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EVALUATION OF *IN VITRO* ANTI-DIABETIC AND ANTI-LIPIDEMIC ACTIVITY OF MEDICINAL PLANTS

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Abstract

Diabetes mellitus is a severe chronic disease that extends lifelong. During the past few years, some research has been done on medicinal plants to isolate new bioactive drugs that showed anti-diabetic activity with more efficacy than oral hypoglycemic medicines used in clinical therapy. The present research was carried out for these two traditional medicinal plants (*Ficus benghalensis* and *Psidium guajava)* in their aqueous and methanolic extracts of leaves for evaluating total phenolic and flavonoid content, DPPH assay for antioxidant and *in vitro* anti-diabetic and anti-lipidemic properties by alpha-amylase inhibition assay, glucose uptake assay and lipase inhibition assay. The highest phenolic (59.4±1.35 mg GAE g^{-1} DE) and flavonoid content (32.6±1.20 mg RE g^{-1} DE) was found in methanolic extract of *Psidium guajava* than other extracts. Among the solvents, methanolic extract of *Ficus benghalensis* has more percent scavanged (70.2 %) therefore having more antioxidant potency than other plant's extracts. The amylase inhibition was more in aqueous extract of *Psidium guajava* showed the minimum IC_{50} value (0.151) than other extracts. The relative movement of glucose out of the membrane was maximum inhibited by aqueous extracts of both the plants whereas the methanolic extract showed the minimum inhibition The lipase inhibition was more in methanolic extract of *Psidium guajava* and has the minimum IC_{50} value (39.7) than other plant's extract. The current study suggested *Psidium guajava* could be a better candidate than *Ficus benghalensis* for the treatment of diabetes.

Keywords: *Amylase, Lipase, Psidium guajava, Ficus benghalensis.*

Introduction

Diabetes mellitus is also be known as Diabetes. Diabetes Mellitus (DM) is a multi-step chronic health condition that is triggered by several genetic and/or environmental factors [1-2]. Indeed, this terminology is characterized by the strong frequency of diabetes for a specific genetic profile like in American Indians and Natives of Alaska, that more likely to have diabetes. The World Health Organization (WHO) Global survey reveals that the number of adults living with diabetes has almost diabetes shows that the number of adults living with diabetes has almost increased significantly since 1980 to 422 million adults, [3] and is expected to rise to 693 million by 2045 [4]. The increase is more rapid in Africa and largest in Asia [5,6]. Diabetes is a lifelong condition that causes an increase in blood sugar levels throughout its period. Diabetes mellitus mainly classified as Type 1 diabetes and Type 2 diabetes. Type 1 Diabetes is insulin-dependent in which the body fails to produce insulin, and the patient requires the insulin injection [7]. The Type 2 diabetes is insulin-independent. In this type, the cells are unable to use blood sugar or glucose for energy because cells become insensitive to insulin. The most common type of Diabetes all around the world is Type 2 diabetes and it contributes about 90% of the diabetic population. The prediabetes conditions and Type 2 diabetes now can be seen in a more ratio among the children and younger adults [8,9]. The plants as a source of medicine show a great diversity in healing and curing various types of disease. It has been estimated that almost 80 % of the world's total population depends regularly on traditional medicine and products for their healthcare needs. Many diabetic people in developing countries combine conventional medicine with traditional medicine [10,11,12]. *Medicinal plants* include various herbs, shrubs and trees. The different parts of a plant like leaves, stem, bark, fruit etc. can be used to cure the various type of disease [13]. In countries like India and China where research is going at an excellent pace on medicinal plants, and this research has been increased on diabetes since 1995 in these countries. The bioactive components are favorably stored in plant leaves at a percentage of 20 as compared to other herbal plants. The treatment of diabetes has set a baseline to cure diabetes all around the world as there is a study which shows that around 493 million adult people all around the world will suffer from diabetes by 2030 [14]. Traditional medicinal plants have been used for many years by different cultures around the world for the management of diabetes. In the past three decades, there is no perfect result, despite the significant progress that has been developed in the treatment of diabetes.These therapies have drawbacks such as drug resistance, side effects and toxicity. Sulfonylureas, for example, lose 44 % of potency in patients after 6 years of treatment. It is also believed that the glucose-lowering drugs are not capable of regulating hyperlipidemia [15]. Now a day's many therapies recommend the use of medicinal plants [16]. Most plants contain carotenoids, flavonoids, terpenoids, alkaloids, glycosides and can often have anti-diabetic effects [17].

Ficus benghalensis is an evergreen plant of the family Moraceae. *Ficus benghalensis* (Banyan tree), is a large tree having aerial roots shredded to the ground and this tree is found all over India and grows wild in the lower Himalayas. It is also known for its Ayurvedic medicinal uses and its bark used in Ayurveda for the treatment of diabetes. Different parts of the tree are widely utilized due to medicinal properties [18]. *It is* used in traditional medicine and is considered as effective, economical and safe ethno medicines which are effective and economical [19]. The extracts of this plant in different solvents show a hypoglycemic effect. Various plant parts like bark, leaves, fruits, aerial roots show antidiabetic activity [20]. Glibenclamide is an antidiabetic agent well known for its hypoglycaemic activity and shows lesser insulin secretion than the water-soluble insulin-secreting constituent present in *Ficus* sp [21].

