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Section A: Natural Products & Metabolomics

The Potential Application of Roselle Extracts (*Hibiscus sabdariffa* L.) in Managing Diabetes Mellitus

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ABSTRACT

Objectives: This study investigates the potential application of Roselle Extracts (Hibiscus sabdariffa L.) in managing diabetes mellitus. The previous study shown the impact of Roselle Extracts on blood glucose levels and insulin sensitivity in diabetic, as well as the identification of compounds found in Roselle Calyces as potential Phosphoenolpyruvate carboxykinase (PEPCK) inhibitors for treating type 2 diabetes mellitus. **Methods:** In first we search about the effects of Roselle Extracts on blood glucose levels, insulin sensitivity, pancreatic alpha-amylase and intestinal alpha-glucosidase enzymes inhibition, pancreatic lipase activity, and gene expression related to gluconeogenesis, glycolysis, and lipogenesis in debate. Then we analyzes the physicochemical properties and pharmacokinetics of potential PEPCK inhibitors present in Roselle Calyces using the SwissADME web tool. **Results:** Metformin has high solubility in aqueous liquids and is easily absorbed during digestion. It does not inhibit most cytochrome P450 liver enzymes and cannot cross the blood-brain barrier, making it pharmacokinetically acceptable. Hibiscetin, Gossypetin, and Flavylium are also soluble in aqueous liquids, but their weak digestive absorption limits their effects on blood. However, their effect on secretory enzymes in the digestive tract is well known. On the other hand, 3,4-dihydroxybenzoic acid has higher digestive absorption and greater availability in the blood, making it a potential antidiabetic compound. Beta-Sitosterol has the highest skin absorption among the studied compounds, while Flavylium compounds show poor skin permeability. Both Metformin and Beta-Sitosterol do not inhibit cytochrome P450 liver enzymes, which is beneficial for their pharmacological profiles. These findings offer valuable insights into the potential uses and limitations of these compounds in various therapeutic applications. **Conclusions:** The previous findings suggest that Roselle Extracts and its identified compounds could play a significant role in managing diabetes mellitus. The Physicochemical Properties and pharmacokinetic properties of these compounds can guide the development of new drugs and formulations, ultimately improving patient outcomes and overall healthcare.

***Keywords**: Roselle, PEPCK inhibitors, Hibiscetin, Gossypetin*

INTRODUCTION

Diabetes Mellitus: Diabetes mellitus has been identified as a major global health concern by the World Health Organization (WHO) in recent years. This chronic metabolic disease is characterized by elevated blood sugar levels due to the body's inability to produce sufficient insulin or utilize it effectively. Over time, diabetes can lead to severe complications, particularly affecting the cardiovascular system, increasing the risk of heart attacks and other cardiovascular diseases [1, 2].

Roselle (Hibiscus sabdariffa): Throughout history, people have relied on medicinal plants as a remedy for various ailments. Hibiscus sabdariffa, commonly known as Roselle, is a plant widely recognized for its medicinal properties. Roselle (Hibiscus sabdariffa) is one such plant that has been proven to possess potent phytochemicals responsible for its medicinal benefits. A study conducted by Adefolalu et al. (2020) revealed that Hibiscus sabdariffa contains significant phytochemical components, including alkaloids, which have hypoglycemic, hyperlipidemic, and hepatotoxic properties, making it an effective treatment for diabetes. Roselle is rich in phytochemicals, including polyphenols, anthocyanins, organic acids, and polysaccharides, which have various therapeutic properties, such as hypoglycemic, hyperlipidemic, hepatotoxic, antioxidant, cardiac depressant, and anti-inflammatory effects [2, 3].

Phenolic Compounds in Roselle: Roselle, a popular edible flower, is well-known for its rich content of phenolic compounds, which are responsible for its attractive color and various health benefits. These compounds include total phenolic compounds (TPC), total flavonoid compounds (TFC), and total anthocyanin compounds (TAC). The phenolic compound content of roselle has been studied extensively over the last decade, with variations observed in the total phenolic compound levels as TPC, TFC, and TAC. These differences can be attributed to sample conditions such as cultivars, growing environments, and processing treatments, as well as analytical methods used for extraction and analysis [4,5].

