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Phytochemical Profiling, GC-MS Analysis, *In-vitro*, and *In-silico* Aldose Reductase Activity of Coccinia Indica (L.) Fruit Extract: A Step Towards the Management of Hyperglycemia

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Coccinia indica (L.) fruit (CIFs) is a mounting, herbaceous, branching latitude vascular domestic plant in Asian countries. It has gained popularity as a medicinal remedy for reducing elevated blood glucose levels. This study aimed to assess the phytochemical composition of CIFs, encompassing its liquid, solid, and gaseous constituents using Gas chromatography and Mass spectroscopy (GC/MS). Additionally, the research sought to investigate the aldose reductase inhibitor (ARI) activity through *in-vitro* assays utilizing CIFs extracts obtained with polar and nonpolar solvents. The findings revealed that the ethanolic extract exhibited a substantial yield of 33.9%, surpassing the aqueous (18.6%), chloroform (11.3%), and petroleum ether (9.2%) extracts. Moreover, the ethanolic extracts displayed the highest concentration of phenolic compounds (12.5 ± 0.84 mg gallic acid equivalent (GAE)/g) and flavonoids (78.4 ± 3.6mg quercetin equivalent (QE)/gm) in dry extract content. GC-MS analysis of the ethanolic extracts unveiled a total of 23 distinct compounds. The *in-vitro* assessment of aldose reductase inhibitor activity conducted on partly purified bovine lens samples highlighted the exceptional performance of the ethanolic solvent, demonstrating a remarkable 78.46% ARI activity at an inhibitory concentration (IC_{50}) value of 2.34 µg ml^{-1}. The aqueous extract also exhibited significant ARI activity at 76.88% at an IC_{50} value of 3.88 µg ml^{-1}. Furthermore, the *in-silico* molecular docking and Density Functional Theory (DFT) studies of the peak compound 4H-Pyran-4-one, 2,3- dihydro-3,5-dihydroxy-6- methyl (DDMP) were carried out to determine the spectroscopic and biological insights. The results suggest that CIFs hold significant potential as effective agents with ARI properties, making it a promising candidate for managing diabetes mellitus and its associated complications.

Keywords: *Coccinia Indica L*, Diabetes Mellitus, GC-MS, Aldose Reductase inhibitor, Molecular Docking, DFT

INTRODUCTION

Contemporary investigation on herbal medicine mainly focuses on traditional folklore medicine, which is non-toxic and safer for humans. Indian conventional medicine systems, including Ayurveda, quoted thousands of effective herbal plants against numerous diseases. One such medicinal plant, *C. indica,* is an indigenous plant of Asia and India. It belongs to the Cucurbitaceous family, which is more

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cultivated in southern India (Andhra Pradesh and Tamilnadu).*C. indica* fruits are widely used for cookeries. In our traditional medicine system, the fruit of *C. indica* is the popular anti-diabetic and hypoglycaemic medicine for its phytochemistry and pharmacology. The primary focus of the current study is to delve into the phytochemical components found within the fruit extract of *C. indica* and its *in-vitro* ARI activity. For that, it is mandatory to screen the phytocomponents to understand the pharmacological activity of the medicinal plants. The fruits are green while ripe and turn red over time. It is slippery, pulpy, primarily oval, with a diameter of 2.5 cm and 5.0 cm in length, and has numerous oblong seeds of about 7mm long and thickened margin. *C. indica* mainly grows in dry, hot, and shady places [1,2]. *C. indica* leaf extracts are widely used to treat fever, wounds, asthma, cough, inflammations, *etc*. [3]. Further investigation was carried out on GS-MS analysis. GC/MS is a very compatible technique often used for identifying and quantifying phytochemicals [4,5]. Almost all parts of the plants (roots, stems, and leaves) are traditionally used for treating allergies, eye infections, insect bites, bronchitis, jaundice, burns, skin eruption, syphilis, *etc*. [6].

C. indica leaves are mainly reported to contain compounds like flavonoids and phenols with significant antioxidant activity. The *C. indica* fruits are also said to have insulin tropic and anti-glycation properties [7]. The fruit of *C. indica* is mainly used for culinary purposes, which is natural medicine. *C. indica* is an essential herb with recognized biological activities against free radicals and aldose reductase inhibition. Various therapeutic plants are studied to find novel compounds which having hypoglycemic [8,9] and hypolipidemic activity [10]. *C. indica,* also named *C.grandis* and *C.cardifolia* have reported the presence of phytocompounds like alkaloids [11], flavonoids, carotenoids, triterpenoids, and lipids [12,13]. *C. indica* possesses hypoglycaemic activity in alloxan-induced diabetic animals, reducing lipid peroxidation and controlling phagocytosis. The polysaccharides present in the fruits of *C. indica* are accountable for its hypoglycaemic activity in the animal diabetic and human models [14]. It was reported that *C.grandis* lowers the blood glucose level by increasing the glycogen level in the liver and reducing the phosphorylase activity. In the animal model, the hypoglycemic effect is achieved by blocking glucose-6-phosphate a key enzyme in glucose metabolism.

Aldose reductase (AR) [EC1.1.1.21], serves as a crucial enzyme in the sorbitol pathway that facilitates the breakdown of glucose into sorbitol, subsequently converting it into fructose [15]. In diabetes mellitus, the sorbitol pathway is activated significantly and utilizes more than 30% glucose compared to 3% of normal conditions. This will result in various microvascular complications [16]. This increased sorbitol concentration in the lens leads to the inflow of liquid, swelling, and causing cataracts [17]. Searching for effective ARIs with antioxidant properties is a practical approach to managing and preventing diabetic complications [18]. Numerous ligands capable of inhibiting AR have been identified, which include quercetin, sorinil, zopolrestat, fidarestat, ponalrestat, tolrestatepalrestat, zenareststt, and resveratrol [19,20]. Since most of the inhibitors have low *in-vivo* efficacy or have contrary effects [21]. Epalrestat is a known drug showing good *in-vivo* development against Diabetes Mellitus (DM) [22]. The *in-vitro* ARI studies strongly suggest that CIFs have pharmacological activity against diabetes and its related complications by inhibiting the bovine lens aldose reductase activity.