Psidium guajava (Guava), a member of the Myrtaceae family that originates in Mexico and now grown in South America, Europe, Africa, and Asia). It has been extensively utilized and recognized in Peru since pre-Columbian times, according to archaeological evidences *Psidium guajava occurs* in all of the world's tropical and subtropical regions, adapting to a variety of climatic environments but preferring dry climates [22]. Genus *Psidium* has nearly 150 species, most of trees are fruit-bearing. *Psidium guajava* is used for the treatment of a number of diseases in different parts of the world, e.g. as an anti-inflammatory, used in the treatment of gastroenteritis, diarrhea, diabetes, high blood pressure, caries, wounds, pain relief and fever reduction. Several researchers have researched into the anti-diabetic effects of *Psidium guajava* bark, leaves, and fruits, and assessed some medicinal plants amongst which *Psidium guajava* is one with excellent anti-diabetic activity. The main habitual use known is anti-diarrhea. Other reported uses include gastroenteritis, dysentery, antibacterial colic pathogenic germs of the intestine [23]. The present study set an objective to evaluate the antioxidant properties and *in vitro* amylase inhibition by the plant extracts. Consequently, this providing a scientific basis for the use of this plant in traditional medicine.

Methods

Plant Material Collection

The leaves of the plant *Psidium guajava* and *Ficus benghalensis* were collected from the district Solan region of Himachal Pradesh (fig. 1).

Extract Preparation

Methanol and water were taken as polar and non-polar solvents for extraction purposes. For this 10g of plant powder dissolved in 100 ml of the respective solvents and then kept for 48 hrs in a rotatory shaker. The extracts were filtered and kept for drying at 30°C. The dried extract was scrapped and refrigerated for further use at 4°C [24,25].

Phytochemical Analysis

Phytochemical analysis is done by both qualitative and quantitative methods.

Qualitative Phytochemical Analysis

The leaves were analyzed for various qualitative methods of phytochemicals. The presence of alkaloids was determined by the Mayer test. Borntrager's test was performed to determine the presence of glycosides [26]. Sodium hydroxide and lead acetate tests were performed to show. The presence of flavonoids showed by [27]. Molish test was used to determine the presence of carbohydrates. The analysis of terpenoids was done by the Salkowski test. Froth test determines the presence of Saponin. Phenols presence showed by ferric chloride test.

Quantitative method

The quantitative phytochemical analysis was done for the total phenolic count and flavonoids content.

Total phenolic determination

Total phenolic content of each plant extract was determined by [28] with some modifications and taking gallic acid as standard and the assay performed in a microtitre plate. Test sample was taken in 2.5µl, 5µl, 7.5µl and 10µl concentrations and was diluted with distilled water to make a final volume. Subsequently, the solution was mixed with 10µl of fresh Folin Ciocalteu reagent and after 5 min 100µl of Na_2CO_3 (7.5%) solution was added with 130µl of distilled water. The plates were incubated for 90 minutes at 30°C. The absorbance was measured at 750nm. The results were expressed as Gallic acid equivalents (mg gallic acid/g dried extract).

Total flavonoids determination

The total flavonoid content was determined by [29.30] with some modifications and taking quercetin acid as standard, the assay performed in a microtitre plate. The plant extracts samples were taken in different 2.5µl, 5µl, 7.5µl and 10µl concentrations and the final volume were prepared adding more distilled water. To each sample 10µl of $NaNO_2$ solution (5%) was added. After 5 minutes, 10µl of an $AlCl_3$ solution (10%) was added and allowed to stand for 6 minutes at room temperature. After that 100µl of NaOH

solution (1M) was added to the mixture and absorbance was measured at 510nm. Rutin was used as a standard for the calibration curve. Total flavonoids content of the extracts was expressed as mg quercetin acid equivalents per gram of sample (mg/g).

***In-vitro* Antioxidant Assays**

DPPH Radical Scavenging Activity

The ability to scavenge DPPH radical was determined by using DPPH method by [31,32] with some modifications. 200µl of plant extract (1mg/ml) is reacted with DPPH (50µl) and then placed in a dark place for 30 min. Ascorbic acid (1mg/ml) was taken as standard and the readings of the samples were taken at 517nm. The percentage inhibition for the scavenging of the DPPH was evaluated using following equation:

Percentage scavenged (1%) = [(control A – sample A)/ control A] ×100

Where sample A is the absorbance of the test compound and control A is the absorbance of the control reaction (which contains all of the reagents except the test compound).

***In-vitro* Antidiabetic Assay**

Amylase Inhibition Assay

The amylase inhibition activity was estimated according to the protocol with some minor modifications [33]. A starch solution (500mg/25ml) was made by dissolving it on 0.4 M NaOH and kept for 5min at heating (100°C). The volume raised to 100ml by the addition of distilled water while maintaining its pH at 7. Plant extract was dissolved in acetate buffer and different concentrations were made. The pH of acetate buffer adjusted to 6.5. 20µl of the substrate added to 10µl of a sample in microwell plate which is followed by the addition of 10µl α-amylase solution(50µg/ml) and left for 15min incubation at room temperature.0.1M HCl (40µl) was used to stop the reaction which is followed by the addition of 100µl of 1Mm iodine solution. The optical density was measured at 650 nm.

Following equation has used to check amylase inhibitory activity:

Amylase inhibitory activity (%) = {1 - (OD2-OD1) / (OD4-OD3) X100}

Lipase Inhibition Assay

The lipase inhibition activity was estimated according to the protocotol with some modifications [34]. The substrate solution was prepared in 9 ml of 0.1 M TES buffer (pH 7.0) by dissolving the lecithin (10 mg), sodium cholate (5 mg) and glycerol trioleate (80 mg). Different plant extract concentrations were prepared in 0.1 M TES buffer. To the sample (20 µl) and substrate solutions (20 µl) in microplate wells, 10 µl of lipase solution (20 µg/ml) was added and incubated for 30 min at 37 °C.