Phenolic Acids in Roselle: Roselle contains various phenolic acids, such as hydroxybenzoic acids (gallic acid, protocatechuic acid, syringic acid, vanillic acid, genistic acid, and 4-hydroxybenzoic acid) and hydroxycinnamic acids (chlorogenic acid, caffeic acid, ferulic acid, p-coumaric acid, 3-p-coumaroylquinic acid, 5-p-coumaroylquinic acid, 3-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, and 5-O-caffeoylquinic acid). Other phenolic acids identified in roselle calyx include neochlorogenic acid, cryptochlorogenic acid, methyl digallate, methyl chlorogenate, dihydroferulic acid-4-O-glucuronide, methyl chlorogenate isomer II, 5-O-caffeoyl shikimic acid, ethyl chlorogenate, and ethyl chlorogenate isomer II [6, 7].

Flavonoids in Roselle: Roselle contains various flavonoids, including flavan-3-ols (epicatechin, (+)-catechin, (−)-epigallocatechin, and (−)-epigallocatechin gallate); flavonols (kaempferol and kaempferol 3-O-rutinoside); myricetin (myricetin 3-sambubioside); quercetins (quercetin 3-O-glucoside, quercetin 3-sambubioside, quercetin 3-rutinoside, and quercetin pentosylhexoside); rutin; quercitrin; and flavanones (hesperitin and hesperidin). Quercetins are the most frequently reported flavonoids in roselle calyx tissue[8, 9].

Anthocyanins in Roselle: Various studies have isolated individual anthocyanin compounds from roselle calyx, revealing glycosylated anthocyanins with three or more sugar moieties and octanoyl derivatives like delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside. The color of roselle calyx is determined by the content of anthocyanins, with genotype accounting for 60–80% of the variation in calyx color. Dark-red calyx color roselle varieties have specific anthocyanin levels and higher antioxidant activity compared to light-red or white cultivars lacking red pigments [10, 11].

Organic Acid and Volatile Compounds: The highest organic acid content, mainly hibiscus acid and its derivatives, was found in roselle extracts using the MAE (microwave-assisted extraction) method, with a total content of 70 mg/g. Other organic acids identified in roselle calyx include citric acid, malic acid, tartaric acid, ascorbic acid, hydroxycitric acid, hibiscus acid, and oxalic acid. Several studies have reported the volatile content of roselle tissue, comparing cultivars from different countries and extraction methods [12, 13].

Inhibitory Effects of Roselle Extract on Digestive Enzymes: Roselle extract inhibited pancreatic lipase in a non-competitive manner with an IC50 value of 0.84 mg/mL. It exhibited mixed-type inhibition on both α-glucosidase and α-amylase with IC50 values of 0.59 mg/mL and 1.93 mg/mL, respectively. Fluorescence quenching assays confirmed the binding of roselle extract to enzyme proteins. Rats pre-treated with roselle extract at doses of 50 and 100 mg/kg body weight (bwt) showed significant reductions in fat absorption and improvements in fat excretion through feces. In vivo studies revealed that roselle extract effectively suppressed the increase in blood glucose after starch consumption, while its effects on maltose and sucrose consumption were relatively weak. The presence of various phytochemicals in roselle extract makes it a potential candidate for treating obesity and type 2 diabetes by inhibiting digestive enzymes [14].

Roselle and Hypoglycemic Effects: Hibiscus sabdariffa, commonly known as roselle, has been studied for its potential hypoglycemic effects. In vitro and in vivo studies have shown that roselle extracts can help

regulate blood glucose levels by inhibiting carbohydrate-digesting enzymes like α-amylase and α-glucosidase. These enzymes play a crucial role in postprandial hyperglycemia, a significant concern for diabetic patients. Research indicates that roselle calyx extracts can inhibit these enzymes, delaying glucose absorption and helping maintain stable blood glucose levels [1-3].

Roselle and Insulin Sensitivity: Roselle extracts have also been found to improve insulin sensitivity, a critical factor in diabetes management. Studies demonstrate that polyphenolic roselle calyx extracts can enhance insulin-stimulated glucose uptake in various cell lines, such as rat aortic smooth muscle A7r5 cells and 3T3F442A adipocytes. These extracts regulate insulin signaling pathways, including the phosphorylation of insulin receptor substrate-1 (IRS-1) and phosphatidylinositol 3-kinase (PI3K), crucial for insulin sensitivity [15].