Diabetes mellitus (DM) is a chronic illness characterized by two forms (T1DM and T2DM). It has been documented that around 90% of suffer from T2DM and 10% from T1DM [23]. A major endocrine disorder has posed medical encounters globally and also develops complicated metabolic threats in patients. As in chronic conditions, elevated levels of lipids as well as oxidative stress, can damage diverse organs, mainly blood vessels, the central nervous system, the eyes, and the kidneys. The World Health Organization (WHO) reported that DM will cause more than expected and a high death rate. 387 million people are pretentious to this disease, and it is estimated that 640 million people will be affected by 2040 [24]. The application of DFT in drug discovery has gained attention in recent decades due to its cost-effectiveness and remarkable precision in forecasting the physiochemical and molecular properties of both natural and synthesized compounds [25-28]. The active compounds To determine the inhibitory activity of aldose reductase of the active compound in the CIFs extract polar (ethanol and water) and nonpolar (petroleum ether and chloroform) solvents. The literature survey reveals an evaluation of the effectiveness of the active components within the CIFs extract and its ARI activity yet to be reported. In light of this observation, our present work deals with *in-vitro* and *in-silico*

activity of phytocompounds within the CIFs extract a novel drug for diabetes mellitus management.

MATERIALS AND METHODS

Fruit and Extraction Preparation

Fresh fruits of *C. indica* were gathered from an agricultural field near Cheyyar and Arni, Tamilnadu. The plants were authenticated by Professor Dr..P. Jayaraman, Director, Plant Anatomy Research Centre, West Tambaram, Chennai, and the voucher specimens are stored in the library for future reference. The fruits are washed thoroughly, cut into pieces, dried in a hot air oven for 4-5 days, separated and crushed using a grinder to make fine powder, and stored in a container for the following analysis [29]. The prepared powder fruit of *C. indica* was cramped in the muslin cloth. It is used for extraction by soxhlet extraction apparatus using water and petroleum ether, chloroform ethanol and water for 24 h separately. It was concentrated through a rotary evaporator and subsequently subjected to freeze-drying in a lyophilizer until dry was obtained [30], preserved in a container free from contamination, and utilized for the phytochemical analysis, GC-MS studies, and ARI activity. The percentage yield was calculated for the dried extracts.

Chemicals

All the standard chemicals were procured from Sigma-Aldrich and HiMedia Laboratories Pvt Ltd from Bangalore and Mumbai, India respectively.

Phytochemical Analysis

The petroleum ether, chloroform, aqueous, and ethanol fruit extract of *C. indica* underwent qualitative phytochemical screening using standard methods to identify a wide range of phytocomponents [31]. The extract determined the existence or non-existence of several active principle compounds like flavonoids, phenols, fixed oils, glycosides, alkaloids, tannins, saponins, amino acids, carbohydrates, fats, and proteins.

Ascertain Total Phenolic Content (TPC) and Flavonoid Content (TFC)

To ascertain the TPC of CIFs extracts, the folin-ciocalteu's phenol reagent technique was employed following standardization with gallic acid [32]. In brief, 0.5 ml of extract dissolved in Dimethyl sulfoxide (DMSO) followed by adding 0.5 ml of folin-ciocalteu's phenol reagent was vigorously shaken and neutralized with 0.5 ml of sodium hydroxide (NaOH). Distilled water (DW) was introduced to attain a total measurement of 5 ml, and the mixture was maintained at Room Temperature (RT) for 90 min with intermittent shaking. The optical density was measured at 760 nm along with the control sample (without extract). The amount of TPC was then measured as milligrams of gallic acid equivalent per gram of dry extract. The aluminum chloride colorimeter method determined the TFC of all four extracts with specific alterations [33]. In a concise, 250 µl of extract dissolved in DMSO was assorted with 1 ml of DW, followed by 150 µl of sodium nitrate, then 75 µl of 10% aluminum chloride solution was introduced and kept RT for 5 minutes. The final measurement was adapted to 2.5 ml with DW after adding 1 ml of NaOH, shaken vigorously, and RT was held for 5 min. After the mixture was measured at 510 nm, the amount of TFC was calculated as mg of quercetin/g of dry extract.

GC-MS Spectrometry

The GC-MS analysis was conducted utilizing an integrated GC-MS instrument (436-GC Bruker) equipped with HP-5 fused silica capillary column**.** This approach is configured to handle both GC and MS. A 1 µl aliquot of sample was introduced into the column through a Programmable Temperature Vaporizing (PTV) injector, set at a temperature of 275 °C. The Column temperature was set to 60 ºC for 5 min to initiate the program and increased to 300 ºC by 8 ºC min^{-1}. Helium is used as carrier gas (1.5 ml min^{-1}). The mass spectroscopy was operated in Ionization Modes (EI) mode with the mass score set at 200 ºC.GC peak areas calculated the present age of constituents in the plant's taken medicinal part. GC-MS data are handled using JEOL software and peak compounds with retention time were identified based on the National Institute of Standard and Technology (NIST), WILEY, and DUKE's database library.

