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RESEARCH ARTICLE

# Phytochemical and *In-Silico*Analysis of the PotentAntidiabetic Compound from Cactus (*Opuntia ficus–indica*) cladode

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## ABSTRACT

The present investigation aimed to identify the bioactive compounds in*Opuntia ficus* cladode and their pharmaceutical importance, which was evaluated through phytochemical and in-silico screening. The cladode's phytochemical screening revealed alkaloids, tannins, flavonoids, phenols, proteins, terpenoids,glycosides, steroids, saponins etc. A total of 25 compounds were identified in the gas chromatography and mass spectrometry (GC-MS) analysis, and an active metabolite 2-Thophene carboxylic acid-5methyl was selected for in-silico docking study againstfour human protein targets namely, Human Estrogen receptor (PDB ID: 3ERT) (anti-cancer), HIV-1 envelope glycoprotein (PDB ID: 4CC8) (anti-HIV), Human C-reactive protein (PDB ID: 1GNH) (anti-inflammation) and Human 11beta-hydroxysteroid dehydrogenase type I (11beta-HSD1) (PDB ID: 1XU7) (anti-diabetes) respectively *via* Schrodinger version 9.3. Among the four target proteins, the potent inhibition observed against Human 11beta-hydroxysteroid dehydrogenase type I (11beta-HSD1) (PDB ID: 1XU7) is involved in type 2 diabetes. Therefore, *Opuntia ficus*cladodes can be explored in managing type 2 diabetes.

**Key words:** *Opuntia ficus* indica; GC-MS; Ligand-based docking; Type-2 diabetes

## INTRODUCTION

The Cactaceae family includes *Opuntia ficus-indica*, also calledNopal or Prickly pear (Beatriz N. Guedes et al, 2023).This plant has long been used traditionally to treat various illnesses, including diabetes, especially in Mexico and Central America(Sáenz *et al.,* 2004). Scientific studies have validated these conventional beliefs, showing that *Opuntia ficus*-indica has hypoglycemic effects in human and experimental animals. Studies have shown that the ingestion of stems can lead to a decrease in blood glucose levels in individuals with non-insulin-dependent diabetes mellitus (NIDDM). Research involving patients with NIDDM found that ingesting 500g of *O. ficus indica* in various preparations (entire broiled, blended broiled, blended crude, or blended crude and heated) resulted in a statistically significant decrease in glycemia after 120 and 180 minutes. The hypoglycemic effect appears independent of heating or blending during preparation(Frati *et al.,* 1990). Several potential mechanisms have been proposed to explain the anti-diabetic properties of *Opuntia ficus-indica.* These include the role of dietary fiber in reducing

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glucose absorption in the intestine and inhibiting α-glucosidase activity, which can postpone the release of glucose. Water extracts of fresh nopal stems have shown significant decreases in blood glucose levels and exhibit α-glucosidase inhibitory activity(Hwang *et al.,*2017). *Opuntia ficus-indica* contains a complex array of chemical constituents, including polysaccharides, flavonoids, sterols, and various other bioactive compounds (Alqudah, S.M., *et al*., 2024). Given the intricate chemical profile of *Opuntia ficus*-indica and the demonstrated anti-diabetic effects, metabolomic mining represents a valuable approach to comprehensively identify and characterize the specific metabolites responsible for its therapeutic actions (Beatriz N. Guedes et al, 2023). Understanding the complete metabolome and its interaction with biological systems can lead to developing novel and effective anti-diabetic treatments with potentially fewer side effects than existing pharmacological agents. The increasing global prevalence of diabetes underscores the importance of exploring natural resources like *Opuntia ficus-indica*to discover new therapeutic strategies(Hwang *et al.,*2017).

Furthermore, a study on Wistar rats with induced hyperglycemia demonstrated the normoglycemic activity of *Opuntia ficus indica* stem extracts (Alqudah, S.M., *et al*., 2024). The acetone extract of OFI showed a high efficiency in controlling blood glucose levels, comparable to Metformin(Frati *et al.,*1990). Considering the growing demand for the development of economical, eco-friendly plant compounds with unique antidiabetic activity, the current study was performed with the objective of untapping the bioactive compounds and *in silico* docking against target potential for the treatment of diabetes.

