ORIGINAL ARTICLE

Molecular and pharmacokinetic properties of the histidine decarboxylase inhibitors from clove

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Abstract

Introduction: Histidine decarboxylase is widely distributed in the gastrointestinal mucosa, mast cells, cerebrospinal fluid, bone marrow, and certain actively growing tumors. This enzyme has been studied till date of various organisms, but the mechanism of action had not been understood. In the present study, the inhibitory activity of flavonoids on purified histidine decarboxylase enzyme from bacterial source using a methanolic extract of clove was examined. **Materials and Methods:** Phytochemical screening of methanolic extract of clove was performed for the presence of various bioactive constituents. Further, the purification of flavonoids present in the spice extract was carried out by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) to isolate and identify the different flavonoids present in methanolic extract of clove. The inhibition of the enzyme was studied by checking the activity of an enzyme in the presence of varying concentrations of inhibitor. Dixon plot was plotted to determine the concentration of the inhibitor at which it inhibits the enzyme. **Results and Discussion:** Phytochemical screening of the clove extract revealed the presence of flavonoids in maximum in comparison to the other constituents. TLC and HPLC were carried out, which indicated the presence of ellagic acid, gallic acid, kaempferol, and quercetin in the methanolic extracts of the clove sample. The concentration of the clove extract in inhibiting the enzyme was found to be 0.9 µmoles from Dixon plot. All the components identified in HPLC quantification were subjected to software's like Molinspiration and SwissADME to study the molecular properties, drug-likeness, and pharmacokinetics. **Conclusion:** In the present study, histidine decarboxylase and the various medical aspects in which the enzyme plays a role and where the function of the enzyme can be altered to treat the disease is highlighted to dictate their ability to be a promising lead compound.

Key words: Clove, Dixon plot, flavonoids, molinspiration, pharmacokinetics, SwissADME

INTRODUCTION

Most spices utilized in India have great economic importance. Spices are the most economic sources of polyphenolic compounds such as flavonoids, phenolic compounds, anthocyanins, phenylpropanoids, and anthraquinones. Clove is an important spice in India which is valued for its flavoring and medicinal properties. Clove is an unopened flower bud of clove tree (*Syzygium aromaticum*). It belongs to the Myrtaceae family native from the Maluku islands in east Indonesia but is also cultured in several parts of the world. Most of the biological activities of clove are due to the presence of secondary metabolites such as phenolics and flavonoids. Clove contains 70% eugenol and other major constituents being β-caryophyllene, vanillin, crategolic acid, tannins, and flavonoids. It is employed in dentistry as anodyne, as carminative, and anthelmintic.

Histamine is a metabolically active, low molecular weight amine, which is naturally occurring in plants, animals, and microbes. It is an imidazole derivative produced by the decarboxylation of the essential amino acid, histidine. This reaction is catalyzed by the enzyme histidine decarboxylase.

The partial inhibition of the enzyme could be a possible option as an alternative to antihistamines to prevent the allergic reaction. Flavonoids present in the methanolic extract of traditional spices would be a potent inhibitor for this enzyme.

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Several scientific reports have described the inhibitory effect of spices on a variety of microorganisms, although variation for the resistance of different microorganisms to a given spice and of the same microorganism to different spices has been observed.[1]

In a recent study, the presence of spice and their components in the culture medium of *Enterobacter aerogenes* caused a delay in producing biogenic amines and that the higher concentrations of the spices were inhibitory to cell growth was reported.[2]

MATERIALS AND METHODS

Chemicals

Acacia powder, acetone, benzene, chloroform, ethyl acetate, ferric chloride, glacial acetic acid, hydrochloric acid, liquor ammonia, methanol, nitric acid, oxalic acid, potassium iodide, potassium sodium tartrate, sodium carbonate, and sulfuric acid were procured from Fisher Scientific. Histidine, potassium acetate, quercetin, and sodium hydroxide were procured from HiMedia. Alizarin red S was procured from Chemie Pvt. Ltd. Mercuric chloride was procured from Merck. Dragendroff's reagent was procured from Nice Chemicals. n-butanol was procured from Qualigens by Thermo Fisher. Aluminum chloride, copper sulfate, histamine, nickel sulfate, and ninhydrin were procured from S D Fine Chem Ltd. Quercetin was procured from Sigma Aldrich. α-naphthol was procured from Thomas Baker.

Plant Source

Random sampling was done by collecting clove buds and green cardamom pods (*Elettaria cardamomum*, belongs to the family, Zingiberaceae) from the Reliance fresh supermarket, Bengaluru, Karnataka.

Sample Preparation

The seed samples were finely powdered using the mixer grinder and 20% methanol extract was prepared by stirring the finely powdered sample for 30 min on a magnetic stirrer with 70% methanol. The stirred samples were then centrifuged at 10,000 rpm for 10 min and the supernatant obtained was used for further analysis and experiments.

Phytochemical Tests

The fresh methanolic extracts were subjected to various phytochemical screening for the detection of a range of plant constituents, characterized for their possible bioactive compounds. The phytochemical tests were performed according to the references.[3,4]

Quantitative Estimation of Flavonoids

0.2–1.0 mL aliquots of the standard quercetin solution were pipetted out. The volume of each tube was made up to 2 mL with methanol. 2.0 mL of methanol was taken as blank. To this reaction mixture, 0.1 mL of 10% $AlCl_3$ was added followed by 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. The tubes were then incubated for 30 min at room temperature and the absorbance was read at 415 nm at the end of the incubation period. The flavonoid content was measured as mg quercetin equivalent/g of extract.[5]

Purification of Flavonoids

Partial purification of flavonoids by thin-layer chromatography (TLC)

TLC was performed to partially purify the methanolic extracts for flavonoids. The solvent system used was n-butane:ethyl acetate:distilled water in the ratio of 5:10:15. The sample was spotted on the TLC plates and was allowed to run with the above solvent system. After completion of the run, the plates were air dried and sprayed with a mixture of 3% boric acid and 10% oxalic acid. The plates were then visualized under the ultraviolet (UV) transilluminator for green fluorescence spots for the indication of flavonoids.[6]