Preparation and Determination of aldose Reductase Activity (AR) and Inhibition (ARI) Assay of Bovine Lens Homogenate

The fresh diseases-free bovine eyes were collected from

the local slaughterhouse on the day of the experiment. The procedure involved extracting the lenses from the eyes, followed by washing with both fresh water and saline solution. Transparent lenses were combined and suspended at a pH of 7.4 with 0.1 ml of phosphate buffer. Afterward, the suspension underwent centrifugation in a refrigerated centrifuge at 5000 rpm for 10 min, resulting in the collection of the supernatant, which was then refrigerated for future analysis. AR activity was determined activity a sample cuvette containing 0.7 ml, 0.1 ml, and 0.1 ml of 0.06 M phosphate buffer, NADPH, and lens supernatant respectively, and a reference cuvette, and the reaction mixture was adjusted to 6.8 pH. Then 0.1 ml of DL glyceraldehyde (substrate) was mixed to start the enzymatic reaction in the sample cuvette but not on the reference cuvette (ensuring the sample cuvette containing the final volume has 1.0 ml, and reference cuvette containing 0.9 ml), and the Optic Density (O.D) was measured at 30 seconds intervals for 3 min at 340 nm. AR activity was measured and expressed as ΔOD /min/mg of protein. For determining the ARI activity of different extractions, a stock solution was prepared by dissolving the plant extract in phosphate buffer saline (PBS).0.1 ml of each different plant extract solution having final concentration (25, 50, 75, 100, 200 and 300 µg ml^{-1}). Control cuvettes contain (0.1 ml, 0.7 ml, and 0.1 ml of lens supernatant, phosphate buffer, NADPH, and various concentrations of quercetin (25, 50, 75, 100, 200 and 300 µg ml^{-1})). Experimental cuvettes contain (0.1 ml, 0.7 ml, and 0.1 ml of lens supernatant, phosphate buffer, NADPH, and diverse concentrations of different fractions of CIFs extract (25, 50, 75, 100, 200, and 300 µg ml^{-1}), the 0.1 ml of substrate solution was mixed to initiate the reaction in all the cuvettes, which was calculated at 340 nm for 3 min at 30 s intervals. For finding the ARI activity of different concentrations of extraction solvents, with quercetin as standard. The calculation of aldose reductase inhibitory activity involved creating a graph that plotted log dose concentration against the percentage of inhibition, IC_{50} values were calculated for all samples.

Computational Details

The theoretical approach was conducted utilizing the DFT/B3LYP/6-311++G(d,p) level of theory within the Gaussian 09W software [34-37]. The Chemcraft program [38] was employed to visualize the optimized geometric features and electronic characteristics of DDPM. The electronic spectra of the titled compound were simulated using a time-dependent DFT (TD-DFT) approach [39-42]. The target protein aldose reductase crystal structure with PDB ID of 3G5E was recovered from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB). GaussView 6.0 was employed to draw the DDPM structure [43], which was then rehabilitated into PDB format to refine as a ligand. Molecular docking studies were performed using AutoDock [44], while the $LigPlot^{+}$ program generated 2D protein-ligand interaction [45].

Statistical Analysis

The outcomes are presented as triplicate mean± standard error mean (SEM). The least square regression line of the logarithmic was analyzed by correlation coefficient r^2. For evaluation of all data, two-way ANOVA followed by Bonferroni post-test was performed. SPSS Statistics Version 10 (SPSS, Inc. Chicago, III., USA) software [46] was utilized for all statistical measurements, with significance denoted by $P < 0.05$.

RESULT AND DISCUSSION

Phytochemical Analysis

This work extracts four solvents, and the yield is represented in Table 1. The CIFs yield was related to its dried weight, ranging from 9.2% (Petroleum ether) to 33.9% (Ethanol). The Ethanolic extraction has a high yield when compared to aqueous (18.6%), chloroform (11.3%), and petroleum ether (9.2%). The variation in the outcomes in polar and nonpolar solvents might be attributed to the diversity of phytocompounds present in the extract of *C. indica* fruit, encompassing different degrees of solubility. The ethanolic and aqueous extract reported a high yield, suggesting intermediate-to-high polar compounds like phenols and flavonoids. TPC was expressed as mg of GAE/g of dry CIFs extract. TFC was expressed as mg of QE/g dry CIFs extract. Phenolic compounds are very effective sorbitol pathway enzymes involved in diabetic complications [47]. TPC and TFC of the four solvent extraction of CIFs is presented in Table 1 maximum volume of TPC was obtained in ethanolic solvent (12.5 ± 0.84 mg of GAE/g dried extract)

Table 1. Result of Percentage Yield, TPC, TFC Different Fraction of Fruit Extract of *C. indica.* (Mean ± SEM)

Extraction solvent	Yield (%)	TPC (mg GAE/g dry extract)	TPC (mg QE/g dry extract)
Petroleum ether	9.2	1.4 ± 0.12	34.2 ± 4.8
Chloroform	11.3	2.6 ± 0.23	44.2 ± 3.8
Ethanol	33.9	12.5 ± 0.84	78.4 ± 3.6
Aqueous	18.6	6.8 ± 0.11	72.5 ± 5.5

Table 2. Qualitative Analysis of the Phytochemical from the Different Extracts of *C. Indica* Fruit

S.No	Phytocomponents	Petroleum ether	Chloroform	Ethanol	Aqueous
1.	Steroids	-	-	+	+
2.	Terpenoids	+	+	+	-
3.	Saponins	+	+	+	-
4.	Flavonoids	-	-	+	+
5.	Alkaloids	-	-	+	+
6.	Carbohydrates	-	-	-	-
7.	Tannins	-	-	+	-
8.	Glycosides	-	-	+	+
9.	Phenols	-	-	+	+
10.	Proteins	-	-	-	-
11.	Amino acids	-	-	-	-
12.	Fats	-	-	+	-

(Presence '+' or Absence '-').

followed by aqueous, chloroform, and petroleum ether (6.8 ± 0.11 mg, 2.6 ± 0.23, and 1.4 ± 0.12 of GAE/g dried extract). The maximum volume of TFC was obtained in ethanolic solvent (78.4 ± 3.6, 72.5 ± 5.5, and 44.2 ± 3.8g of QE/g dried extract). The quantification of TFC showed many polyphenols and flavonoids in CIFs with increasing solvent polarity. The significant difference in the different solvents can be attributed to the contradictions of phytocompounds in the CIFs.