Roselle Extracts and Blood Glucose Levels: Numerous studies have investigated the effects of roselle extracts on blood glucose levels in various animal models of diabetes. These studies utilized different types of roselle extracts, including aqueous, ethanolic, and flavonoid-rich or polyphenolic extracts, prepared from calyces, flowers, or a combination of these plant parts. Several studies have shown the hypoglycemic effects of aqueous extracts from roselle calyces or flowers in diabetic animals. For example, aqueous extracts made with roselle calyces reduced blood glucose levels in rats with diabetes induced by alloxan, streptozotocin, or fructan (16) Researchers have also observed the hypoglycemic effects of ethanolic extracts from roselle calyces or flowers in diabetic animals. For instance, ethanolic extracts made with roselle calyces reduced blood glucose levels in rats with T1DM induced by streptozotocin[17]. Flavonoid-rich or polyphenolic extracts have also been shown to have hypoglycemic effects in diabetic animals. For example, polyphenolic extracts from roselle calyces reduced blood glucose levels in rats with T2DM induced by streptozotocin (18). Some studies have explored the effects of roselle extracts combined with other products. For instance, hydroalcoholic extracts of roselle flowers and Carum carvi showed enhanced hypoglycemic effects in alloxan-induced diabetic rats [19].

Roselle Extracts and Insulin Sensitivity: Several studies have demonstrated the ability of roselle extracts to improve insulin sensitivity in diabetic animals. These extracts have been shown to increase insulin sensitivity by reducing insulin resistance and indirectly alleviating insulin resistance through body fat reduction. Roselle aqueous extracts have been shown to increase insulin sensitivity in diabetic animals. For example, aqueous extracts made with roselle calyces improved insulin sensitivity in rats with T2DM induced by a high-fructose diet. Ethanolic extracts from roselle calyces or flowers have also been reported to improve insulin sensitivity in diabetic animals. Ethanolic extracts made with roselle calyces increased insulin sensitivity in rats with T1DM induced by streptozotocin. Flavonoid-rich or polyphenolic extracts have been shown to have insulin-elevating effects in diabetic animals. For example, polyphenolic extracts from roselle calyces increased serum insulin levels in rats with T2DM induced by streptozotocin [17-20].

Roselle Calyces Compounds as Potential PEPCK Inhibitors: Phosphoenolpyruvate carboxykinase (PEPCK) is the rate-controlling enzyme in gluconeogenesis. In diabetic individuals, altered rates of gluconeogenesis are responsible for increased hepatic glucose output and sustained hyperglycemia. Insulin can inhibit this pathway by suppressing the transcription of the enzyme PEPCK. The investigation of Roselle Calyces (Hibiscus sabdariffa L.) chemical compounds as potential PEPCK inhibitors is significant in the context of type 2 diabetes mellitus treatment. As the need for timely adaptation and application of in silico approaches in pharmaceutical research increases, the overall efficiency of drug discovery is expected to improve. Roselle Calyces (Hibiscus sabdariffa L.), known for treating various diseases and conditions, contains flavonoids and alkaloids like protocatechuic acid, quercetin, anthocyanin, β-sitosterol, pectin, and wax. In silico docking has been employed to investigate the potential activity of Roselle Calyces (Hibiscus sabdariffa L.) chemical compounds against PEPCK compared to Metformin as the standard compound. In one in silico study, the docking scores for Quercetin, Hibiscetin, Gossypetin, and Protocatechuic Acid, four Roselle Calyces compounds, showed better potential as PEPCK inhibitors compared to Metformin [21].

MATERIAL AND METHODS

Predicting Physicochemical Properties and Pharmacokinetics: The SwissADME web tool is a valuable resource for drug development researchers as it provides a user-friendly and efficient platform to predict essential physicochemical properties and pharmacokinetic parameters. This online tool, accessible without registration at http://www.swissadme.ch, employs reliable predictive models such as BOILED-Egg and iLOGP to facilitate its operations. Its ease of use and accurate predictions have significantly contributed to numerous research findings, as demonstrated in studies[22-26].