## MATERIALS AND METHODS

### *Source of plant samples and chemicals*

Cladode and fruit of Opuntia were collected from the Agricultural College and Research Institute campus, Madurai. The identification was done at the Department of Plant Sciences, Madurai Kamaraj University, Madurai. Analytical grade chemicals for preparing the stock solutions were purchased from Sigma-Aldrich chemicals and Himedia Laboratories, India.

### *Phytochemical screening*

After the cladodes were gathered from the field and the thrones were manually removed, they were cleaned with tap water and dried before being milled into a fine powder. Seven distinct solvents—hexane, chloroform, diethyl ether, ethyl acetate, methanol, ethanol, and water—were used to get the crude extracts. Using a conventional methodology, the crude extracts were examined for the presence of active phyto components, including proteins, terpenoids, alkaloids, tannins, flavonoids, phenols, reducing sugars, glycosides, carbohydrates, and saponins (Mansoori *et al*., 2020). Wagner's and Dragendroff's assays were used to detect alkaloids.Tannins, flavonoids, and phenols were detected using ferric chloride, lead acetate, and ferric chloride test respectively. A sugar reduction test was also carried out. Ninhydrin and Millon's assays were used to determine whether proteins were present. To find terpenoids and steroids, the Salkowaski and copper acetate assays were used. The froth tests were used to detect saponins. The Molishch and Legal tests were used to check for glycosides and carbohydrates.

### *Determination of total phenolic contents*

The Folin Ciocalteu method (Elizabeth Rojas-Ocampo

**Fig.1. Cactus cladode and solvent extracts**

*et al.,* 2021) was used with minor adjustments to determine the total phenolic contents in the in various solvent extracts of cladode and fruit, viz., hexane, ethyl acetate, 40% ethanol, and water. To the aliquot of 0.25 mL, 1.25 mL Folin-Ciocalteu reagent was added, diluted ten times. Then, 1 mL of sodium carbonate (7.5%) was added. The mixture was incubated in the dark for 30 min. The absorbance was measured at 765 nm against a blank. The total phenolic contents were expressed as gallic acid equivalents (GAE) in milligrams per 100 g dry material.

***Determination of flavonoid contents***

The flavonoid compounds content wasascertained in various solvent extracts ofcladode and fruit, viz., hexane, ethyl acetate, 40% ethanol, and water,as described by Chougui*et al.,* 2013. In summary, 1.5 mL of extract was added to 1.5 mL of $AlCl_3$ reagent (2%). After 30 min of incubation in the dark, the absorbance was read at 430 nm against a blank. Quercetin was used as a standard for the calibration curve. The results are expressed as mg equivalent of quercetin (QE) per 100 g of dry matter.

***Gas Chromatography – Mass Spectrometry Analysis (GC-MS)***

The extract was prepared according to the protocol Rahul et. al. (2012) described with appropriate modification. Five (5) g of the powdered cladode was soaked in 25 mL of methanol for 48 hours. The extract was centrifuged at 5000 rpm for 10 min, then filtered through Whatman filter paper No. 41 along with 0.5 g sodium sulphate to remove any sediments and traces of water in the filtrate, and finally passed through a polyvinylidenedifluoride membrane filter of 0.22 micron (PDVF, Agilent Technologies, India).

***Analysis of the extract through GC-MS***

GC-MS analysis, the methanol cladode extract of *Opuntia ficus* – indica was performed using a Shimadzu QP2020 (Shimadzu, Japan) gas chromatographconnected to a mass spectrometer. GC was equipped with Rx Sil 5MS capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) consisting of a stationary phase 5% phenyl and 95% methyl polysiloxane. The injection was carried out in splitless mode at an injector temperature of 260°C. Helium gas was a carrier gas with a 1.0 mL/min flow rate. The oven temperature programming was as follows: the initial oven temperature was held at 70°C for 2.0 min, and then increased to 210°C at a rate of 20°C/min, and then increased to 290°Cat a rate of 10 °C/min held for 5 min. The ion source and transfer temperature were 230°C and 280°C, respectively.

***Identification of the compounds***

GC-MS, which detected the various compounds present in the methanol extract of the cladode. Identification and interpretation of each compound's mass spectrum was carried out using National Institute Standard and Technology (NIST) database available in the instrument. The spectrum of the unknown components was compared with those of the known components stored in the NIST library.

***In silico analysis of the biomolecules present in Cactus cladode methanolic extract***

The names, molecular formula, molecular weight, and structure of the compounds in the methanol cladode extract were identified using GC-MS analysis, and the NIST library was ascertained via PubChem database and Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp.kegg).