Phenolic compounds are crucial to the antioxidant properties due to the scavenging capacity of the hydroxyl group. Polyol enzymes are the more effective phenolic compounds in diabetic complications [48-50]. Flavonoids and tannins have significant antioxidant activity and a broad range of therapeutic activity, including anti-inflammatory, anti-allergenic, anti-aging, and anti-cancer activity [51]. Flavonoids are also competent in treating people with diabetes by inhibiting the enzyme aldose reductase. These results for TPC and TFC agree with the report, and fruit extracts possess a strong anti-glycan effect [52,53]. The fruit extract of *C. indica* is a good source of TPC and TFC, which contribute to the inhibition of AR involved in the sorbitol pathway. The obtained results of qualitative phytochemical analysis of the different extracts in the fruit of *C. indica* are shown in Table 2. The ethanolic CIFs extract showed the presence of flavonoids, fats, phenols, fixed oils, tannins, carbohydrates, glycosides, saponins, alkaloids, proteins, and amino acids. The aqueous extract also shows phytochemicals like ethanolic extract without Terpenoids, tannins, saponins, and fats. Chloroform and petroleum ether extract show the presence of saponins, glycosides, Terpenoids, and saponins, respectively. The abounded phytoconstituents obtained

from the ethanolic CIFs extract having different pharmacologically significant compounds leads to be identified.

Aldose Reductase Activity Profile

Under diabetic conditions, Aldose reductase is crucial in converting glucose into sorbitol while simultaneously converting the coenzyme NADPH to NADP+. This enzymatic process is implicated in developing complications associated with DM [54]. The inhibition of AR by herbal constituents is the best therapeutic strategy for diabetic complications [55]. The present work assessed the *in-vitro* inhibitory potential of the isolated phytocompound DDPM on AR activity, using quercetin as the standard reference. The IC_{50} values were calculated for solvent extracts of CIFs that display over 50% inhibition, and the agreeing information exists in Table 3 and Fig. 1.

All four solvent extracts were found to exhibit the bovine lens AR activity with IC_{50} values of these extractions. The ethanolic extraction was found to exhibit the strongest ARI activity with a mean IC50 of 2.34 µg ml^{-1} compared to the aqueous extract, which showed mean IC50 values of 3.88 µg ml^{-1}, chloroform extract showed a mean IC50 value of 6.72 µg ml^{-1} and petroleum ether extract showed a mean IC50 values of 7.26 µg ml^{-1}. In contrast, the positive control quercetin showed a mean IC_{50} values of 2.05 µg ml^{-1}. The ethanol and aqueous extract were additionally effective in ARI, as specified by its lower IC_{50} values [IC_{50} (2.34 µg ml^{-1})] and [IC_{50} (3.88 µg ml^{-1})], respectively, in comparison with other extracts.

The CIFs extract of ethanol and aqueous showed effective ARI activity, which can treat diabetic complications in the beginning stage. The ARI activity of pharmacological compounds present in the plant source is found to be phenols and flavonoids, which are the main constituents to reduce the AR enzyme activity in glucose metabolism [56]. The reported phytochemicals can potentially reduce oxidative stress by scavenging free radicals and reducing cell damage [57]. It is reported that the TPC and TFC in the fruit extracts possess an anti-glycan solid effect. This supports our study that our fruits of *C. indica* hold high amounts of TPC and TFC.

GC-MS

These pharmacologically active phenols and flavonoid compounds were identified using GC-MS. The ethanolic extract is performed alone based on the maximum content of TPC and TFC obtained, shown in Table 1. The spectrum and chromatogram of peaks were visualized in Fig. 2. The spotting of the compound within the sample was achieved by comparing the peaks in the chromatogram with that database available in the National Institute of Standard and

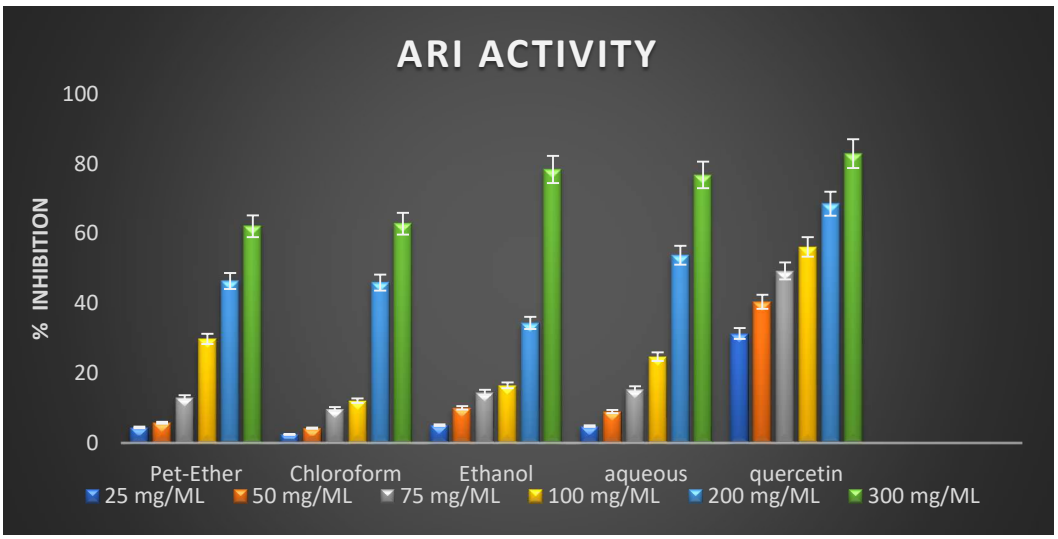

Fig. 1. Aldo reductase inhibitory activity of *Coccinia indica* fruit of various extracts with quercetin as standard. The triplicate value of results was expressed as the mean ± SD.