Purification of flavonoids by high-performance liquid chromatography (HPLC)

The HPLC system (Agilent Technologies Company) was equipped with a dual lamp binary system, UV detector, C18 column (i.e., 4.6 mm × 150 mm, 5 µm) and data were integrated by Agilent Chem Station software. Standards and sample extracts were analyzed using the following gradient program (A. 100% acetonitrile B HPLC grade water 0 min, 5% A: 10 min, 15% A: 20 min, 25% A: 30 min, 35% A: 40 min, 45% A: 50 min 55% A). The flow rate was 0.8 ml/min and injection volume was 20 µl. The samples were detected at 280 nm by analyzing the peaks obtained and the retention time of individual peaks was used to identify polyphenols by comparing with standard polyphenols such as caffeic acid, kaempferol, quercetin, gallic acid, ferulic acid, and ellagic acid.[7]

Inhibition of histidine decarboxylase

The pure cultures of Lactococcus were sonicated. The resultant supernatant containing the enzyme, after centrifugation was subjected to ammonium sulfate precipitation to partially purify the enzyme. The partially purified enzyme was further purified by ion-exchange chromatography and molecular exclusion chromatography. The purified enzyme was further taken ahead for inhibition studies. The enzyme was allowed to interact with the flavonoids present in the spice extracts. It was then allowed to catalyze the decarboxylation of histamine and its activity was determined. The results were then inferred in order to identify the type of inhibition by

varying the concentrations of the enzyme and the substrate keeping the concentration of the inhibitor constant.

Determination of In silico pharmacokinetics and bioactivity

Molinspiration Cheminformatics was used for calculating important drug-like properties such as logP, polar surface area, number of hydrogen bond donors, number of hydrogen bond acceptors, number of rotatable bonds, volume, and number of violations from rule of five. It was also used to predict bioactive scores against important drug targets such as G-protein coupled receptor (GPCR) ligand, kinase inhibitors, ion channel modulators, nuclear receptors, protease inhibitors, and enzyme inhibitors.[8]

Determination of absorption, distribution, metabolism and excretion (ADME) properties

It is necessary that a potent drug molecule reaches its target in sufficient concentrations and is bioactive for a period long enough to bring about a biological response. The assessment of absorption, distribution, metabolism, and excretion is necessary in the process of drug development. SwissADME is a tool which gives free access to predictive models for physicochemical properties, pharmacokinetics, drug-likeness, and medical chemistry friendliness. It provides an easy efficient input and interpretation and is a user-friendly interface. The physicochemical parameters give a global description of the structure.[9]

RESULTS AND DISCUSSION

The role played by the flavonoids in inhibiting the activity of the enzyme histidine decarboxylase was studied. The methanolic extracts of clove were used as the source of flavonoids. The extracts were purified by TLC and HPLC to identify the different flavonoids present. The inhibition studies were performed which gave positive results explaining the ability of flavonoids to act as a partial inhibitor of the enzyme histidine decarboxylase.

Phytochemical Screening

Phytochemical screening is the qualitative analysis of components present in plant products. Phytochemicals are non-nutritive plant compounds with health benefits.[10] They are bioactive compounds produced by plants as a defense against predators. These phytochemicals have a range of applications in cosmetics, pharmaceuticals, etc., making the screening test mandatory. The methanolic extract of clove buds indicated the presence of carbohydrates, alkaloids, and phenolic acids, including tannins, flavonoids, terpenoids, saponins, and coumarins. Studies have revealed the presence of reducing sugars, tannins, saponins, terpenoids, steroids, and glycosides in methanolic extracts of *S. aromaticum*.[11] Another study revealed the presence of alkaloids and flavonoids along with tannins, saponins, steroids, glycosides, and terpenoids [Table 1] [Figure 1].[12]

Quantitative Estimation of Flavonoids

Aluminum chloride forms stable acid complexes with C-4 keto groups and either the C-3 or C-5 hydroxide group of flavones and flavanols. It also forms liable complexes with ortho dihydroxide groups in A/B rings of flavonoids.[13] The methanolic extracts of clove and green cardamom had a flavonoid content of 0.0262 mg/g of quercetin equivalent and 0.0131 mg/g of quercetin equivalent, respectively. Green cardamom had a comparatively lesser amount of flavonoids due to which the clove extracts were taken for further studies. Previous studies reveal the flavonoids content in cloves to be 3.00 ± 0.09.[14] The clove extract was found to have total flavonoid content of 75.97 ± 0.01 μg QE/g.[15]

Table 1: Phytochemical analysis of clove and green cardamom

Phytochemical test	Clove	Green Cardamom
Test for carbohydrates		
Fehling's test	+	–
Molisch's test	–	–
Benedict's test	–	–
Test for proteins		
Biuret test	–	–
Xanthoproteic test	–	+
Ninhydrin test	–	–
Millon's test	–	–
Test for fixed oils and fats		
Filter paper test	–	–
Test for alkaloids		
Dragendroff's test	+	–
Mayer's test	–	–
Wagner's test	–	–
Test for phenolics and tannins		
Ferric chloride test	++	+
Test for flavonoids		
Sodium hydroxide test	+++	+
Lead acetate test	+++	+
Test for Terpenoids	++	+
Test for steroids		
Liebermann–Burchard reaction	–	–
Salkowski reaction	–	–
Test for glycosides		
Keller–Kiliani test	–	–
Legal test	–	–
Borntrager's test	–	–
Foam test	+	–
Test for coumarins		
Alkali test	+	–

Figure 1: Thin-layer chromatography of methanolic extracts of clove and green cardamom Lane 1 – Extract from green cardamom, Lane 2 – Extract from clove

Purification of Flavonoids

Partial purification by TLC

Flavonoids are universal within plant kingdoms and most common pigments next to chlorophyll and carotenoids. They occur as glycosylated derivatives. Due to their structure, they have UV absorbing properties. The methanolic extract of clove was subjected to TLC, wherein all the spots were colorless in daylight but exhibited fluorescence under UV light [Figure 1]. Three spots were observed, of which one showed green fluorescence, whereas the other spots showed deep blue and dull green fluorescence, respectively.