***Screening of ligands for pharmacological activity***

The three-dimensional (3D) structures of the Biomolecules with enzymatic inhibitory activity were obtained from PubChem compound-specific database. The pharmacological activity for the selected structures was screened using the QikProp module from the Schrodinger 9.3 and Prediction of Activity Spectra for Substances (PASS).

***Ligand–based docking using GLIDE (Grid-based Ligand Docking with Energetics)***

To carry out glide analysis, 3D conformation structures of the ligands for antidiabetic propertywere obtained from Pubchem in .sdf format. The receptors were prepared, grid generation was done, the ligands were prepared, and finally, docking analysis was carried out.

***Analysis of the docking result***

GLIDE XP visualization of Schrodinger software was used in viewing the docking results and incorporated them into the project table as an XP descriptor file in .xpdes format. The analyzed results were saved in .csv format.

***Screening of the identified compound for pharmacological activity***

From the list of compounds in table 2, the highest peak area (%) compound was screened for

various pharmacologicalactivities. The respective 3D conformation structure and biological activity were obtained from PDB and PASS database, respectively (Table 3).

### *Statistical analysis*

Data are expressed as mean ± standard deviation from three separate observations. For total phenol compounds assays (phenol and flavonoid) a way ANOVA test was used to analyze the significance of the difference between various extracts studied ($P < 0.05$).

## RESULTS AND DISCUSSION

### *Phytochemical analysis of Cactus cladode*

The *Opuntia indica*ficus cladode was subjected to preliminary Phytochemical screening using different qualitative tests. The results indicated the presence of alkaloids, tannins, flavonoids, phenols, proteins, terpenoids, reducing sugars, glycosides, carbohydrates, steroids, and saponins as shown in Table 1.

### Total Flavonoid content analysis (TFC)

Total flavonoid analysis refers to determining the total amount of flavonoids present in a sample. This analysis is essential because flavonoids are known for their antioxidant and other health-promoting properties. TFC is typically determined, often involving aluminum chloride, and results are expressed as equivalents of a standard flavonoid, like catechin or quercetin.

Cactus cladode and fruit possess higher health-promoting properties in terms of high antioxidant potential in ethyl acetate fraction thanwith other solvent fractions.

### *GC-MS analysis of the cladode of Cactus*

The methanol cladode extract of cactus was subjected to GC-MS analysis using Shimadzu QP2020 gas chromatography mass spectrometry. The compounds identified are recorded in Table 2.