Table 3. Result of Inhibitory Effect of the *Coccinia Indica* Fruit Extract on Bovine Lens Aldose Reductase (Mean ± SEM)

Extract	Concentration (μg ml^{-1})	Inhibition (%)	IC_{50} (μg l^{-1})
Petroleum ether	300	62.13	7.26
	200	46.44	
	100	29.86	
	75	13.07	
	50	5.80	
	25	4.45	
Chloroform	300	64.86	6.72
	200	46.0	
	100	12.12	
	75	9.78	
	50	4.22	
	25	2.45	
Ethanol	300	78.46	2.34
	200	34.44	
	100	16.56	
	75	14.55	
	50	10.06	
	25	5.12	
Aqueous	300	76.88	3.88
	200	53.85	
	100	24.76	
	75	15.44	
	50	9.04	
	25	4.81	
Quercetin	300	82.98	2.05
	200	68.65	
	100	56.22	
	75	49.34	
	50	40.45	
	25	31.34	

Technology Mass Spectral Database (NISTMS) and Duke's Database library and depicted briefly in Table S1 (Supplementary materials). The ethanolic extract of CIFs identified 23 peaked compounds with the standard mass spectra data library. The pharmacologically active compounds were found to be D-Alanine, N-propargyloxy carbonyl-, dodecyl ester, 4H-Pyran-4-one, 2,3-dihydro -3,5-dihydro-6-mrthyl, 5-Hydroxymethylfurfural, n-Hexadecanoic acid, 10 (E), 12 (Z)-Conjugated linoleic acid, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, 13, 27-Cycloursan-3-one, γ-Sitosterol, Betulinaldehyde, Lupeol. The polyphenols obtained are natural antioxidants, and flavonoids are known to have active ARI activity. The compounds identified in the ethanolic extract of CIFs have been subjected to numerous plants [58,59]. The high amount of TPC, TFC, and other phytoconstituents in the extract of *C. indica* fruits supports our findings that they are significant pharmaceutically active composites known to inhibit Aldose reductase. However, additional experimental evidence will help subject the biological activity to anti-diabetic drug development.

Molecular Docking

Molecular docking is a valuable computational technique for assessing the binding affinity between small molecule ligands, macromolecule proteins, and DNA [60-65]. AutoDock4 facilitated the molecular docking analysis in this study between the small molecule DDPM and the macromolecule aldose reductase enzyme to determine the binding affinity. The X-ray crystal structure of aldose reductase with PDB ID: 3G5E was recovered from PDB, a significant target for numerous diabetes mellitus. The binding energy, hydrogen bonding, and hydrophobic interactions of DDPM with 3G5E were presented in Table 4 and pictorially shown in Fig. 3. The findings revealed that DDPM creates strong hydrogen bonding with amino and carboxyl groups of the active site amino acids, including leucine 195, glutamine 192, threonine 191, and glutamic acid 193, with a binding energy of 3.34, 3.05, 2.91, 3.03 and 2.73 Å. Additionally, hydrophobic interactions were raised between the phenylalanine and asparagine. These findings confirm the DDPM potential as an antagonist for the aldose reductase enzyme.

Geometrical Parameters

The optimized geometric parameters of DDMP were simulated at the DFT/B3LYP 6-311++G(d,p) basis set. From the structural perspective, DDPM contains a hydroxyl group

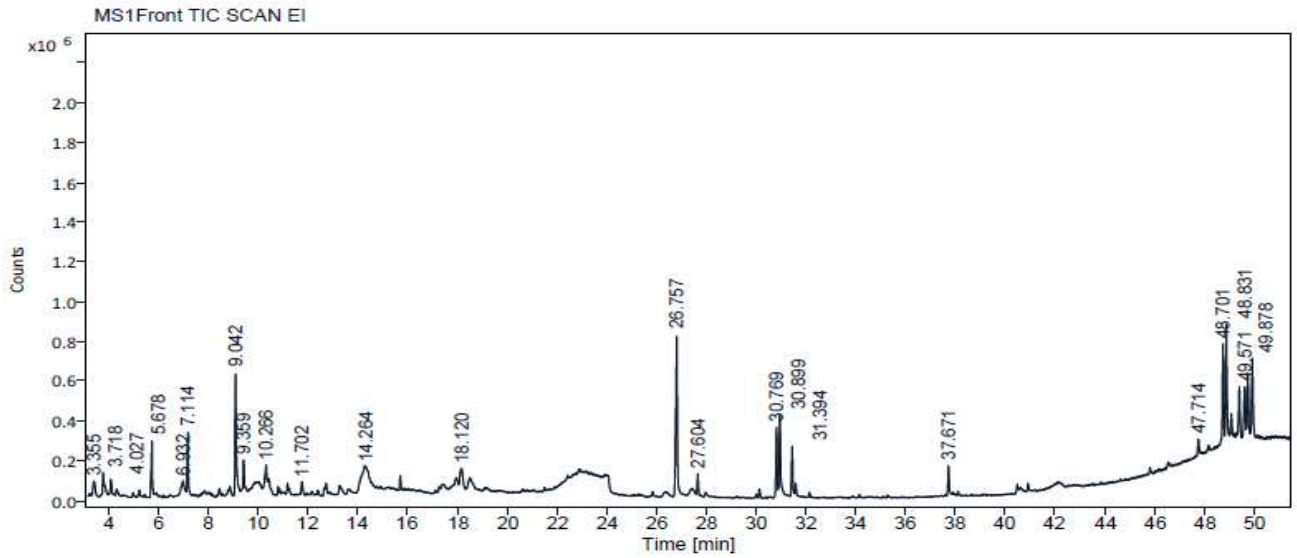

Fig. 2. Chromatogram representation of ethanolic extract of *C. indica* fruit by GC-MS.