Method: To determine the efficacy of Hibiscus sabdariffa L in managing diabetes mellitus, a comprehensive literature search was conducted using various databases, including PubMed, ScienceDirect, and Scopus. The search strategy involved incorporating relevant keywords related to herbal and traditional

medicine, as well as diabetes. By surveying the main outcomes of these studies, we then calculated the Physicochemical Properties and Pharmacokinetics of antidiabetic compounds found in Hibiscus sabdariffa L using the SwissADME database's online software. This investigation aimed to better understand the potential of H. sabdariffa L for managing diabetes mellitus.

RESULTS

Physicochemical and pharmacological properties of the studied compounds

In the investigation of the physicochemical and pharmacological properties of the studied compounds, various aspects such as molecular weight, solubility, polarity, and others were analyzed. The properties of metformin, 3,4-dihydroxybenzoic acid, Hibiscetin, Gossypetin, Flavylium, and Beta-Sitosterol were compared.

Metformin, with a molecular weight of 129 Daltons, was found to be slightly lighter than 3,4-dihydroxybenzoic acid, which has a molecular weight of 154 Daltons. Among the studied compounds, metformin exhibited the highest solubility in aqueous liquids, making it a candidate for biological applications. Interestingly, metformin did not show inhibitory effects on most cytochrome P450 liver enzymes.

Molar refractivity (MR) plays a crucial role in understanding the relationship between a molecule's size, shape, and its physicochemical properties. While metformin had the lowest Molar refractivity (MR) value, indicating high polarity, the highest MR index was observed in the most hydrophobic compound, Beta-Sitosterol. The three compounds Hibiscetin, Gossypetin, and Flavylium were found to be less hydrophobic, which means they have weak digestive or skin absorption and high polar surfaces that may reduce their possibility of passing through biological membranes and their availability in the patient's blood. Their heavy molecular weight also contributes to reduced gastrointestinal absorption and availability in the blood.

Although Hibiscetin, Gossypetin, and Flavylium compounds do not dissolve as highly as metformin in blood and other physiological fluids, they may have a higher impact on secretory enzymes in the digestive tract. Their effects could potentially be more pronounced in the blood when administered through injection. On the other hand, the 3,4-dihydroxybenzoic acid compound, with its light molecular weight, makes it easier to digest and more soluble in biological fluids. This increases its availability in the blood, suggesting that it could have good digestive absorption when consumed orally and exhibit therapeutic effects. The study of these compounds' physicochemical properties provides valuable insights into their potential applications in managing diabetes mellitus. The findings suggest that metformin, 3,4-dihydroxybenzoic acid, and other compounds may have varying degrees of efficacy depending on their properties, such as solubility, polarity, and molecular weight, which influence their digestive absorption, bioavailability, and interactions with biological systems (**Tables 1, 2**).

Pharmacokinetic properties of the studied compounds

We have calculated that the digestive absorption of Hibiscetin, Gossypetin, and Beta-Sitosterol is relatively low compared to other compounds in the study. Conversely, 3,4-dihydroxybenzoic acid, Quercetin, and Flavylium compounds like Metformin exhibit higher digestive absorption rates. A crucial aspect of this research is the ability of certain compounds to cross the blood-brain barrier, a protective mechanism that separates the central nervous system from the rest of the body, allowing only specific substances to pass through. Among the studied compounds, only Flavylium compounds have been found to cross this barrier, which could have significant implications for their potential effects on the central nervous system.

P-glycoprotein 1 is an important transporter protein that helps maintain cellular homeostasis by expelling toxic or unwanted substances. Our findings suggest that Flavylium compounds are subject to P-glycoprotein 1.