The optical density was recorded at 550 nm using a microplate reader

Lipase inhibitory activity (percent) was calculated as follows:

Lipase Inhibition (%) = {1 - (OD2-OD1)/(OD4-OD3)X100}

Glucose uptake assay

The glucose uptake assay was performed as described with some modifications [34]. For this methanolic and aqueous extract (100mg/ml) of the plant was prepared. The present experiment comprised of a one-sided sealed dialysis tube(12000MW, Himedia) into which 1 ml of 22 Mm D-glucose in 0.15 M NaCl and 1 mL extract (100 mg/ml) or control (water) was taken. The opposite end was then sealed and the membrane was placed into a beaker containing 45 ml, 0.15 M NaCl. The beaker was then kept into an orbital shaking incubator at 37°C and a speed of 100 rotations per minute. The movement of glucose into the solution was recorded every half an hour. The current experiment was done in three replications and the movement was monitored for three hours.

Statistical Analysis

All the experiments were done in triplicates for each sample and the results expressed as mean ± SD. IC_{50} values were calculated by linear regression.

Results

Qualitative analysis of Phytochemical Screening Test

Phytochemical screening test showed that Phenols, Flavonoid, Alkaloids, Terpenoids and glycosides are present in all plants. Amino acid is present in *Ficus benghalensis* except *Psidium guajava.* Saponins are

present in only *Psidium guajava* extract (Table 1).

Total Phenolic and Flavonoids estimation

Phenols and flavonoids are important Phytochemical which give many health benefits and help on may disease treatment. Total phenolic content was calculated from the standard curve of gallic acid using the equation: y= 0.741x+0.1326, while total flavonoid content was calculated using the standard curve of quercetin using the equation: y=0.965x+0.0362 contents. Total phenolic content obtained was in the range of 15.12 to 59.4 mg GAE g^{-1}. The highest phenolic content was achieved by methanol extract (59.4±1.35 mg GAE g^{-1} DE) of *Psidium guajava* than other extracts (fig. 2a and Table 2). Total flavonoid content was in the range of 8.10 to 32.6 mg QE g^{-1}. The highest flavonoid content was found in methanol extract (32.6±1.20 mg RE g^{-1} DE) of *Psidium guajava* than other extracts (fig. 2b and Table 2).

Antioxidant activity

The antioxidants in the DPPH assay inhibit the oxidation reaction by converting free radicals to stable states, and this assay has been extensively utilized to investigate the antioxidant properties of natural products from microbial sources and plants. All the extracts exhibited good antioxidant properties, which varied with the type of solvents. Methanolic extract of *Ficus benghalensis* has more %inhibition (70.2 %) than other extracts. Therefore, the methanolic extract has more free radical scavenging activity (fig. 3 and Table 3). Ascorbic acid was used as a standard, which exhibited % inhibition (87 %) for both plant samples respectively.

***Invitro* Antidiabetic Analysis**

Amylase inhibition assay

From many years In Ayurveda, several plant extracts with known anti-diabetic effects have been discovered and extensively utilized. In the present study, two medicinal plant's antidiabetic properties were checked and analyzed by using *in vitro* antidiabetic assay. Alpha-amylase is responsible for postprandial glucose levels various plant extracts with alpha-amylase inhibitory activity are being studied which can decrease postprandial blood glucose levels, making it an important and innovative therapeutic target for the treatment of diabetes mellitus. In the present study, it is observed that the aqueous extract of *Psidium guajava* has the minimum IC_{50} value (0.151) than other plants extract as mentioned in Table 4. Therefore, *Psidium guajava* can be a more potent plant than *Ficus benghalensis* for the treatment of Diabetes.

Lipase Inhibition Assay

Obesity is a major factor for increasing rates of cardiovascular disease, non-alcoholic fatty liver metabolic syndrome, and non-insulin-dependent diabetes. Hydrolysis of dietary lipid into fatty acid and 2-monoacylglycerol by pancreatic lipase is essential before it gets absorbed by the intestines. thus, inhibiting digestive enzymes, α-amylase and pancreatic lipase might prove effective in diabetes treatment. In the present study, it is observed that the methanolic extract of guava has the minimum IC_{50} value (39.7) than other plants extract as shown in Table 4. Therefore, *Psidium guajava* can be a more potent plant than *Ficus benghalensis* for the treatment of Diabetes.

Glucose uptake assay

Fig. 4a and 4b is given with the results of the glucose diffusion assay which showed that both the extracts of the plant significantly inhibit the glucose activity. So according to the table, the aqueous extracts of both the plants showed the maximum inhibition whereas the methanolic extract showed the minimum inhibition as shown in Table 5 and Table 6.

Discussion

Diabetes is now the third human "killer" after cancer and cardiovascular diseases, due to its high prevalence, morbidity and mortality [35].The chronic hyperglycemia of diabetes is the long-term damage that is associated with inflammation and loss of multiple organs [36]. Herbal medicines and plant components with none or less toxicity with no side effects and are notable

therapeutic options for the treatment of this disease around the world [37].