Table 4. Molecular Docking Studies of DDPM with 3G5E

Ligand	PDB ID	Binding energy	Hydrogen bonding	Bond distance (Å)	Hydrophobic interactions
DDPM	3G5E	-5.94	Leucine 195	3.34	Phenylalanine 315
			Glutamine 192	3.05, 2.91	Aspargine 292
			Threonine 191	3.03	
			Glutamic acid 193	2.73	

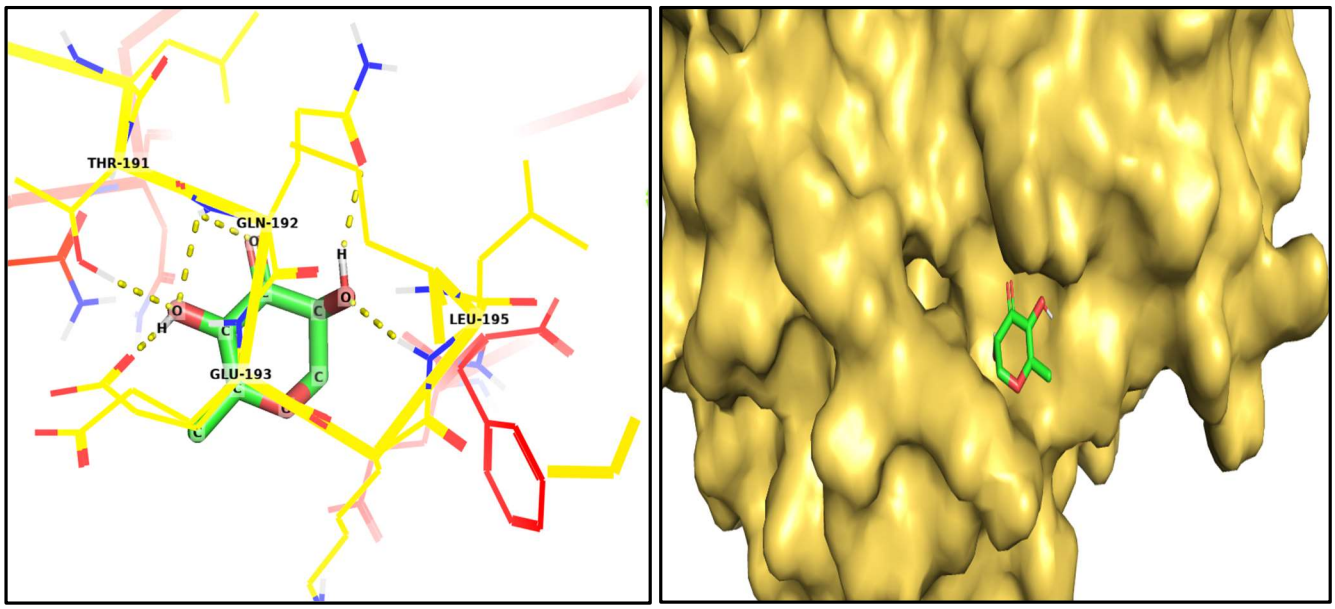

Fig. 3. 3D PymoL view of DDPM with aldose reductase enzyme.

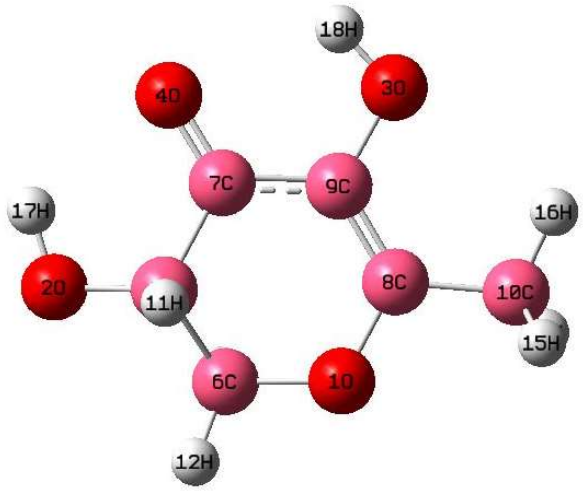

Fig. 4. Optimized molecular structure of DDPM.

(OH) in para and ortho positions and a keto group (C=O) in the meta position concerning the methyl (CH_3) group. The present research findings reveal that the DDMP electronic structure exhibits four protuberant bond lengths and six different bond angles. Figure 4 depicts the optimized molecular structure, while Table 5 lists the geometrical parameters of DDPM. The bond distance of C_7=O_4 was calculated at 1.261 Å, which is 0.132 and 0.176 Å lower than single-bonded C_9-O_3 (1.393) and C_5-O_2, confirming the double bond characteristics. The hydroxyl group (OH) bond length was 0.985 and 0.983 Å for O_2-H_{17} and O_3-H_{18}. The bond length of (CH) in the methyl group fell in the 1.091-1.097 Å range. At the same time, the bond distance of CH in the aromatic ring was 1.088 Å. The results unveiled simulated bond angles of O-C-C and C-O-C, which were spanned from 110.4-122.3° and 117°, respectively. Likewise, the bond angle of O-C-H was determined to lie between 105.4-110.4°. In contrast, the C-C-H and H-C-H angles were exhibited in the range of 108.1-112.7 and 107.1-110.1°, respectively. Additionally, the bond angles of carbon atoms notably fell between 110.5-125.3. Compared with other bond lengths, O_2-H_{17} and O_3-H_{18} were reduced due to the impact of electronegative oxygen.