Purification by HPLC

HPLC is a dominant analytical technique used for separation and characterization of flavonoids. The relatively high molecular mass and intrinsic features of hydrophobic flavonoid aglycones and hydrophilic flavonoids glycosides favor HPLC. HPLC also offers a singular chance to separate simultaneously all components together and even in their low concentrations. Various standards such as caffeic acid, gallic acid, ellagic acid, quercetin, and kaempferol were run using the acetonitrile:water system. The sample was then run to identify the various flavonoids present in the extract with respect to the standards. The reference of the standards indicated the presence of ellagic acid, gallic acid, kaempferol, and quercetin in the methanolic extracts of the clove sample [Figure 2 and Table 2].

Inhibition of Histidine Decarboxylase

The enzyme was isolated by sonication and purified by various purification steps such as ammonium sulfate precipitation, ion-exchange chromatography, and molecular exclusion chromatography. The enzyme activity was assayed after each purification step was carried out. Inhibition studies were further performed using this purified enzyme. The incubation of the enzyme with the inhibitor causes binding of the inhibitor to specific sites of the enzyme, decreasing its efficiency of binding to the substrate and thus decreasing the concentration of the product. Overall, a decrease in enzyme activity can be observed. The activity of enzyme in the presence of varying concentrations of inhibitor was determined and a reciprocal plot, Dixon plot was plotted to determine the concentration of the inhibitor at which it inhibits the enzyme. Clove inhibited the reaction at 0.9 µmoles. An inhibitor which inhibits reactions at nanomoles concentration is said to be a very good inhibitor, whereas the ones inhibiting the reaction at millimoles concentration is said to be a very bad inhibitor. The inhibitory concentrations in our study are found to be at µmoles concentration, indicating that it partially inhibits the enzyme and does not cause complete inhibition of the enzyme. According to previous studies, water extracts of clove showed considerable inhibitory action. Approximately 40% of the activity was inhibited by the water extract of clove. The ethanol extract of clove almost inhibited 65% of the activity of histidine decarboxylase [Figure 3].[2]

Determination of *Insilco* Pharmacokinetics and Bioactivity Score

Molinspiration, a web-based software, was used to obtain parameters such as MiLogP, topological polar surface area (TPSA), and drug-likeness. MiLogP is calculated by the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors. MiLogP parameter is used to check good permeability across the cell membrane. TPSA is related to hydrogen bonding potential of compound. The calculation of volume developed at Molinspiration is based on group contributors. The number of rotatable bonds measures molecular flexibility. It is a very good descriptor of absorption and bioavailability of drugs. Through drug-likeness data of molecule, it can be checked for molecular properties and structure features in respect to known drugs [Figure 4-7] [Table 3].[16]

Bioactivity of the drug can be checked by calculating the activity score of GPCR ligand, ion channel modulator, nuclear receptor legend, kinase inhibitor, protease inhibitor, and enzyme inhibitor. All the parameters were checked with the help of the software Molinspiration drug-likeness score online (www.molinspiration.com). Calculated drug-likeness score of each compound was compared with the specific activity of each compound, and the results were compared with standard drugs. For organic molecules, the probability is if the bioactivity score is (>0), then it is active, if (−5.0–0.0), then moderately active, if (<−5.0) then inactive [Table 4] [Figure 8-11].[16]

Determination of ADME Properties

The methanolic extract of clove was found to contain ellagic acid, gallic acid, kaempferol, and quercetin based on the

standards by performing HPLC. All the flavonoids identified by HPLC were subjected to SwissADME software to determine its drug-likeness and pharmacokinetics in the form of SMILES. The bioavailability radar of all the flavonoids fell within limits in all the properties except for saturation wherein the molecules exceeded the limit required for a probable drug molecule. All the flavonoids obeyed the Lipinski's rules with the molecular weight ranging between 170 and 302 g/mol, which is <500; the number of hydrogen bond acceptors and donors is well within 10 and 5, respectively, and the log $P < 5$ in all molecules. The partition coefficient between n-octanol and water gives a classical description of lipophilicity.[9] The consensus log P o/w gives us the average of log P obtained by various different models. The solubility of a molecule in water greatly facilitates activities related to drug development and, more importantly, the handling and formulation of the drug.[17] In the case of oral administration, solubility becomes a major factor influencing absorption.[18] The values predicted are the decimal logarithm of molar solubility in water (log S). Three different models are employed to determine solubility,

Table 2: Results of HPLC of clove sample

Retention time (min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	WO5 (min)	Response factor
1.507	29.649	4.369	0.0	0.2	0.11	0.000
2.127	5743.684	304.957	7.5	17.1	0.22	0.000
2.670	742.494	67.631	1.0	3.8	0.22	0.000
2.987	2692.788	184.744	3.5	10.4	0.18	0.000
3.667	1185.226	18.225	1.5	1.0	1.10	0.000
5.800	119.303	3.423	0.2	0.2	0.68	0.000
6.670	88.444	1.679	0.1	0.1	1.26	0.000
9.330	38.484	0.847	0.1	0.0	0.99	0.000
16.617	11.629	0.195	0.0	0.0	1.00	0.000
18.173	15.812	0.366	0.0	0.0	0.71	0.000
21.703	1400.526	28.443	1.8	1.6	0.69	0.000
22.313	1870.614	72.306	2.4	4.1	0.36	0.000
22.6	1887.953	85.646	2.5	4.8	0.47	0.000
23.197	2601.346	82.024	3.4	4.6	0.66	0.000
23.900	3938.051	43.860	5.1	2.5	0.86	0.000
28.090	687.570	8.102	0.9	0.5	1.26	0.000
31.457	335.590	4.806	0.4	0.3	1.11	0.000
32.897	672.881	13.470	0.9	0.8	0.78	0.000
33.573	559.277	12.793	0.7	0.7	0.90	0.000
35.130	1438.206	26.597	1.9	1.5	1.03	0.000
35.797	598.327	14.427	0.8	0.8	0.75	0.000
36.917	660.353	15.909	0.9	0.9	0.79	0.000
37.507	1570.465	21.213	2.1	1.2	1.70	0.000
39.633	884.587	18.348	1.2	1.0	1.12	0.000
41.420	11601.731	153.398	15.2	8.6	0.81	0.000
49.613	494.099	4.819	0.6	0.3	1.70	0.000
50.863	696.044	6.785	0.9	0.4	1.66	0.000
53.997	39.042	1.275	0.1	0.1	0.64	0.000
55.463	241.357	3.686	0.3	0.2	1.22	0.000
57.023	60.543	2.023	0.1	0.1	0.62	0.000
58.497	2429.621	44.552	3.2	2.5	0.71	0.000
60.523	859.082	11.235	1.1	0.6	1.27	0.000
62.410	457.642	8.574	0.6	0.5	0.79	0.000
64.403	70.568	2.196	0.1	0.1	0.52	0.000
65.800	29765.875	509.894	38.9	28.6	0.60	0.000
Total	76488.865	1782.815	100.0	100.0		