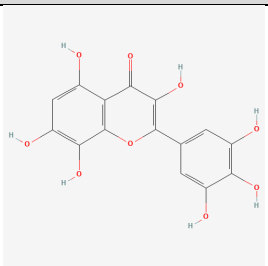
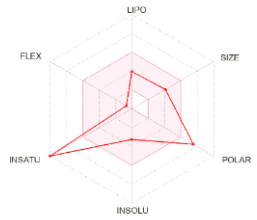
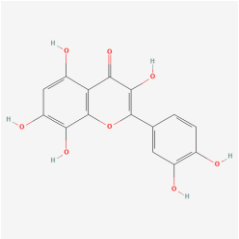
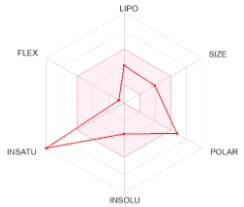
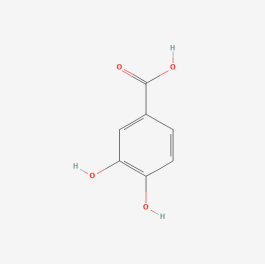
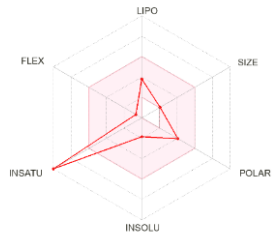
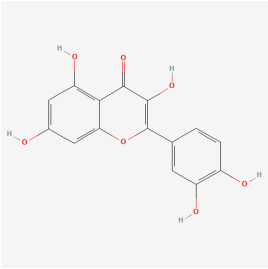
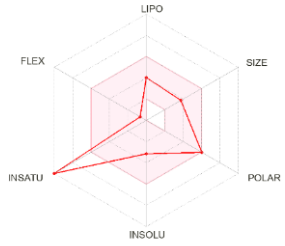
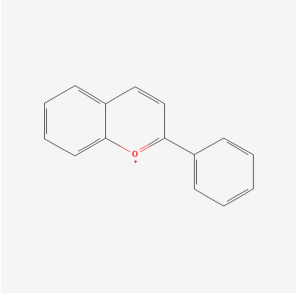
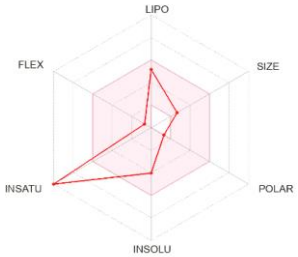
Regarding skin absorption, we highlight Beta-Sitosterol as the most effective compound among those studied. Skin absorption is a critical factor to consider when developing topical medications or formulations, and Beta-Sitosterol's high skin absorption rate may contribute to its potential use in various topical applications. Conversely, Flavylium compositions demonstrate poor skin absorption, which may limit their potential use in topical applications. The remaining compounds in the study do not show notable skin permeability.

Interestingly, Metformin and Beta-Sitosterol do not inhibit cytochrome P450 liver enzymes, which play a crucial role in drug metabolism. The absence of inhibitory effects on these enzymes by Metformin is a beneficial aspect of its pharmacological profile. The physiological properties of the studied compounds provide valuable insights into their potential uses and limitations in various therapeutic applications (**Table 3**).

DISCUSSION

A previous study investigated the effects of Roselle Extracts on blood glucose levels and insulin sensitivity in diabetic animals, while we focus on the compounds found in Roselle Calyces as potential PEPCK inhibitors for treating type 2 diabetes mellitus[21]. Firstly, various studies have demonstrated that Roselle

Table 1. Introduction of the studied compounds.

Computed Name	IUPAC Name	CID PubChem	Chemical structure	Radar scale of physicochemical properties
Hibiscetin	3,5,7,8-tetrahydroxy-2-(3,4,5-trihydroxyphenyl) chromen-4-one	15559735		
Gossypetin	2-(3,4-dihydroxyphenyl) -3,5,7,8-tetrahydroxychromen-4-one	5280647		
3,4-Dihydroxybenzoic acid	3,4-dihydroxybenzoic acid	72		
Quercetin	2-(3,4-dihydroxyphenyl) -3,5,7-trihydroxychromen-4-one	5280343		
Flavylium	2-phenylchromenylium	145858		