Most of the previous work has shown the effects of hypoglycemic medicinal plants containing in the diabetes treatment of diabetes. The most important active ingredients obtained from medicinal plants in treating diabetes are flavonoids, tannins, phenolics, and alkaloids [38]. Most The efficacy of these compounds shows the importance of the anti-diabetic properties of these plants [37]. Results showed that methanol extracts yield greater quantities of active compounds as compared to aqueous solvent extracts and also possessed good antioxidant and antidiabetic activities. The preliminary phytochemical screening tests might be helpful in the identification of the pharmacologically bioactive components. Results obtained in this study indicated the presence of alkaloids, carbohydrates, flavonoids, phenols, saponins and proteins. The presence of these secondary metabolites in the extract of *Psidium guajava* and *Ficus benghalensis* is in agreement with the previous reports [39].

Many studies have shown that phytochemicals such as phenolics and flavonoid compounds found in different herbs are well known for their antioxidant and antidiabetic activity [40]. For this cause, there is an interest in the use of phenolics and flavonoids rich extracts in the treatment of diabetes and its complications. It has been commonly recognized in the past that phenols and flavonoids are two essential phytochemical classes that contribute significantly to plant antioxidant function. Polyphenolic compounds such as flavonoids, phenolic acids and tannins are thought to be the main contributors to the antioxidant activity of medicinal plants. The antioxidant effects of polyphenolic compounds are due to their redox properties, which makes them act as reducing agents, singlet oxygen quenchers and hydrogen donors [41].

Antioxidants have been reported to minimize the risk of diabetes onset [42] increase glucose imbalance [43] and improve some of the related complications [44]. Oxidative stress has been linked with the pathogenesis and development of multiple degenerative diseases, including naturally occurring and chemically-caused diabetes mellitus [45]. In addition to the increased development of free radicals, antioxidant defense mechanisms are impaired by diabetes mellitus [46]. The antioxidant properties of methanolic extracts of *Psidium guajava* and *Ficus benghalensis* were determined utilizing DPPH assays in present analysis. The methanolic extract of *Psidium guajava* leaves reported substantial antioxidant activity in all of the experiments conducted, suggesting high antioxidant properties of extract. The presence of a high amount of polyphenolic compounds in the methanolic extract of *Psidium guajava* may be the reason for antioxidant activity. These results are similar to the earliest study where similar results were reported in the case of *Psidium guajava* leaf extracts [47]. It was also observed that most polar extraction solvents showed more antioxidant activity and phenolic content as compared to less polar extraction solvents. It indicates that the polar solvents are important in extracting phytoconstituents with more antioxidant activity and phenolic content which is in agreement with an earlier study [48]. Earlier also several studies have reported a strong correlation between the presence of phenolic contents and the antioxidant potential of the plants [49,50]. Other researcher have also shown such type of favorable positive correlation between antioxidant activity and phenolic content [51,52]. The effect of antioxidants on DPPH radicals is due to their hydrogen donating ability [53]. Further, the present antidiabetic activity also revealed that the *Psidium guajava* extracts showed the highest amylase and lipase inhibitory activity than *Ficus benghalensis*. Deguchi confirmed that in vitro antidiabetic activity of *Psidium guajava* leaves is more in methanolic extract and is efficiently showed the inhibitory effect of glucose utilization when compared with different solvents using specific standard protocols [54]. Therefore present investigation established the

pharmacological evidence to support the anti-diabetic potential of the plant leaves that have antidiabetic and antioxidant activity.

Conclusion

Diabetes is a disorder of metabolism in which high blood sugar (hyperglycemia) abnormally resulting from insufficient levels of the hormone insulin. Treatment of diabetes by natural resources seems to be a promising approach and can be favored for inhibition of alpha-amylase. Further study is required on the isolation and characterization of the principal bioactive compounds of the medicinal plant extracts and that can be safely used in clinical research for long-term administration of the natural plant extracts for type 2 diabetes. The screening of phytochemicals shows the presence of phenols, flavonoids, antioxidant and antidiabetic compounds in both plants. But the *Psidium guajava* extracts showed the highest amylase and lipase inhibitory activity than *Ficus benghalensis*. So results show that guava has better results against diabetes than *Ficus benghalensis.* However, both plants can be studied further for *in vivo* antidiabetic potentials of plants.

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Conflict of Interest

There is no conflict of interest

References

1. Sun, X., Yu, W., & Hu, C. (2014). Genetics of type 2 diabetes: insights into the pathogenesis and its clinical application. *BioMed research international*, *2014*.
2. Kaul, N., & Ali, S. (2016). Genes, genetics, and environment in type 2 diabetes: implication in personalized medicine. *DNA and cell biology*, *35*(1), 1-12.
3. WHO. Diabetes Programme. WHO. [(accessed on 27 January 2019)];2019 Availableonline: https:// www.who.int/diabetes/en/
4. Cho, N., Shaw, J. E., Karuranga, S., Huang, Y. D., da Rocha Fernandes, J. D., Ohlrogge, A. W., & Malanda, B. (2018). IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes research and clinical practice*, *138*, 271-281.
5. Zhou, B., Lu, Y., Hajifathalian, K., Bentham, J., Ferreccio, C., ... & Miranda, J. (2016). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4· 4 million participants..
6. Nanditha, A., Ma, R. C., Ramachandran, A., Snehalatha, C., Chan, J. C., Chia, K. S., ... & Zimmet, P. Z. (2016). Diabetes in Asia and the Pacific: implications for the global epidemic. *Diabetes care*, *39*(3), 472-485.
7. Chen, L., Magliano, D. J., & Zimmet, P. Z. (2012). The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. *Nature reviews endocrinology*, *8*(4), 228-236.
8. Deshmukh, C. D., & Jain, A. (2015). Diabetes mellitus: A review. *Int. J. Pure App. Biosci*, *3*(3), 224-230.
9. Wong, C. Y., Al-Salami, H., & Dass, C. R. (2017). Potential of insulin nanoparticle formulations for oral delivery and diabetes treatment. *Journal of controlled release*, *264*, 247-275.
10. Musila, W., Kisangau, D., & Muema, J. (2002). Conservation status and use of medicinal plants by traditional medical practitioners in Machakos District, Kenya. *National Museums of Kenya*, *22*, 12-18.
11. Mahwasane, S. T., Middleton, L., & Boaduo, N. (2013). An ethnobotanical survey of indigenous knowledge on medicinal plants used by the traditional healers of the Lwamondo area, Limpopo province, South Africa. *South African Journal of Botany*, *88*, 69-75.