Medicinal plants are the source of potent drugs,

### Total phenolic content estimation using Folin-Ciocalteu assay (TPC)

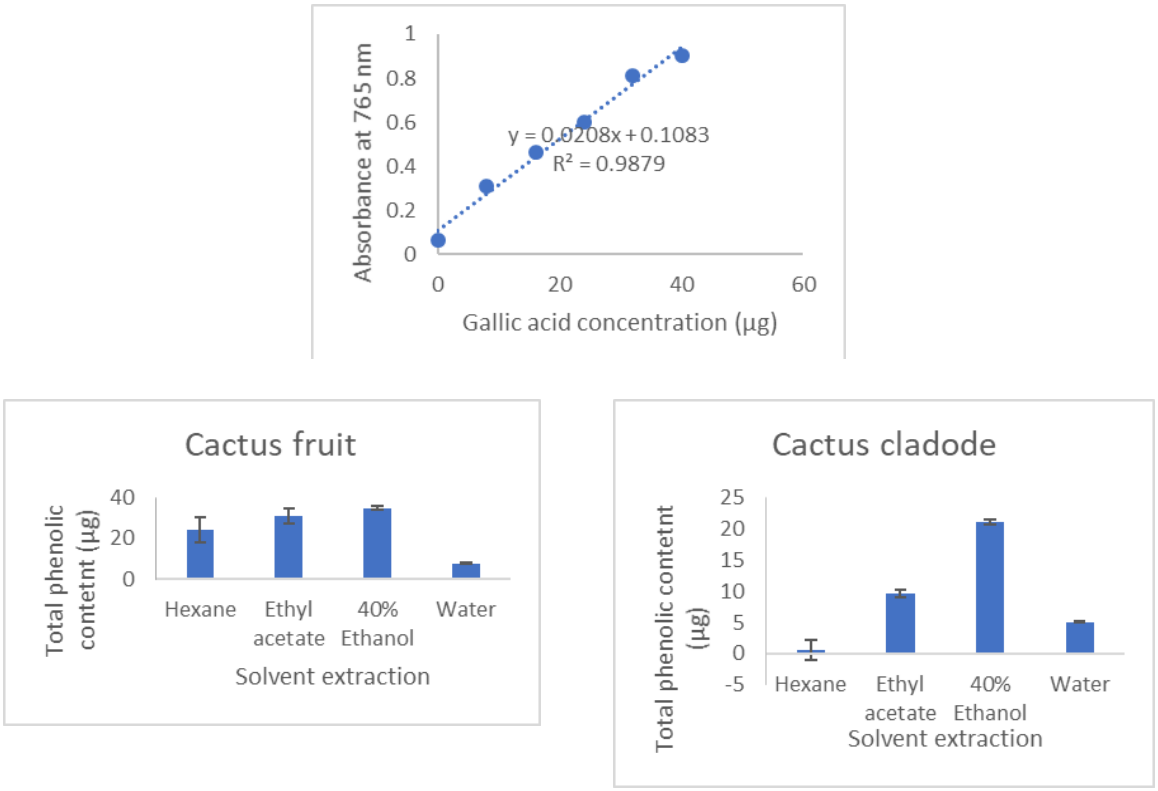
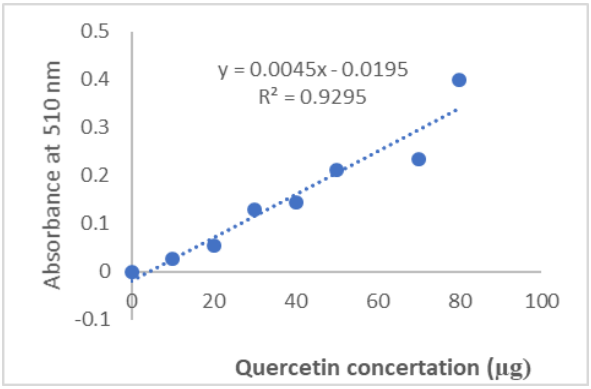



**Figure 2. Total phenolic estimation by the Folin-Ciocalteu method in Opuntia plant fruits and Cladodes. A. The standard graph uses gallic acid as a standard polyphenol. B. Cactus fruit; C. Cactus cladode.**

**Table 1. Phytochemical analysis of Cactus cladode methanol extract**

| | | Solvent extracts | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Test | Reagents | Hexane | Chloro form | Diethyl ether | Ethyl acetate | Methanol | Ethanol | Water |
| Alkaloids | Dragendroff | + | + | + | - | - | - | + |
| | Wagner | + | + | + | - | + | - | + |
| Reducing sugars | Benedict | + | - | + | + | + | + | + |
| Proteins | Ninhydrin | + | + | + | + | + | + | + |
| | Millon's reagent | + | + | + | + | + | + | + |
| Terpenoids | Salkowski test | + | - | + | + | + | + | - |
| | Copper acetate test | - | + | + | - | - | + | + |
| Steroids | Salkowski test | + | + | + | + | - | - | - |
| Tannins | Ferric chloride | - | - | + | + | + | + | + |
| Phenols | Ferric chloride | + | + | + | + | + | + | + |
| Flavonoids | Lead acetate | - | - | + | + | - | + | - |
| Saponins | Froth test | - | - | - | - | - | + | + |
| | Foam test | - | - | - | - | - | + | + |
| Carbo hydrates | Molisch's test | + | - | + | + | + | + | + |
| Glycosides | Legal's test | - | + | - | - | + | + | + |