HPLC: High-performance liquid chromatography

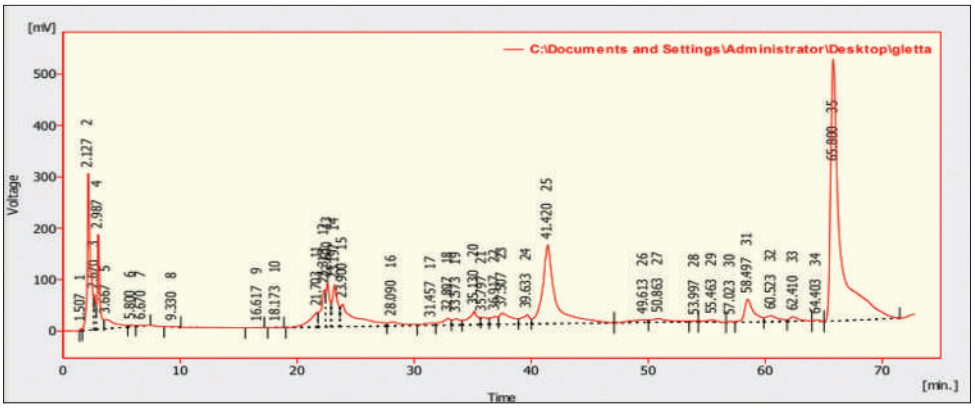

Figure 2: High-performance liquid chromatography of clove sample

Figure 3: Inhibition of histidine decarboxylase by clove extract

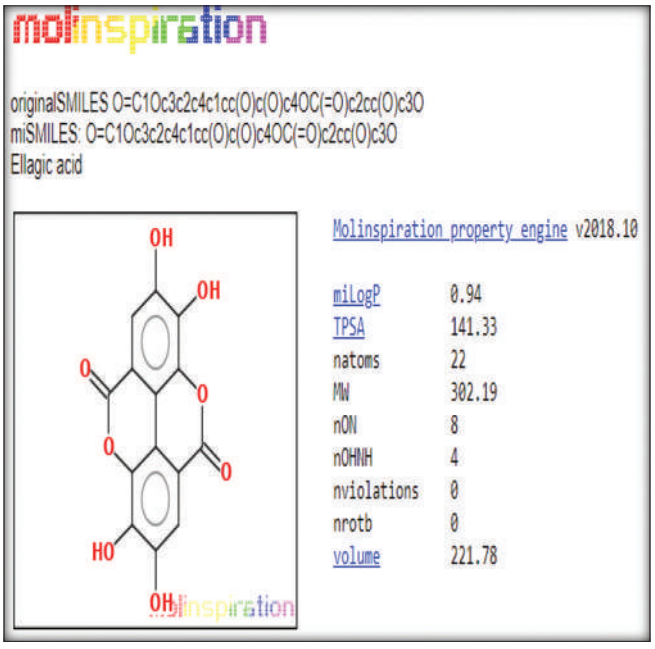

Figure 4: Molecular properties of ellagic acid

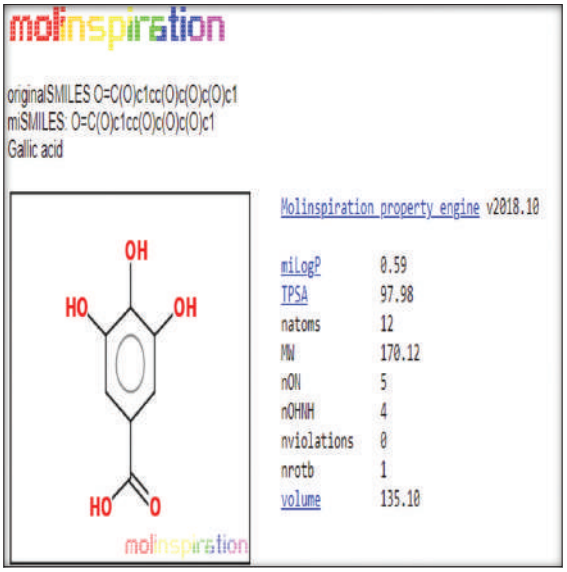

Figure 5: Molecular properties of gallic acid

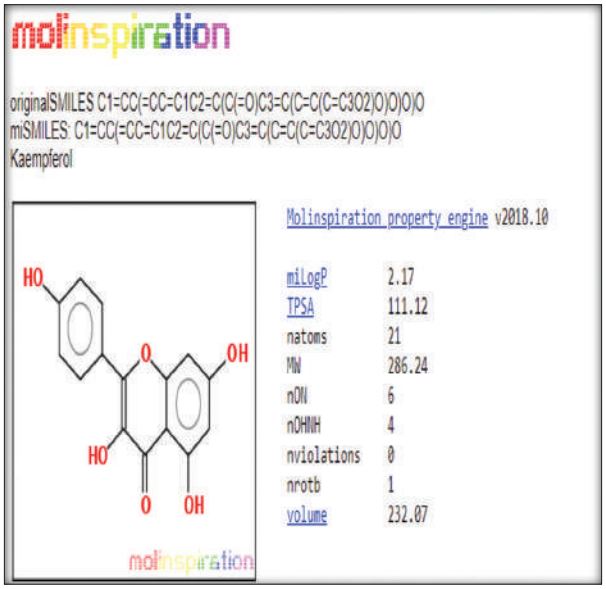

Figure 6: Molecular properties of kaempferol

i.e., ESOL model;[19] model adapted by Ali *et al*.[20] and the third one developed by SILICOS-IT. All the models predict that molecules of interest to be water soluble [Table 5].