12. Kinyanjui, M. J., Latva-Käyrä, P., Bhuwneshwar, P. S., Kariuki, P., Gichu, A., & Wamichwe, K. (2014). An inventory of the above ground biomass in the Mau Forest ecosystem, Kenya. *Open Journal of Ecology*, *2014*.
13. Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in pharmacology*, *4*, 177.
14. Chan, C. H., Ngoh, G. C., & Yusoff, R. (2012). A brief review on anti diabetic plants: Global distribution, active ingredients, extraction techniques and acting mechanisms. *Pharmacognosy reviews*, *6*(11), 22.
15. Dey, L., Attele, A. S., & Yuan, C. S. (2002). Alternative therapies for type 2 diabetes. *Alternative medicine review*, *7*(1), 45-58.
16. Kooti, W., Moradi, M., Ali-Akbari, S., Sharafi-Ahvazi, N., Asadi-Samani, M., & Ashtary-Larky, D. (2015). Therapeutic and pharmacological potential of Foeniculum vulgare Mill: a review. *Journal of HerbMed Pharmacology*, *4*(1), 1-9.
17. Afrisham, R., Aberomand, M., Ghaffari, M. A., Siahpoosh, A., & Jamalan, M. (2015). Inhibitory Effect of Heracleum persicum and Ziziphus jujuba on Activity of Alpha-Amylase. *Journal of Botany*, *2015*.
18. Gayathri, M., & Kannabiran, K. (2008). Antidiabetic and ameliorative potential of Ficus bengalensis bark extract in streptozotocin induced diabetic rats. *Indian journal of clinical Biochemistry*, *23*(4), 394-400.
19. Singh, R. K., Mehta, S., Jaiswal, D., Rai, P. K., & Watal, G. (2009). Antidiabetic effect of Ficus bengalensis aerial roots in experimental animals. *Journal of ethnopharmacology*, *123*(1), 110-114.
20. Sharma, S., Chaturvedi, M., Edwin, E., Shukla, S., & Sagrawat, H. (2007). Evaluation of the phytochemicals and antidiabetic activity of Ficus bengalensis. *International journal of diabetes in developing countries*, *27*(2).
21. Misbah, H., Aziz, A. A., & Aminudin, N. (2013). Antidiabetic and antioxidant properties of Ficus deltoidea fruit extracts and fractions. *BMC complementary and alternative medicine*, *13*(1), 1-12.
22. Gutiérrez, R. M. P., Mitchell, S., & Solis, R. V. (2008). Psidium guajava: a review of its traditional uses, phytochemistry and pharmacology. *Journal of ethnopharmacology*, *117*(1), 1-27.
23. Grover, J. K., Yadav, S., & Vats, V. (2002). Medicinal plants of India with anti-diabetic potential. *Journal of ethnopharmacology*, *81*(1), 81-100.
24. Dulta, K., Thakur, K., Virk, A. K., Thakur, A., Chauhan, P., Kumar, V., & Chauhan, P. K. (2021a). Comparison of different solvents for Antioxidant and Antibiogram Pattern of Bergenia ciliata rhizome Extract from Shimla district of Himachal Pradesh. *Jordan Journal of Biological Sciences*, *14*(1).
25. Thakur, A., Singh, S., & Puri, S. (2021). Nutritional evaluation, Phytochemicals, Antioxidant and Antibacterial activity of Stellaria monosperma Buch.-Ham. Ex D. Don and Silene vulgaris (Moench) Garcke: wild edible plants of Western Himalayas. *Jordan Journal of Biological Sciences*, *14*(1).
26. Evans W.C, Trease and Evans. (2002). WB Saunders Harcourt Publishers Ltd. ; 292:357-375.
27. Siddiqui A.A, Ali, M. (1997). Practical pharmaceutical chemistry. CBS Publishers & Distributors.
28. Laily, N., Kusumaningtyas, R. W., Sukarti, I., & Rini, M. R. D. K. (2015). The potency of guava Psidium guajava (L.) leaves as a functional immunostimulatory