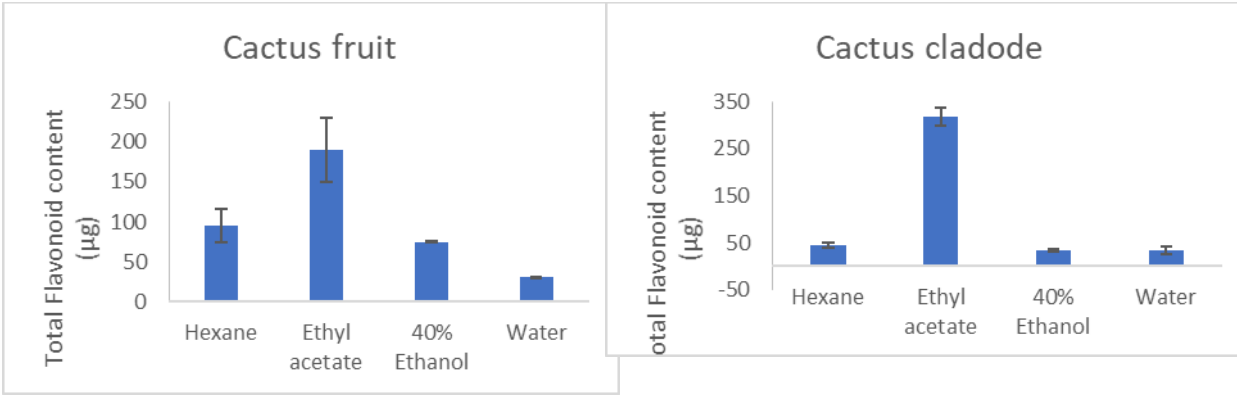




**Figure 3. Total flavonoid estimation by Aluminium chloride method in Opuntia plant fruit and cladode. A. Standard graph using Quercetin as a standard flavonoid. B. Cactus fruit; C. Cactus cladode**

***Methanol extract of the cladode:***

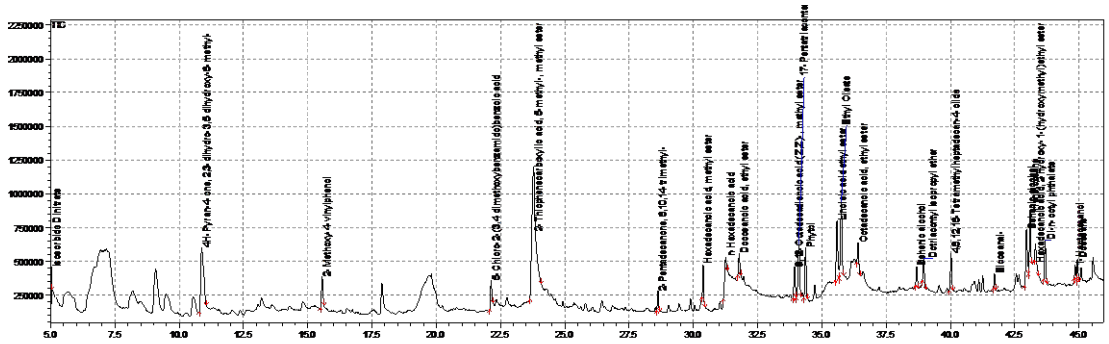


**Table 2. Compounds identified in the methanol extract of cactus cladode by GC-MS**

| RT (min) | Name of the compound | Peak area (%) | Molecular weight (g/mol) | Molecular formula |
|---|---|---|---|---|
| 5.02 | Isosorbide Dinitrate | 0.87 | 236 | $C_6H_8N_2O_8$ |
| 10.86 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydrdoxy-6-methyl | 10.68 | 144 | $C_6H_8O_4$ |
| 15.55 | 2-Methoxy-4-vinyl phenol | 2.48 | 150 | $C_9H_{10}O_2$ |
| 22.13 | 5-Chloro-2(3,4-dimethoxy benzamido) benzoic acid | 2.04 | 335 | $C_{16}H_{14}ClNO_5$ |
| 23.78 | 2-Thophene carboxylic acid-5-methyl | 27.56 | 156 | $C_7H_8O_2S$ |
| 28.62 | 2-Pentadecanone-6,10,14-trimethyl | 1.30 | 268 | $C_{18}H_{36}O$ |
| 30.38 | n-Hexadecanoic acid | 4.67 | 270 | $C_{17}H_{34}O_2$ |
| 31.78 | Docosanoic acid | 1.58 | 368 | $C_{24}H_{48}O_2$ |
| 33.93 | 9,12-Octadecadienoic acid | 2.31 | 294 | $C_{19}H_{34}O_2$ |
| 34.10 | 17-Pentatriacontane | 5.30 | 490 | $C_{35}H_{70}$ |
| 34.36 | Phytol | 4.00 | 296 | $C_{20}H_{40}O$ |
| 35.58 | Linoleic acid ethyl ester | 5.42 | 308 | $C_{20}H_{36}O_2$ |
| 35.75 | Ethyl oleate | 6.98 | 310 | $C_{20}H_{38}O_2$ |
| 36.40 | Octadecanoic acid ethyl ester | 1.71 | 312 | $C_{20}H_{40}O_2$ |
| 38.96 | Dotriacontyl isopropyl ether | 1.80 | 508 | $C_{35}H_{72}O$ |
| 40.02 | 4,8,12,16-Tetramethyl heptadecan-4-olide | 2.81 | 324 | $C_{21}H_{40}O_2$ |
| 41.72 | Eicosanol | 0.94 | 296 | $C_{20}H_{40}O$ |
| 42.95 | Behenic alcohol | 4.73 | 326 | $C_{22}H_{46}O$ |
| 43.10 | Henicosane | 4.17 | 296 | $C_{21}H_{44}$ |
| 43.31 | Hexadecanoic acid-2 hydroxy -1(hydroxymethyl)ethyl ester | 1.69 | 330 | $C_{19}H_{38}O_4$ |
| 43.69 | Di-n-Octyl Phthalate | 3.22 | 390 | $C_{24}H_{38}O_4$ |
| 44.85 | 1-Heptacosanol | 0.88 | 396 | $C_{27}H_{56}O$ |
| 44.94 | Docosane | 1.37 | 310 | $C_{22}H_{46}$ |