Furthermore, the flavonoids proved to be promising leads as they showed high absorption in the gastrointestinal tract and no permeability across the blood-brain barrier. It is a necessity to known whether the compound is a substrate or not of

the permeability glycoprotein. Permeability glycoprotein possesses a major role of protecting the central nervous system (CNS) from xenobiotics[21] and its overexpression is seen in tumor cells which may lead to multidrug resistant cancers.[22] The inability of the identified flavonoids to act as substrates to permeability glycoprotein indicates that they are safe to be consumed and are not harmful to the CNS. The cytochrome P450 are a superfamily of isozymes involved in drug elimination through metabolic biotransformation.[23] These small molecules can act as a substrate to any one of five isozymes of cytochrome P450.[24,25] Inhibition of any of the isozymes is certainly one of the major causes of pharmacokinetics related drug-drug interactions,[26,27] leading to toxic or other unwanted adverse effects due to lower clearance and accumulation of the drug or its metabolites.[28]

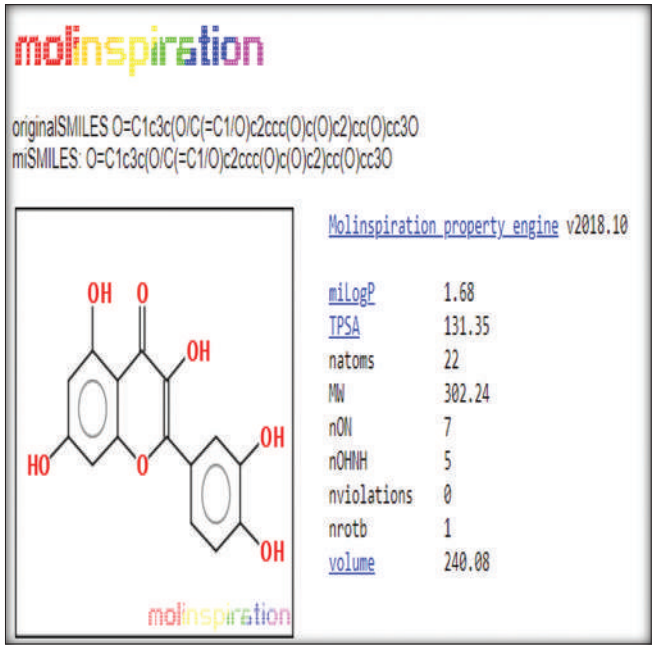

Figure 7: Molecular properties of quercetin

Thus, it is of great importance in drug discovery to predict the propensity with which the molecule will cause significant drug interactions through inhibition of cytochromes P450 and to determine which isoforms are affected. Ellagic acid is found to inhibit only CYPIA2; gallic acid is found to inhibit only CYP3A4, whereas kaempferol and quercetin inhibit CYP1A2, CYP2D6, and CYP3A4. The skin permeability coefficient is calculated using the model adapted from Potts and Guy[29] which correlates K_p with molecular size and lipophilicity. The more negative the value, lesser is the skin permeability.[9] All identified flavonoids are not skin permeable due to their high negative values [Table 6].

Drug-likeness assesses qualitatively the chance for a molecule to become an oral drug with respect to bioavailability. Drug-likeness is established from structural or physicochemical inspections of development compounds advanced enough to be considered oral drug candidates. SwissADME gives access to five different rule-based filters, with diverse ranges of properties inside, of which the molecule is defined as drug-like.[9] The Lipinski (Pfizer) filter is the pioneer rule of five.[30] The Ghose (Amgen), Veber (GSK), Egan (Pharmacin), and Muegge (Bayer) were later adopted.[31-34] The multiple selection methods allow the usage of best fitting results according to the needs of the specific end users.[9] Quercetin along with kaempferol prove very promising as it follows all models of drug-likeness, whereas ellagic acid and gallic acid violate some models. The bioavailability score predicts the probability of the compound to have at least 10% oral bioavailability in rat.[9]

The medicinal chemistry section provides two complementary pattern recognition method which allows the identification of potentially problematic fragments [Table 7]. Pan assay interference compounds (PAINS) are molecular containing substructures showing potent response in assays irrespective of protein targets. The Brenk pattern recognition method

Table 3: Molecular properties of flavonoids

Compound name	Log P	TPSA	Molecular weight(g)	H bond acceptors	H bond donors	Rotatable bonds	Molecular volume
Ellagic acid	0.94	141.33	302.19	8	4	0	221.78
Gallic acid	0.59	97.98	170.12	5	4	1	135.10
Kaempferol	2.17	111.12	286.24	6	4	1	232.07
Quercetin	1.68	131.35	302.24	7	5	1	240.08

Table 4: Bioactivity scores of flavonoids

Compound Name	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Ellagic acid	−0.29	−0.27	−0.01	0.11	−0.18	0.17
Gallic acid	−0.77	−0.26	−0.88	−0.52	−0.94	−0.17
Kaempferol	−0.10	−0.21	−0.21	0.32	−0.27	0.26
Quercetin	−0.06	−0.19	0.28	0.36	−0.25	0.28