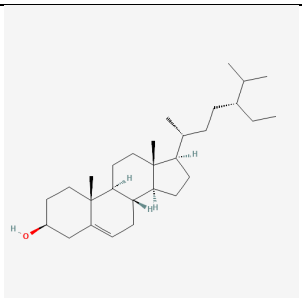
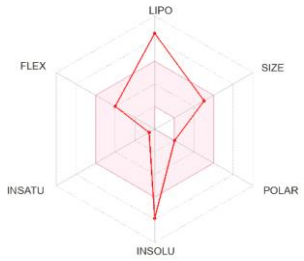
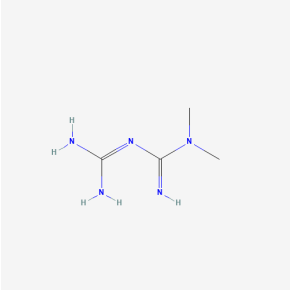
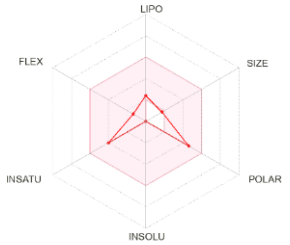
Beta-Sitosterol	(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	222284		
Metformin	3-(diaminomethylidene)-1,1-dimethylguanidine	4091		

Table2. Physicochemical and pharmacological properties of the studied compounds

Name	Hibiscetin	Gossypetin	3,4-dihydroxybenzoic acid	Quercetin	Flavylium	Beta-Sitosterol	Metformin
Formula	C15H10O9	C15H10O8	C7H6O4	C15H10O7	C15H11O+	C29H50O	C4H11N5
MW	334.23	318.24	154.12	302.24	207.25	414.71	129.16
Heavy atoms	24	23	11	22	16	30	9
Aromatic heavy atoms	16	16	6	16	16	0	0
Fraction Csp3	0	0	0	0	0	0.93	0.5
Rotatable bonds	1	1	1	1	1	6	2
H-bond acceptors	9	8	4	7	1	1	2
H-bond donors	7	6	3	5	0	1	3
MR	82.08	80.06	37.45	78.03	66.06	133.23	36.93
TPSA	171.82	151.59	77.76	131.36	13.14	20.23	91.49
iLOGP	1.28	1.33	0.66	1.63	-0.76	5.05	0.34
XLOGP3	1.45	1.81	1.15	1.54	3.51	9.34	-1.27
WLOGP	1.4	1.69	0.8	1.99	4.38	8.02	-1.24
ESOL Log S	-3.25	-3.4	-1.86	-3.16	-4.01	-7.9	0.29
ESOL Solubility (mg/ml)	0.19	0.12	2.14	0.21	0.02	0.00	253.00
ESOL Class	Soluble	Soluble	Very soluble	Soluble	Moderately soluble	Poorly soluble	Highly soluble

Table 3. Pharmacokinetic properties of the studied compounds

	Hibiscetin	Gossypetin	3,4-dihydroxybenzoic acid	Quercetin	Flavylium	Beta-Sitosterol	Metformin
GI absorption	Low	Low	High	High	High	Low	High
BBB permeant	No	No	No	No	Yes	No	No
Pgp substrate	No	No	No	No	Yes	No	No
CYP1A2 inhibitor	Yes	Yes	No	Yes	Yes	No	No
CYP2C19 inhibitor	No	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No	No
CYP2D6 inhibitor	No	Yes	No	Yes	Yes	No	No
CYP3A4 inhibitor	Yes	Yes	Yes	Yes	No	No	No
log Kp (cm/s)	-7.31	-6.96	-6.42	-7.05	-5.07	-2.2	-7.99