and the use of plants as a medicine has been inherited as an important component of the health care system from the ancient period. India is also regarded as the largest producer of medicinal herbs (Sathyaprabha, 2010). *Opuntia ficus-indica* is also used traditionally to treat various illnesses, including diabetes, especially in Mexico and Central America (Saenz et al, 2004).

Successful isolation of the bioactive principle from plant materials largely depends on the type of solvent used in the extraction procedure. The solvents can also contribute to the variation since it has been proven that the extraction of biologically active compounds

from plants depends upon the polarity of the solvent used (Ghosh *et al.,* 2012).

In the present study, the cladode was subjected to qualitative phytochemical screening. Seven different solvents: Hexane, chloroform, diethyl ether, ethyl acetate, methanol, ethanol and water were used. The presence of alkaloids, tannins, flavonoids, proteins, terpenoids, reducing sugars, glycosides, carbohydrates, steroids and saponins was documented and these results were similar to the results of Shimaa Ali *et al.,* 2022.

### *Docking analysis*

Docking analysis was carried out for the compound 2-Thophene carboxylic acid-5methyl (2-carboxy-5-methylthiophene) obtained from the methanolic extract of the cactus cladode for four human protein targets namely, Human Estrogen receptor (PDB ID: 3ERT) (anti-cancer), HIV-1 envelope glycoprotein (PDB ID: 4CC8) (anti-HIV), Human C-reactive protein (PDB ID: 1GNH) (anti-inflammation) and Human 11beta-hydroxysteroid dehydrogenase type I (11beta-HSD1) (PDB ID: 1XU7) (anti-diabetes) respectively *via* Schrodinger version 9.3.

The bioactive compounds obtained from plants, include alkaloids, flavonoids, tannins, phenolic compounds, etc.,are the main drivers of the pharmacological actions of medicinal plants (Hussein and El-Anssary, 2018). The chemical constituents of the plants are the economic source for the synthesizing complex chemical substances (Moniruzzaman Sohag Howlader *et al*., 2016).

A total of 25 compounds were identified from the GC-MS result, the concentration of 2-Thophene carboxylic acid-5methyl (2-carboxy-5-methylthiophene) was found to higher in terms of area %. So far, this compound is not reported from the methanolic extract of the cactus cladode and was found to be a first report. Other therapeutic compounds such as heneicosane, behenic alcohol, pentatriscontene, and docosane were also found.

Gas chromatography-mass spectrometry has been firmly established as a key technological platform for identifying secondary metabolites in both plant and non-plant species. Its ability to separate complex organic molecules with high efficiency (Zhang *et al.,* 2012), the mass fragmentation pattern based on m/z values, provide a clue to the molecular structure. Additionally, the data library along with the molecular ion peaksignificantly improves the compound identification.

We conducted the in-silico analysis using the biologically active secondary metabolites identified from the GC-MS screening. Among the chosen targets, the highest binding affinity of -5.25 kcal/mol wasobserved withdiabetes target Human 11beta-hydroxysteroid dehydrogenase type I (11beta-HSD1), which showed two hydrogen bond interactions (GLN 21 and ARG 252 residues) with 2-Thiophenecarboxylic acid, 5-Methyl-,2-(Methylamino)-2-Oxoethylester. The second highest binding affinity was shown against the anti-HIV target, HIV-1 envelope glycoprotein (-5.14 kcal/mol), with PRO 167 and SER 176 residues involved in hydrogen bond interactions at the active site. Further, 2-Thiophenecarboxylic acid,5-Methyl,2-(Methylamino)-2-Oxoethyl estershowed binding value of -4.55 kcal/mol and -3.46 kcal/mol against cancer target and inflammation target respectively. Further, this compound has a bioavailability score of 0.55 and follows the Lipinski rule of five, which is an important property for the drug's likelihood.