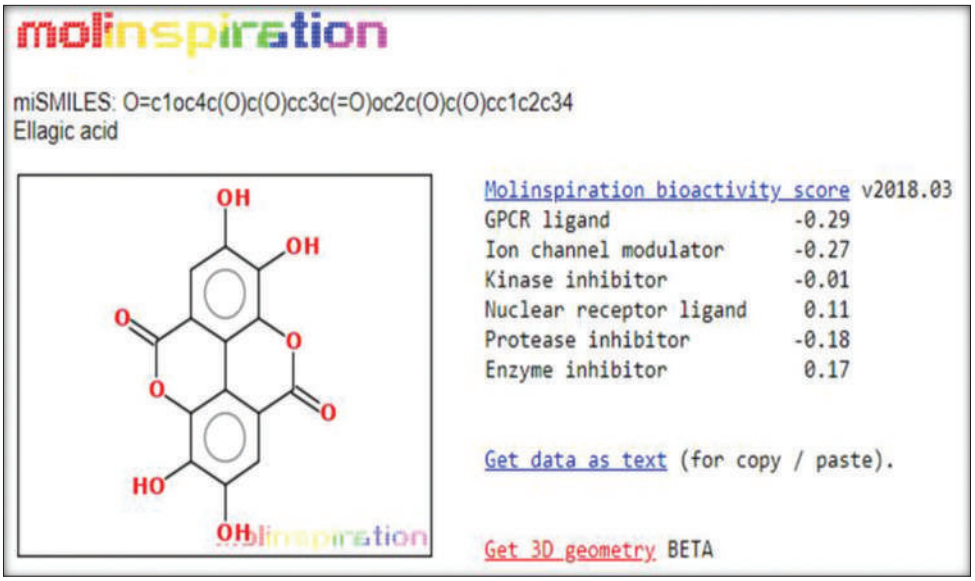

Figure 8: Bioactivity score of ellagic acid

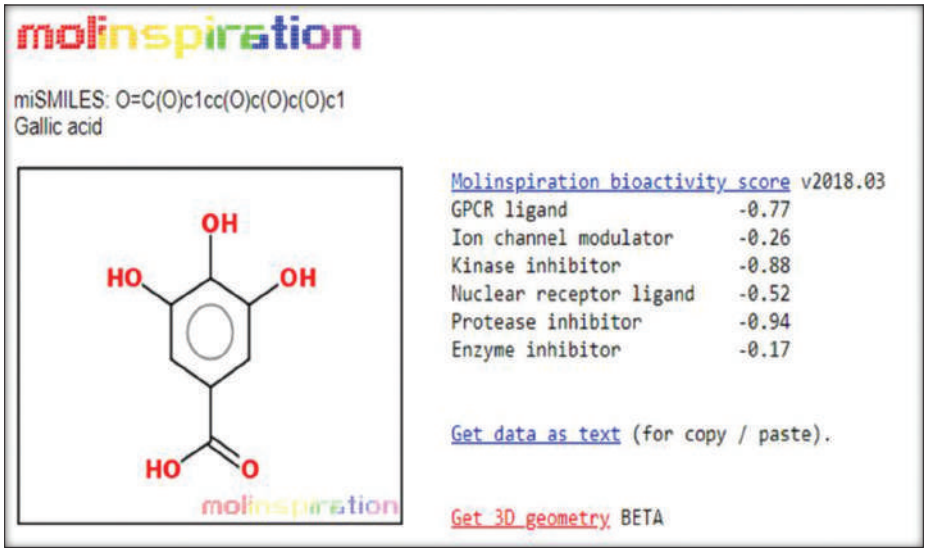

Figure 9: Bioactivity score of gallic acid

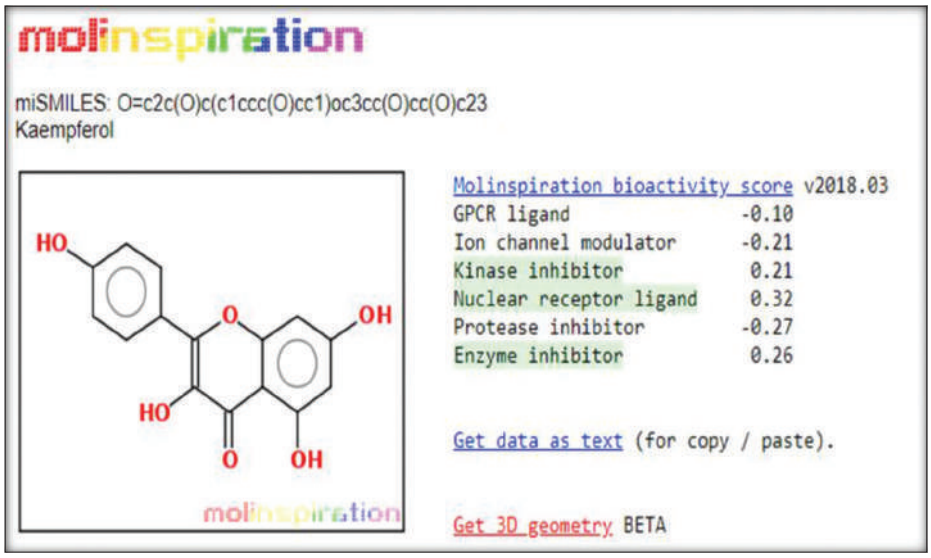

Figure 10: Bioactivity score of kaempferol

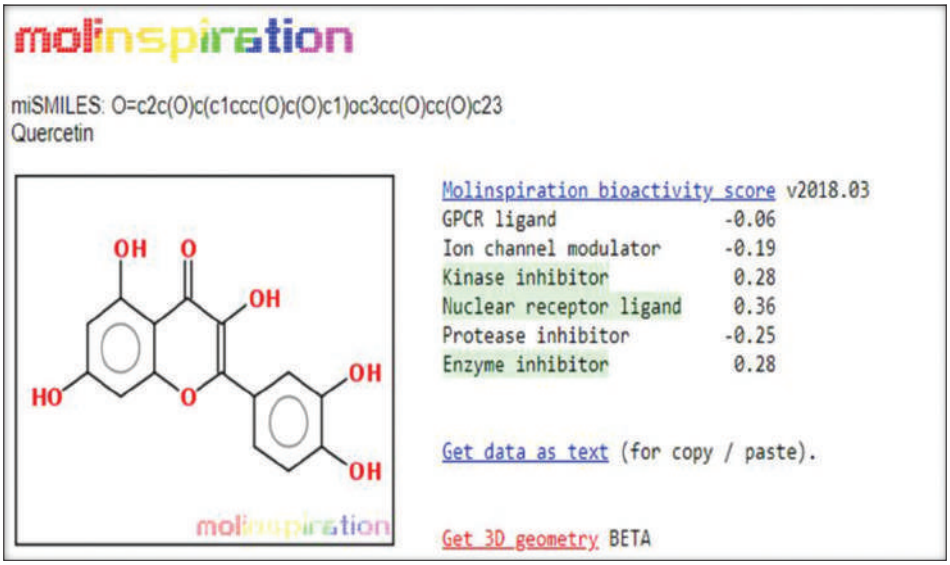

Figure 11: Bioactivity score of quercetin

Table 5: Physicochemical properties of flavonoids

Compound name	Physicochemical properties					
	MW(g/mol)	H bond acceptors	H bond donors	TPSA(Å)	Log P	Log S
Ellagic acid	302.19	8	4	141.34	0.79	–2.94
Gallic acid	170.12	5	4	97.99	0.21	–1.64
Kaempferol	286.24	6	4	111.13	1.70	–3.31
Quercetin	302.24	7	5	131.36	1.63	–3.16

Table 6: Pharmacokinetics of flavonoids

Compound name	Pharmacokinetics								
	GI Absorption	BBB permeability	Pgp substrate	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor	Log K_p(cm/s)
Ellagic acid	High	No	No	Yes	No	No	No	No	–7.36
Gallic acid	High	No	No	No	No	No	No	Yes	–6.84
Kaempferol	High	No	No	Yes	No	No	Yes	Yes	–6.70
Quercetin	High	No	No	Yes	No	No	Yes	Yes	–7.05

Table 7: Drug-likeness of flavonoids

Compound name	Drug-likeness					
	Lipinski's model	Ghose's model	Veber's model	Egan's model	Muegge's model	Bioavailability score
Ellagic acid	Yes	Yes	No	No	Yes	0.55
Gallic acid	Yes	No	Yes	Yes	No	0.56
Kaempferol	Yes	Yes	Yes	Yes	Yes	0.55
Quercetin	Yes	Yes	Yes	Yes	Yes	0.55

helps identify fragments that are putatively toxic, chemically reactive, metabolically unstable, and possess properties of poor pharmacokinetics.[35] The PAINS identified catechol to be a problematic fragment in all molecules except kaempferol, whereas the Brenk method identifies catechol, coumarin, and polycyclic aromatic hydrocarbons as problematic fragments in case of ellagic acid and only catechol are said to be the trouble maker in case of quercetin and gallic acid. Kaempferol seems to be the most promising molecule in the absence of any problematic fragments. The lead-likeness is