Electronic Properties

The electronic spectrum was theoretically modeled using the TD-DFT approach based on the B3LYP/6-311++G(d,p) basis set. Table 6 presents a comprehensive overview of

Table 5. Theoretical Geometrical Parameters of DDPM

Bond length (Å)	Theoretical	Bond length (Å)	Theoretical
O_1-C_6	1.478	C_5-H_{11}	1.103
O_1-C_8	1.385	C_6-H_{12}	1.088
O_2-C_5	1.437	C_6-H_{13}	1.096
O_2-H_{17}	0.985	C_7-C_9	1.438
O_3-C_9	1.393	C_8-C_9	1.365
O_3-H_{18}	0.983	C_8-C_{10}	1.487
O_4-C_7	1.261	C_{10}-H_{14}	1.097
C_5-C_6	1.523	C_{10}-H_{15}	1.096
C_5-C_7	1.521	C_{10}-H_{16}	1.091
Bond angle (°)	**Theoretical**	**Bond angle (°)**	**Theoretical**
C_6-O_1-C_8	117	C_6-C_5-C_7	109
O_1-C_6-C_5	110.4	C_6-C_5-H_{11}	109.9
O_1-C_6-H_{12}	105.4	C_5-C_6-H_{12}	112.7
O_1-C_6-H_{13}	108.5	C_5-C_6-H_{13}	109.5
O_1-C_8-C_9	121.3	C_7-C_5-H_{11}	108.1
O_1-C_8-C_{10}	113.4	C_5-C_7-C_9	117
C_5-O_2-H_{17}	107.6	H_{12}-C_6-H_{13}	110.1
O_2-C_5-C_6	108.8	C_7-C_9-C_8	121.7
O_2-C_5-C_7	110.7	C_9-C_8-C_{10}	125.3
O_2-C_5-H_{11}	110.4	C_8-C_{10}-H_{14}	110.5
C_9-O_3-H_{18}	107.8	C_8-C_{10}-H_{15}	110
O_3-C_9-C_7	117.8	C_8-C_{10}-H_{16}	110.5
O_3-C_9-C_8	120.5	H_{14}-C_{10}-H_{15}	107.1
O_4-C_7-C_5	120.7	H_{14}-C_{10}-H_{16}	109.1
O_4-C_7-C_9	122.3	H_{15}-C_{10}-H_{16}	109.6

the DPPM electronic characteristics and its significant contributions. From the theoretical findings, three distinct bands emerged, with a prominent peak observed at 316 nm with a substantial contribution of H→L (85%). Additionally, medium peaks at 304 and 250 nm significantly contribute to HOMO-1→LUMO (71%) and HOMO-1→LUMO (15%) transition, respectively, as shown in Fig. 5. The energy gaps were assessed and illustrated in Fig. 6 to evaluate the compound's chemical stability. The energy map of HOMO-LUMO displays positive and negative phases in green and red hues, respectively [66]. According to the data in Table S2

Table 6. Simulated Electronic Parameters of DDPM

λ_{max}	E (eV)	f	Major contribution
316	3.92	0.1273	H→L (85%)
204	4.07	0.0206	H-1→L (71%)
250	4.95	0.0165	H-1→L (15%)

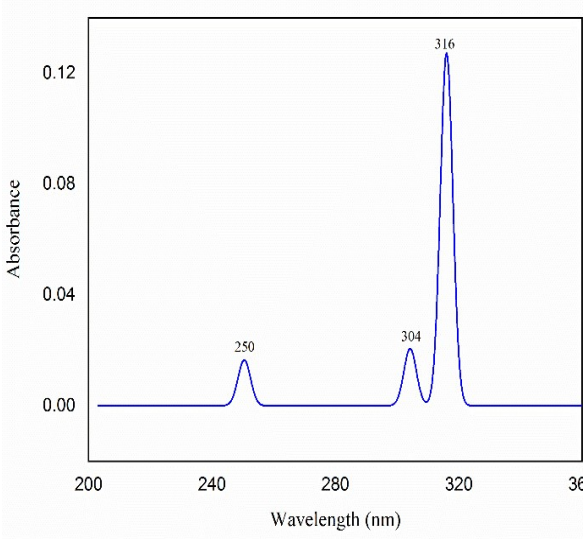

Fig. 5. Simulated electronic spectrum of DDPM.

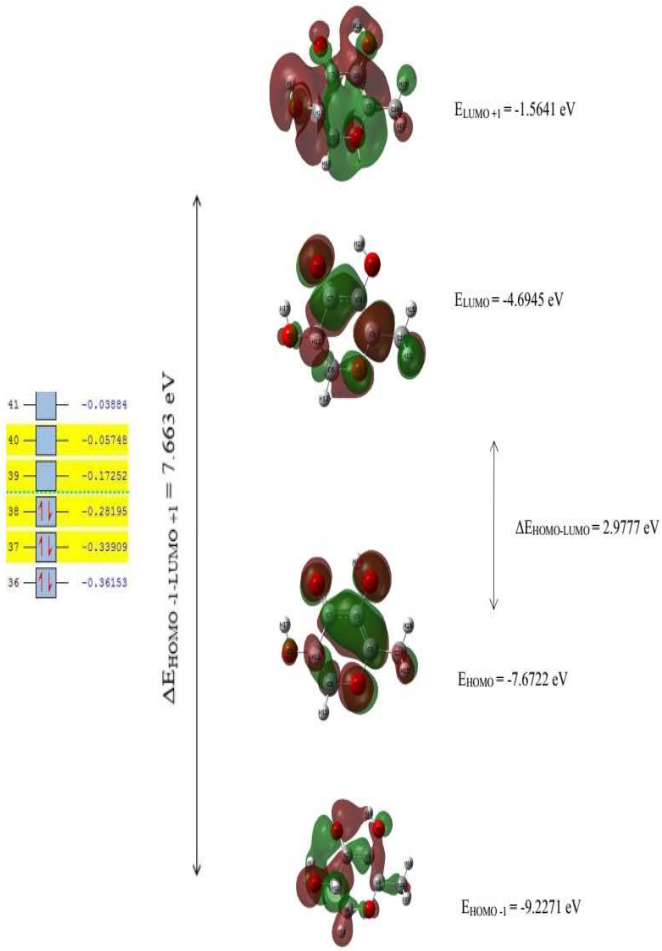

Fig. 6. HOMO-LUMO plot of the DDPM.