Based on the above docking results, Thiophenecarboxylic acid,5-Methyl,2-(Methylamino)-2-Oxoethyl ester is predicted as the best anti-diabetic compound and may be considered as a drug-like molecule for further investigation to develop as a drug.

### *Conclusion*

The medicinal/pharmacological property of the cactus cladode is attributed to the presence of various bioactive compounds. The findings recorded in the present research will be helpful in designing an anti-diabetic drug. Therefore, this study supports the management of type-2 diabetes with the plant-based compound as they are practical, easily accessible, and have no known adverse effects.

### *Acknowledgement*

The authors are grateful to the Tamil Nadu Agricultural University for sanctioning the University research project and the Centre for Plant Molecular Biology and Biotechnology (CPMB&B), TNAU, Coimbatore, for their support throughout this research.

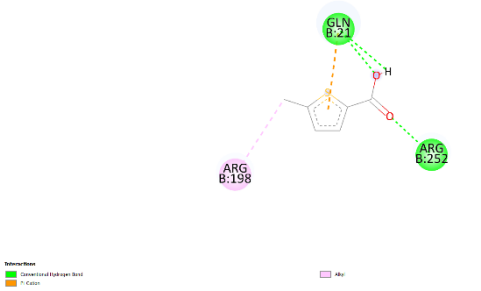
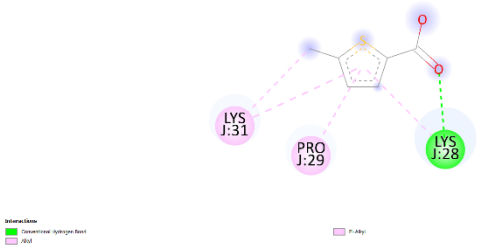
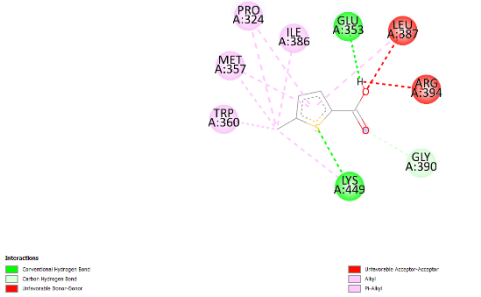
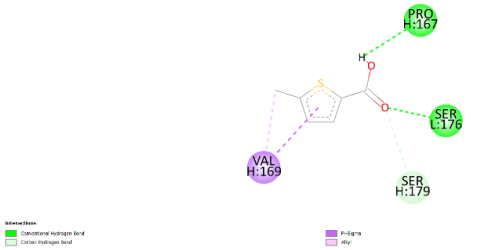
### *Conflict of Interest*

The authors hereby declare no conflict of interest.

### *Ethics statement*

No specific permits were required for the described

**Table: Information of binding affinity and the 2D interaction diagram**

| Ligand (Pubchem ID) | Protein | Lowest Binding Energy | Interaction diagram |
|---|---|---|---|
| 2-Thiophenecarboxylicacid,5-Methyl-,2-(Methylamino)-2-Oxoethylester(9ci)(3471009) | 1XU7/Human 11beta-hydroxysteroid dehydrogenase type I (11beta-HSD1) | -5.25 |   |
| | 1GNH/ Human C-reactive protein | -3.46 |   |
| | 3ERT /Human Estrogen receptor | -4.55 |   |
| | 4CC8/HIV-1 envelope glycoprotein | -5.14 |   |

field studies because no human or animal subjects were involved in this research.

***Consent for publication***

All the authors agreed to publish the content.

***Competing interests***

There were no conflicts of interest in the publication of this content.

***Author contributions***

Idea conceptualization, writing original draft – Vellaikumar, Experiments – Vellaikumar, Saranya, reviewing and editing – Saranya andPreethieswari.