ingredient. *Procedia chemistry*, *14*, 301-307.
29. Sahreen, S., Khan, M. R., & Khan, R. A. (2017). Evaluation of antioxidant profile of various solvent extracts of Carissa opaca leaves: an edible plant. *Chemistry Central Journal*, *11*(1), 1-7.
30. Dulta, K., Thakur, K., Sharma, P. C. A., & Chauhan, P. K. (2021b). IMPACT OF DIFFERENT SOLVENTS ON PHYTOCHEMICAL PROFILING AND ANTIOXIDANT ACTIVITY OF CARICA PAPAYA LEAF FROM NORTHWESTERN HIMALAYAN REGION.
31. Venkatachalam, R. N., Singh, K., & Marar, T. (2012). Phytochemical screening in vitro antioxidant activity of Psidium guajava. *Free Radicals and Antioxidants*, *2*(1), 31-36.
32. Bohara, P., Dulta, K., Chauhan, P., Thakur, K., Seth, A., & Chauhan, P. K. (2021). IMPACT OF DIFFERENT SOLVENTS ON PHYTOCONSTITUENTS, ANTIOXIDANT AND FTIR ANALYSIS OF DIPLAZIUM ESCULENTUM LEAF EXTRACT FROM HIMALAYAN REGION.
33. Patil, M. M., Anand, T., Ilaiyaraja, N., & Khanum, F. (2017). In-vitro antioxidant and anti-obesity properties of Bauhinia Variegata. *Def Life Sci J*, *2*, 128-132.
34. Gallagher, A. M., Flatt, P. R., Duffy, G. A. W. Y., & Abdel-Wahab, Y. H. A. (2003). The effects of traditional antidiabetic plants on in vitro glucose diffusion. *Nutrition research*, *23*(3), 413-424.
35. Li, W. L., Zheng, H. C., Bukuru, J., & De Kimpe, N. (2004). Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *Journal of ethnopharmacology*, *92*(1), 1-21.
36. Lyra, R., Oliveira, M., Lins, D., & Cavalcanti, N. (2006). Prevention of type 2 diabetes mellitus. *Arquivos Brasileiros de Endocrinologia & Metabologia*, *50*(2), 239-249.
37. Gupta, P. D., & De, A. (2012). Diabetes mellitus and its herbal treatment. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, *3*(2), 706-721.
38. Mamun-or-Rashid, A. N. M., Hossain, M. S., Hassan, N., Dash, B. K., Sapon, M. A., & Sen, M. K. (2014). A review on medicinal plants with antidiabetic activity. *Journal of Pharmacognosy and Phytochemistry*, *3*(4), 149-159.
39. Bhaskara Rao, K. V., Ojha, V., Preeti, Kumar, G., & Karthik, L. (2014). Phytochemical composition and antioxidant activity of Ficus benghalensis (Moraceae) leaf extract. *Journal of Biologically Active Products from Nature*, *4*(3), 236-248.
40. Singh, G., Pathania, R., Khan M. A., Tonk, R. K., Kumar, D., & Dash, A. K. (2021). Identification and quantification of some natural compounds of *pinus gerardiana* leaf extract and its antimicrobial and antioxidant activities. *Pharmcology Online*, *(2)*, 333-351.
41. Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in plant science*, *2*(4), 152-159.
42. Montonen, J., Knekt, P., Järvinen, R., & Reunanen, A. (2004). Dietary antioxidant intake and risk of type 2 diabetes. *Diabetes care*, *27*(2), 362-366.
43. Ylönen, K., Alfthan, G., Groop, L., Saloranta, C., Aro, A., Virtanen, S. M., & Botnia Research Group. (2003). Dietary intakes and plasma concentrations of carotenoids and tocopherols in relation to glucose metabolism in subjects at high risk of type 2 diabetes: the Botnia Dietary Study. *The American journal of clinical nutrition*, *77*(6), 1434-1441.
44. De Young, L., Yu, D., Bateman, R. M., & Brock, G. B. (2004). Oxidative stress and antioxidant therapy: Their impact in diabetes-associated

erectile dysfunction. *Journal of andrology*, *25*(5), 830-836.
45. Kumar, M., Guleria, S., Chawla, P., Khan, A., & Kaushik, R. (2020). Severity, types, factors affecting and strategy to overcome obesity. *Plant Archives*, *(20)*, 657-672.
46. Kumar, M., Guleria, S., Chawla, P., Khan, A., Modi, V. K., Kumar, N., & Kaushik, R. (2020). Anti-obesity efficacy of the selected high altitude Himalayan herbs: in vitro studies. *Journal of Food Science and Technology*, *57*(8), 3081-3090.
47. Sultana, C., Kundo, N., Islam, S., Ahmed, R., Afrin, S., Saqueeb, N., & Ibne Wahed, M. I. (2020). Antioxidant, analgesic and antimicrobial activities of different fractions from methanolic extract of Psidium guajava L. Leaves. *International Journal of Pharmaceutical Sciences and Research*, *11*, 2733.
48. Barchan, A., Bakkali, M., Arakrak, A., Pagán, R., & Laglaoui, A. (2014). The effects of solvents polarity on the phenolic contents and antioxidant activity of three Mentha species extracts. *Int J Curr Microbiol App Sci*, *3*(11), 399-412.
49. Bhatia, G., Khanna, A. K., Sonkar, R., Mishra, S. K., Srivastava, S., & Lakshmi, V. (2011). Lipid lowering and antioxidant activity of flavones in triton treated hyperlipidemic rats. *Medicinal Chemistry Research*, *20*(9), 1622-1626.
50. Dulta, K., Ağçeli, G. K., Chauhan, P., Jasrotia, R., & Chauhan, P. K. (2021c). A novel approach of synthesis zinc oxide nanoparticles by *bergenia ciliata* rhizome extract: antibacterial and anticancer potential. *Journal of Inorganic and Organometallic Polymers and Materials*, *31*(1), 180-190.
51. Piluzza, G., & Bullitta, S. (2011). Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. *Pharmaceutical biology*, *49*(3), 240-247.
52. Farasat, M., Khavari-Nejad, R. A., Nabavi, S. M. B., & Namjooyan, F. (2014). Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. *Iranian journal of pharmaceutical research: IJPR*, *13*(1), 163.
53. Choi, H. Y., Jhun, E. J., Lim, B. O., Chung, I. M., Kyung, S. H., & Park, D. K. (2000). Application of flow injection—chemiluminescence to the study of radical scavenging activity in plants. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *14*(4), 250-253.
54. Deguchi, Y., & Miyazaki, K. (2010). Anti-hyperglycemic and anti-hyperlipidemic effects of guava leaf extract. *Nutrition & metabolism*, *7*(1), 1-10.