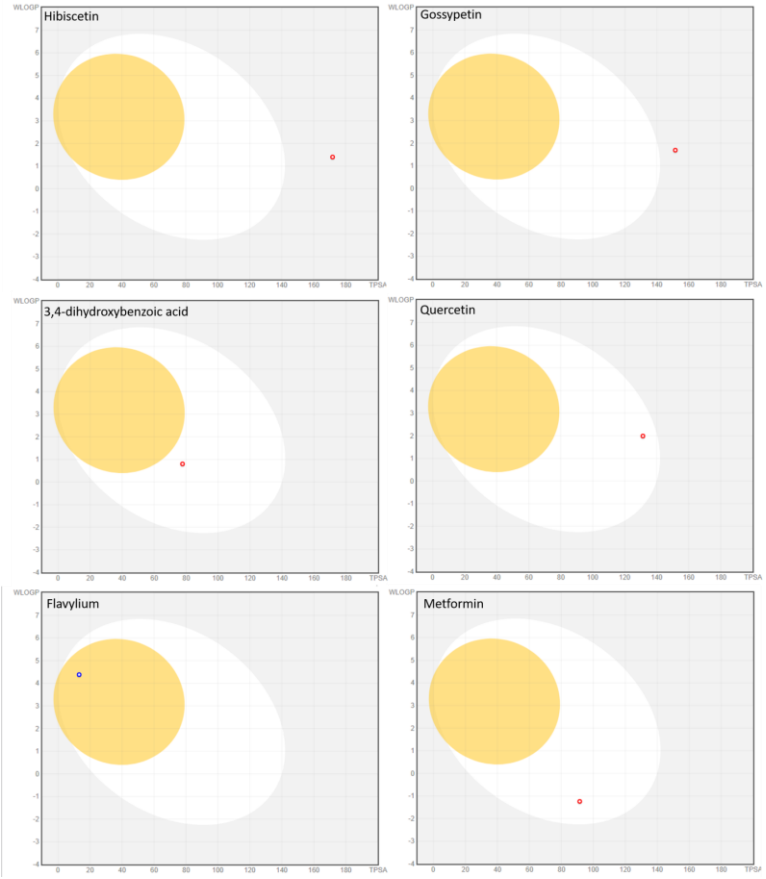


Figure 1. Yellow areas represent compounds that can passively cross the blood-brain barrier. White areas contain compounds that can be passively absorbed by the digestive system. Blue dots indicate compounds that can enter the central nervous system through P-glycoproteins. Red dots signify compounds that can be removed from the central nervous system through glycoproteins.[23]

Extracts can inhibit pancreatic alpha-amylase and intestinal alpha-glucosidase enzymes, reducing carbohydrate absorption and lowering blood glucose levels. Additionally, Roselle Extracts have been observed to inhibit pancreatic lipase activity, contributing to weight loss and improved insulin sensitivity in diabetic animals. The previous study also highlights that Roselle Extracts can modulate gene expression related to gluconeogenesis, glycolysis, and lipogenesis, contributing to blood glucose regulation and insulin sensitivity [2, 3, 15-20]. The previous study identifies four compounds in Roselle Calyces consisting of Quercetin, Hibiscetin, Gossypetin, and Protocatechuic Acid as potential PEPCK inhibitors with better potential than Metformin according to a previous in silico study[21]. This finding contributes to understanding Roselle Calyces' potential in managing diabetes mellitus.

Furthermore, our study analyzes the physicochemical properties and pharmacokinetics of the studied compounds using the SwissADME web tool. The tool helps predict essential properties and pharmacokinetic parameters, contributing to drug discovery efficiency. Our study compares the properties of metformin, 3,4-dihydroxybenzoic acid, Hibiscetin, Gossypetin, Flavylium, and Beta-Sitosterol.

The study's findings reveal that metformin exhibits the highest solubility in aqueous liquids and has a lower molecular weight than 3,4-dihydroxybenzoic acid. The four identified PEPCK inhibitors show varying degrees of solubility, polarity, and molecular weight, which influence their digestive absorption, bioavailability, and interactions with biological systems. Additionally, the study discusses the pharmacokinetic properties of the compounds, including their blood-brain barrier permeability, P-glycoprotein 1 interaction, skin absorption, and cytochrome P450 liver enzyme inhibition. Understanding these properties can guide the development of new drugs and formulations, ultimately improving patient outcomes and overall healthcare.

CONCLUSION

We focus on the potential use of Roselle Extracts (Hibiscus sabdariffa L.) in managing diabetes mellitus. A previous study examines the effects of Roselle Extracts on blood glucose levels and insulin sensitivity in diabetic animals, and we investigate Roselle Calyces compounds as potential PEPCK inhibitors for treating type 2 diabetes mellitus.

Roselle Extracts are found to inhibit pancreatic enzymes and modulate gene expression related to glucose regulation, contributing to improved insulin sensitivity. We study compounds as potential PEPCK inhibitors. The study analyzes the physicochemical properties and pharmacokinetics of the compounds, providing insights into drug development and improving patient outcomes.

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Conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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