## REFERENCES

Alqudah, S.M., Hailat, M., Zakaraya, Z, Abu Dayah, A.A, Abu Assab, M, Alarman, S.M., Awad, R.M., Hamad, M.F., Vicas, L.G., Abu Dayyih, W. Impact of *Opuntia ficus*-indica Juice and Empagliflozin on Glycemic Control in Rats. Current Issues

in Molecular Biology. 2024. 46: 12343–12353. https://doi.org/10.3390/cimb46110733

Beatriz N. Guedes, Faezeh Fathi, Amélia M. Silva, Antonello Santini, M. Beatriz P. P. Oliveira, Eliana B. Souto. 2023. Biopharmaceutical applications of Opuntia fcus-indica: bibliometric map, bioactivities and extraction techniques. European Food Research and Technology, 249:2457–2469. https://doi.org/10.1007/s00217-023-04314-w

Chougui, N., Tamendjari, A., Hamidj, W., Hallal, S., Barras, A., Richard, T., andLarbat, R. 2013. Oil composition and characterisation of phenolic compounds of Opuntia ficus-indica seeds. *Food chemistry.*, ***139*(1-4)**: 796-803.DOI: 10.1016/j.foodchem.2013.01.054

Elizabeth Rojas-Ocampo, Llisela Torrejon-Valqui , Lucas D. Munoz-Astecker, Marleni Medina-Mendoza, Diner Mori-Mestanza, Efraín M. Castro-Alayo. 2021. Antioxidant capacity, total phenolic content and phenolic compounds of pulp and bagasse of four Peruvian berries. Heylion, 7(1-6). https://doi.org/10.1016/j.heliyon.2021.e07787 R

Frati, A. C., Jiménez, E., and Ariza, C. R. 1990. Hypoglycemic effect of *Opuntia ficus indica* in non insulin-dependent diabetes mellitus patients. *Phytotherapy Research.*, ***4*(5)**:195–197. https://doi.org/10.1002/ptr.2650040507

Hwang, S. H., Kang, I.-J., and Lim, S. S. 2017. Antidiabetic Effect of Fresh Nopal ( *Opuntiaficus-indica* ) in Low-Dose Streptozotocin-Induced Diabetic Rats Fed a High-Fat Diet. *Evidence-Based Complementary and Alternative Medicine.*, ***2017*(1)**, 4380721. https://doi.org/10.1155/2017/4380721

Moniruzzaman Sohag Howlader, Sheikh Rashel Ahmed, Khadizatul Kubra and Md. Khairul Hassan Bhuiyan, 2016. Biochemical and phytochemical evaluation of *Stevia rebaudiana*. Asian Journal of Medical and Biological Research, 2 (1): 121-130; Doi: https://doi.org/10.3329/ajmbr.v2i1.27577

Mansoori A, Singh N, Dubey SK, Thakur TK, Alkan N, Das SN and Kumar A (2020) Phytochemical Characterization and Assessment of Crude Extracts from *Lantana camara* L. for Antioxidant and Antimicrobial Activity. Front. Agron. 2:582268. doi: https://doi.org/10.3389/fagro.2020.582268

.Rahul, J. S., Jyoti, S., Shakya, B., and Naz, F. 2012. Effect of *Eucalyptus citriodora* extract on hsp70 expression and tissue damage in the third instar larvae of transgenic Drosophila melanogaster (hsp70-lacZ) Bg9. *Phytopharmacology*, ***3*(2):** 111-121.DOI: https://doi.org/10.3109/00207454.2012.709893

Sáenz, C., Sepúlveda, E., andMatsuhiro, B. 2004. Opuntia spp mucilage's: A functional component with industrial perspectives. *Journal of Arid Environments.*, ***57*(3)**: 275–290. https://doi.org/10.1016/S0140-1963(03)00106-X

Sathyaprabha, G., Kumaravel, S., Ruffina, D., and Praveenkumar, P. 2010. A comparative study on antioxidant, proximate analysis, antimicrobial activity and phytochemical analysis of Aloe vera and Cissus quadrangularis by GC-MS. *J.Pharm Res.*, **3:** 2970-2973.DOI: http://dx.doi.org/10.13005/ojc/320307

Shimaa K. Ali , Sara M. Mahmoud, Samar S. El-Masry, Dalal Hussien M. Alkhalifah, Wael N. Hozzein, Moustafa A. Aboel-Ainin. 2022. Phytochemical screening and characterization of the antioxidant, antiproliferative and antibacterial effects of different extracts of *Opuntia ficus-indica* peel. Journal of King Saud University – Science 34: 102216. https://doi.org/10.1016/j.jksus.2022.102216