Table 1. Phytochemicals analysis in different extracts

Phytochemical tests	Test Name	*Psidium guajava*		*Ficus benghalensis*	
		Methanol	Aqueous	Methanol	Aqueous
Alkaloids	Mayer's test	+ve	-ve	+ve	+ve
Carbohydates	Benedict's test	+ve	+ve	+ve	+ve
Flavanoids	Lead acetate test	+ve	+ve	+ve	+ve
Phenol	Ferric chloride test	+ve	+ve	+ve	+ve
Saponins	Froth test	+ve	+ve	-ve	-ve
Proteins	Ninhydrin test	+ve	+ve	+ve	-ve
Amino acids	Millon's test	-ve	-ve	+ve	+ve

Figure 1. a) *Psidium guajava* b) *Ficus benghalensis* leaves

Table 2. Total phenolic and flavonoid content of different extracts

Plant	Extract type	Phenolic content (mg GAE/g DE)	Flavonoid content (mg RE/g DE)
Psidium guajava	Methanol	59.4 ± 1.35	32.6 ± 1.20
	Aqueous	20.86 ± 1.48	10.66 ± 1.07
Ficus benghalensis	Methanol	34.66 ± 1.69	21.7 ± 1.03
	Aqueous	15.12 ± 1.32	8.10 ± 1.28

Figure 2. a)Total Phenolic Content b) Total Flavonoid Content

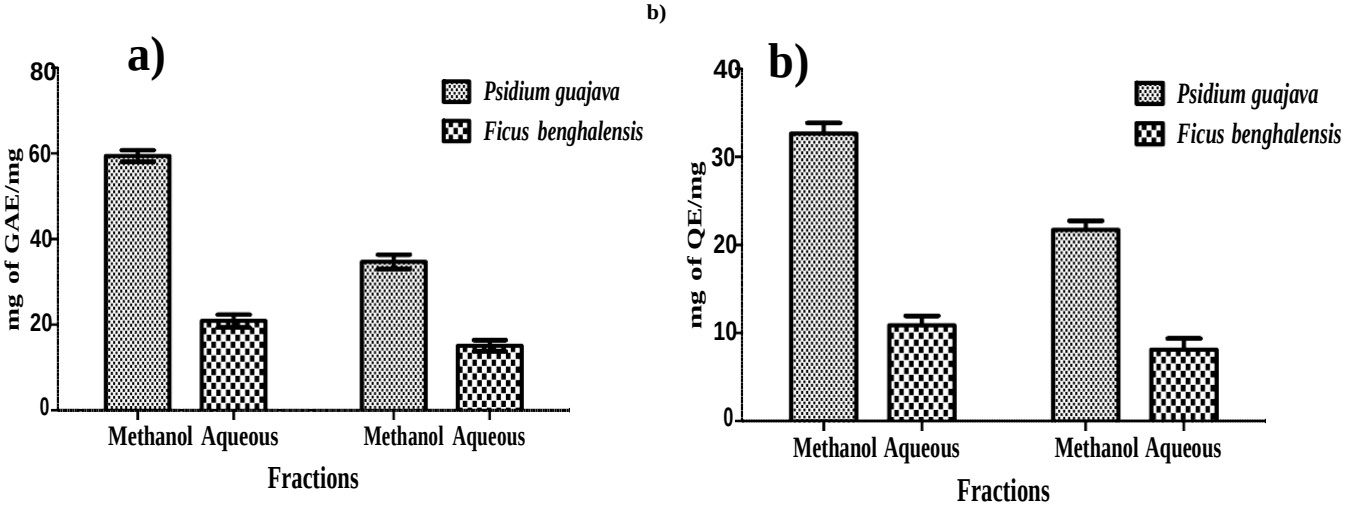

Table 3. Antioxidant activity of different extracts

Plant	Extract type	% Inhibition
Psidium guajava	Methanol	54.8
	Aqueous	41.0
Ficus benghalensis	Methanol	70.2
	Aqueous	53.7
	Ascorbic acid	87.0