(Supplementary materials), the simulated energy gap is around 2.9777 eV and exhibits more excellent chemical stability. Furthermore, Fig. 7 portrays the Density of states (DOS), supporting the predicted energy gap. Occupied orbitals are shown in green in the DOS spectrum, whereas unoccupied orbitals are shown in blue.

MESP

Molecular electrostatic potential surfaces (MESP), were employed to ascertain the sites of nucleophilic and electrophilic regions within the compound. In particular, MESP surface specific to DDPM were created to vividly depict the contrasting colors that highlight variations between electron-rich and poor areas. The technique visually represents the molecular charge distribution, identifying and differentiating these crucial regions within the molecule [67,

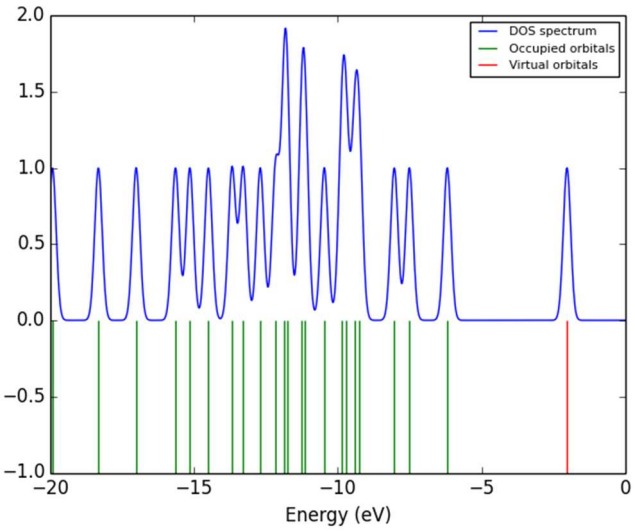

Fig. 7. Simulated DOS spectrum of DDPM.

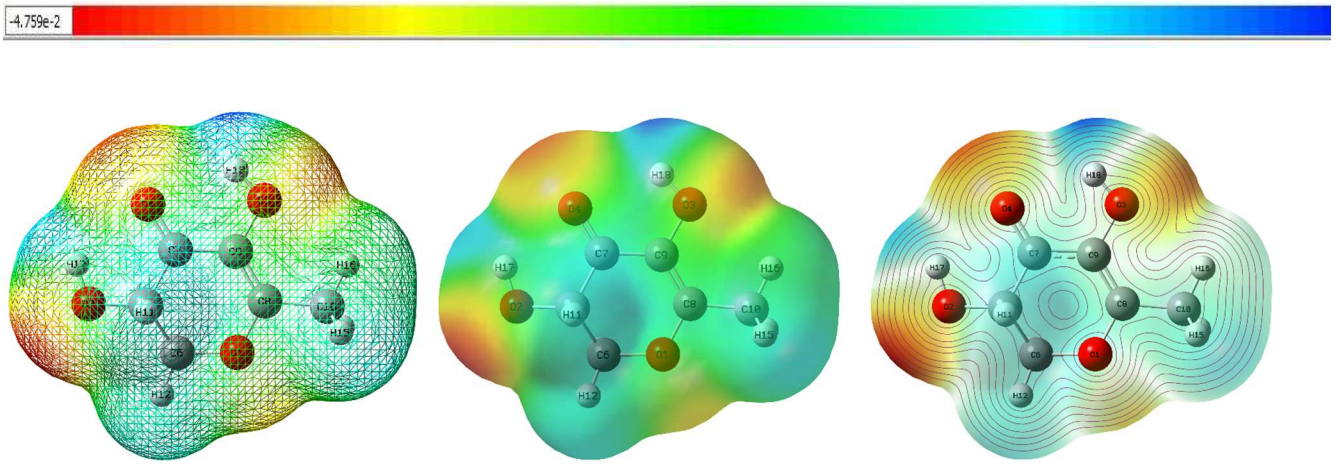

Fig. 8. Total density and Contours map MESP map of DDPM.

68]. The DPPM has a color code for MESP that maps between 7.076 × 10^{-2} to 7.076 × 10^{-2} e.s.u. shown in Fig. 8. Red denotes the most substantial electron repulsion, while white denotes the most potent electron attraction. The resulting graphic shows that the hydrogen atoms, mainly the methyl group hydrogens, are surrounded by electron-poor zones characterized by white color mesh. The red color mesh indicates that electron-rich areas are developing around the oxygen atoms of the carbonyl, methoxy, and hydroxyl groups. These findings suggest a strong correlation between Mulliken charges.

CONCLUSION

Coccinia indica is a famous plant for its safe anti-diabetic properties. In the present study, the GC-MS analysis has provided compelling evidence supporting a diverse range of bioactive compounds in *C. indica* and concluded that the taken CIFs are a promising source with numerous pharmacological activities. The ethanolic and aqueous extracts derived from the CIFs displayed potent inhibitory activity against bovine lens aldose reductase, the correlation between the secondary metabolites obtained from the CIFs and ARI studies suggests that the high content of Phenols and flavonoids in the CIFs extract which driven the effective ARI activities. The phytochemicals identified from GC-MS support the use of CIFs having beneficial pharmacological activity. The MESP analysis confirms the charge distributions, the presence of electronegative atom oxygen impacts the charges associated with the carbon atoms. The molecular docking investigation evidence for DDPM effectiveness in encountering diabetes by targeting the aldose reductase enzyme, exhibiting a binding energy of -5.94 (Kcal mol^{-1}). Additionally, the simulated electronic characteristic, including the energy gap, further validates the chemical reactivity and biological potency of DDPM. Further efforts should made to segregate all the bioactive compounds from the CIFs and access its *in-vitro* ARI activity in animal prototypes. This drives the findings of promising drugs for controlling and managing DM and its complications.

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