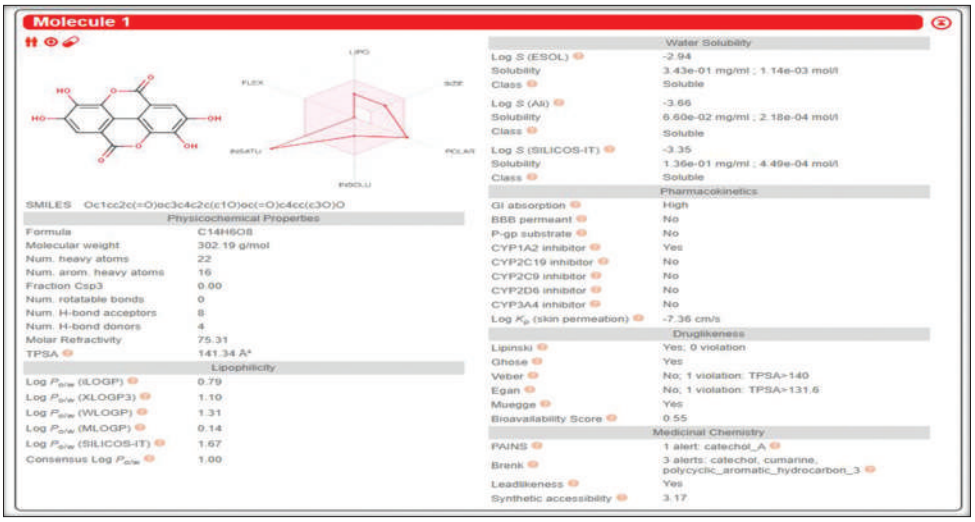

Figure 12: The pharmacokinetics of ellagic acid

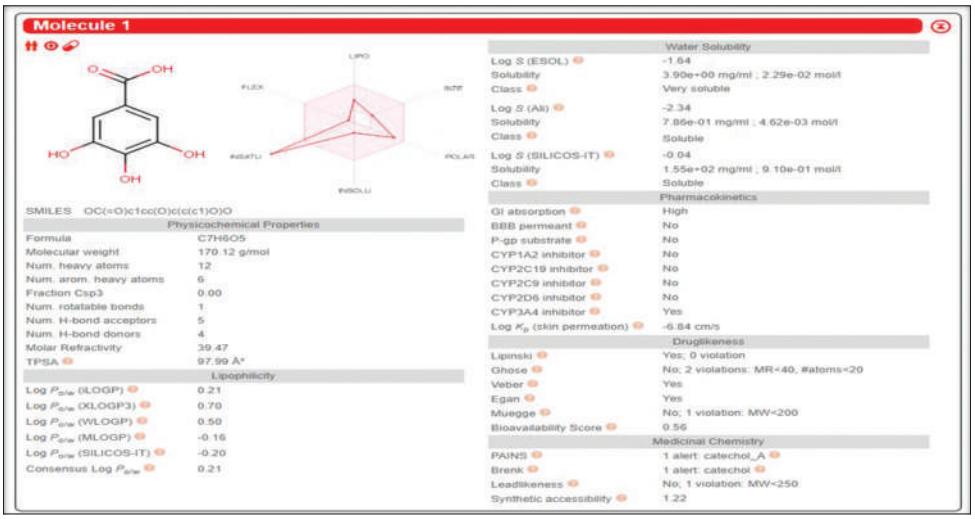

Figure 13: The pharmacokinetics of gallic acid

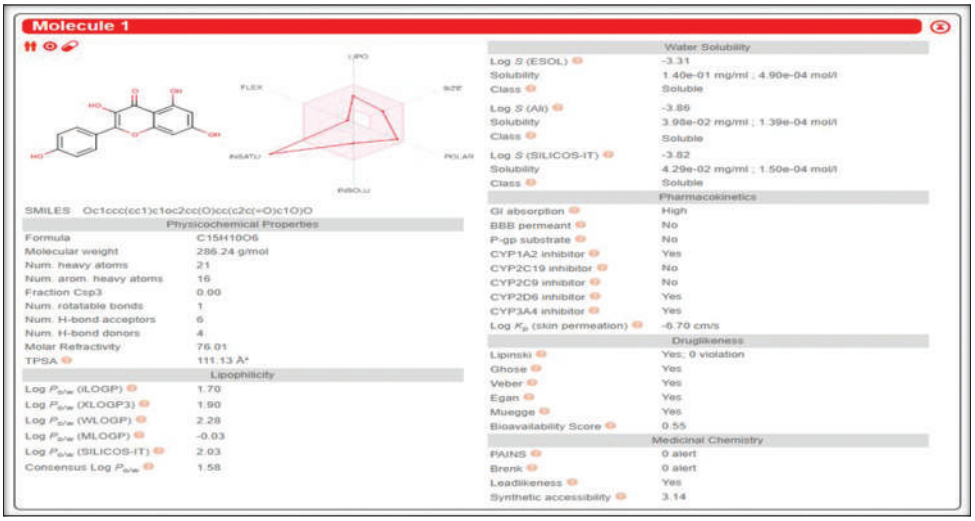

Figure 14: Pharmacokinetic properties of kaempferol

similar to drug-likeness, indicating optimization can make it a suitable molecular entity.[9] This can be achieved by chemical modifications such as increasing size and lipophilicity.[36] Synthetic accessibility is a major factor considered in the

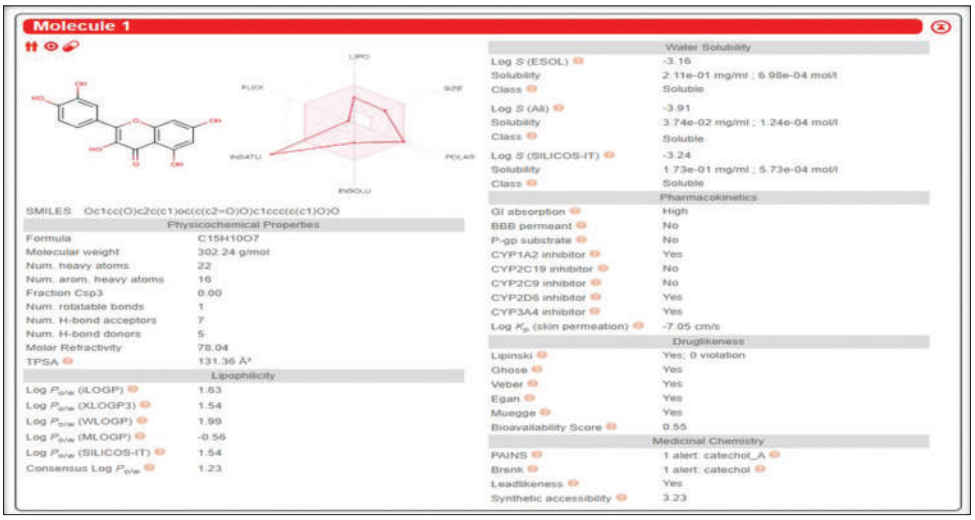

Figure 15: Pharmacokinetic properties of quercetin

Table 8: Medicinal chemistry of flavonoids

Compound name	Medicinal chemistry			
	PAINS	Brenk	Lead-likeness	Synthetic accessibility
Ellagic acid	Catechol alert	Catechol, coumarin, and polycyclic aromatic hydrocarbon alert	Yes	3.17
Gallic acid	Catechol alert	Catechol alert	Yes	1.22
Kaempferol	0 alerts	0 alerts	Yes	3.14
Quercetin	Catechol alert	Catechol alert	Yes	3.23

selection process. It ranges from 1 (very easy) to 10 (very difficult). All molecules except gallic acid possess synthetic accessibility around three making them moderately easy molecules for synthesis and gallic acid seems to be the easiest of all with synthetic accessibility of 1.22 [Figure 12-15] [Table 8].

CONCLUSION

Cloves are rich sources of phenolic compounds such as flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, and hydroxyphenyl propene. Other phenolic acids are also found in clove, they are the caffeic, ferulic, ellagic, and salicylic acids. Flavonoids such as kaempferol, quercetin, and its derivatives (glycosylated) are other flavonoids which are found in lower concentrations in clove. In the present study, the biochemical constituents and the nutritional value of these spices were explained. The procedure involved in the extraction of flavonoids and their phytochemical analysis to determine its various components has been briefed upon. The latest aspects of research about the histidine decarboxylase and the various medical aspects in which the enzyme of interest plays a role and where the function of the enzyme can be altered to treat the disease are highlighted. The inhibition of the enzyme using the spice extract was confirmed and was then continued with the analysis of the flavonoids in spice extracts for the determination of their molecular properties and bioactivity to dictate their ability to be a promising lead compound. The inhibition was a partial one; thus, not completely bringing the activity of the enzyme to a standstill. The medical applications of the spice extracts being traditionally used as a treatment for various ailments and diseases along with the constituents of the spices confer them with these properties have been reviewed for a better understanding of the present-day research.

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