Figure 3. Antioxidant DPPH Assay

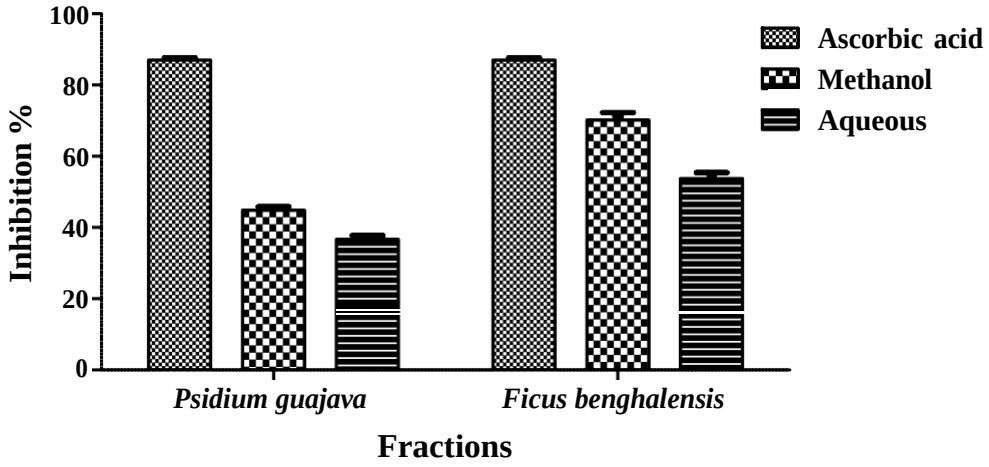

Table 4. IC_{50} value of Amylase and Lipase Inhibition assay

Plant	Extract type	Amylase assay IC_{50} (µg mL^{-1})	Lipase assay IC_{50} (µg mL^{-1})
Psidium guajava	Methanol	0.172	39.7
	Aqueous	0.151	98.3
Ficus benghalensis	Methanol	0.221	240
	Aqueous	0.253	229

IC_{50} = half maximal inhibitory concentration.

Figure 4. Effect of methanolic and aqueous extract on diffusion of glucose out of a dialysis membrane *a) Psidium guajava* b) *Ficus benghalensis*

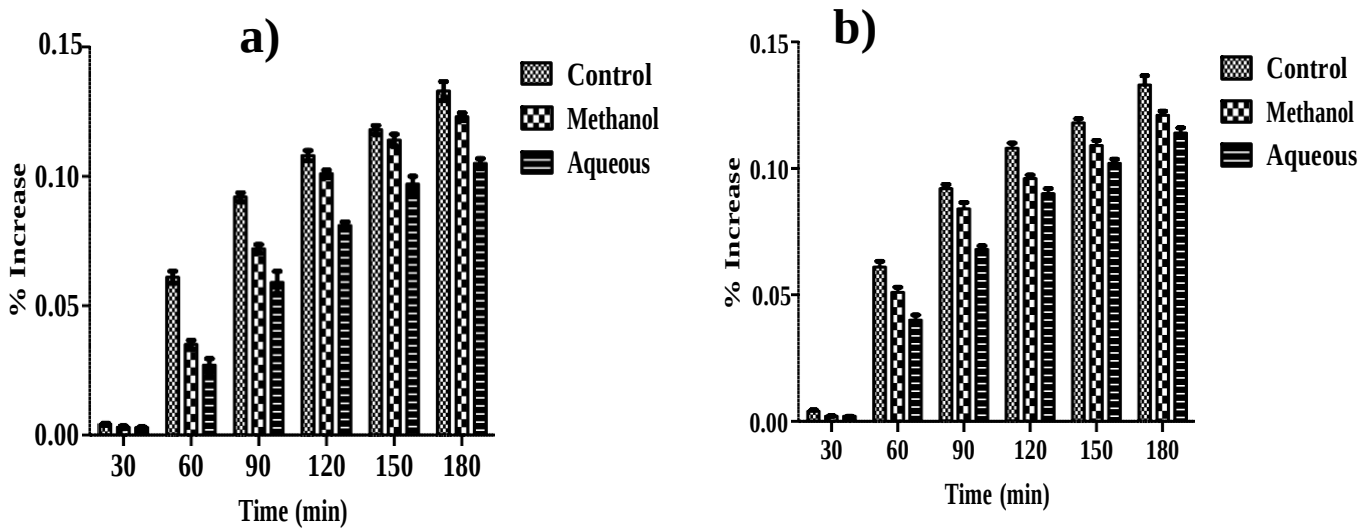

Table 5. Effect of methanolic and aqueous extract of *Psidium guajava* on diffusion of glucose out of a dialysis membrane over 180 minutes.

Time (Minutes)	Control Mean±SEM*	*Psidium* Methanol Mean±SEM*	Relative movement %**	*Psidium* Aqueous Mean±SEM*	Relative movement %**
30	0.004±0.0005	0.0031±0.0005	77.50	0.0029±0.00024	72.50
60	0.061±0.0023	0.035±0.0017	57.37	0.027±0.0026	44.26
90	0.092±0.0017	0.072±0.0017	78.26	0.059±0.0043	64.13
120	0.108±0.0020	0.101±0.0014	93.51	0.081±0.0014	75.00
150	0.118±0.0017	0.114±0.0023	96.61	0.097±0.0030	82.20
180	0.133±0.0037	0.123±0.0015	92.48	0.105±0.0020	78.94

Table 6. Effect of methanolic and aqueous extract of *Ficus benghalensis* on diffusion of glucose out of a dialysis membrane over 180 minutes.

Time (Minutes)	Control Mean±SEM*	Ficus Methanol Mean±SEM*	Relative movement %**	Ficus Aqueous Mean±SEM*	Relative movement %**
30	0.004±0.0005	0.002±0.0002	62.5	0.002±0.0001	50
60	0.061±0.0023	0.051±0.0020	83.60	0.040±0.0020	65.57
90	0.092±0.0017	0.084±0.0026	91.30	0.068±0.0014	73.91
120	0.108±0.0020	0.096±0.0014	88.88	0.090±0.0021	81.48
150	0.118±0.0017	0.109±0.0020	92.37	0.102±0.0017	86.44
180	0.133±0.0037	0.121±0.0017	90.97	0.114±0.0020	85.71