpecker Journal of Pharmacy and Pharmacology*, 2(1), 1–5.
- Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S., & Devasagayam, T. P. (2007). Indian herbs and herbal drugs used for the treatment

- of diabetes. *Journal of Clinical Biochemistry and Nutrition*, 40(3), 163–173. <https://doi.org/10.3164/jcbrn.40.163>
- Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1–2), 55–63. [https://doi.org/10.1016/0022-1759\(83\)90303-4](https://doi.org/10.1016/0022-1759(83)90303-4)
- Narayanan, C. R., Joshi, D. D., Mujumdar, A. M., & Dhekne, V. V. (1987). Pinitol—A new antidiabetic compound from the leaves of *Bougainvillea spectabilis*. *Current Science*, 56(3), 139–141.
- Narkhede, M. B., Ajimire, P. V., Wagh, A. E., Mohan, M., & Shivashanmugam, A. T. (2011). *In-vitro* antidiabetic activity of *Caesalpinia digyna* (R.) methanol root extract. *Asian Journal of Plant Science and Research*, 1(2), 101–106.
- Nie, X., Chen, Z., Pang, L., Wang, L., Jiang, H., Chen, Y., ... & Zhang, J. (2020). Oral nano drug delivery systems for the treatment of type 2 diabetes mellitus: an available administration strategy for antidiabetic phytochemicals. *International journal of nanomedicine*, 10215–10240.
- Odeyemi, S. W. (2015). A comparative study of the *in-vitro* antidiabetic properties, cytotoxicity and mechanism of action of *Albuca bracteata* and *Albuca setosa* bulb extracts.
- Poongothai, G., Sindhu, S., & Sripathi, S. K. *A comprehensive revelation on Pisonia grandis R. Br.*
- Pramanick, D. D., Mondal, M., & Maiti, G. G. (2015). Pollen morphological studies on some members of the family Nyctaginaceae in India. *Asian Journal of Plant Science and Research*, 5(2), 72–76.
- Rosenzweig, C., Solecki, W. D., Romero-Lankao, P., Mehrotra, S., Dhakal, S., & Ibrahim, S. A. (Eds.). (2018). *Climate change and cities: Second assessment report of the urban climate change research network*. Cambridge University Press.
- Sancheti, S., Sancheti, S., & Seo, S. Y. (2010). Evaluation of antiglycosidase and anticholinesterase activities of *Boehmeria nivea*. *Pakistan Journal of Pharmaceutical Sciences*, 23(2), 236–240.
- Thakur, S., Shandilya, M., & Lal, M. (2017). Biosynthesis of nanoparticles using plant extracts. Nova Publisher, 1, 365–382.
- V, T., & Pugazhendy, K. (2017). Ethnobotanical and Phytopharmacological review of *Pisonia alba* Span. *Asian Journal of Pharmaceutical and Clinical Research*, 10(5), 69–71. <https://doi.org/10.22159/ajpcr.2017.v10i5.17356>
- Van de Venter, M., Roux, S., Bungu, L. C., Louw, J., Crouch, N. R., Grace, O. M., Maharaj, V., Pillay, P., Sewnarian, P., Bhagwandin, N., & Folb, P. (2008). Antidiabetic screening and scoring of 11 plants traditionally used in South Africa. *Journal of Ethnopharmacology*, 119(1), 81–86. <https://doi.org/10.1016/j.jep.2008.05.031>
- Vimalavalli, S., & Raji Sugumar, V. (2015). AntiDiabetic Activity of *Pisonia grandis* R. Br. leaves against streptozotocin (STZ) induced diabetes mellitus in rats. *Int. J. Phytopharmacol.*, 6(3), 156–160.
- Wu, Y., Ding, Y., Tanaka, Y., & Zhang, W. (2014). Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *International journal of medical sciences*, 11(11), 1185.
- Yadav, M., Chatterji, S., Gupta, S. K., & Watal, G. (2014). Preliminary phytochemical screening of six medicinal plants used in traditional medicine. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(5), 539–542.