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RESEARCH ARTICLE

EVALUATION OF ANTIOXIDANTS AND MOLECULAR DOCKING STUDIES OF HELICTERES ISORA FRUIT EXTRACTS

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ABSTRACT

The medical plants are widely used by the traditional medicinal practitioners for curing various diseases. The present study aims at evaluate the phytochemical properties and antioxidant sensing activity of the fruit H*elicteres Isora*. For this the fruit parts of the plant were chosen and extracts were prepared using methanol and ethanol. Phytochemical qualitative screening revealed the presence of compounds such as alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids and flavonoids. The antioxidant activity of the plant extracts were performed by DPPH method. The free radical scavenging activity was measured spectrophotometrically as maximum fading power of DPPH at 517nm. All extracts showed different level of antioxidant activity. Molecular docking studies was also done to study the mechanism of action of the active compounds present in the fruit.

Keywords: *Helicteres Isora*, DPPH assay, FRAP assay, Phytochemicals, Molecular docking.

INTRODUCTION

Herbs have always been the principal form of medicine in India and presently they are becoming popular throughout the world, as people strive to stay health in the face of chronic stress and pollution, and to treat illness with medicines that work in the count with the body's own defence. There is a widespread belief that green medicines are healthier and more harmless or safer than synthetic ones. Medicinal plants have been used to cure a number of diseases [1,2]. Through the recovery is slow, the therapeutic use of medicinal plant is becoming popular because of its inability to cause side effects and antibiotic resistant microorganisms [1-3].

The present investigation aims to study the qualitative phytochemical screening of *Helicteres isora* followed by screening of plant extracts as an antioxidant agent. Helicteres isora fruits are used as astringent, stomachic, vermifuge, vulnerary and useful in bowel gripes [4,5]. In tradition, the root juice is claimed to be useful in diabetes, empyema and a favorite cure for snakebite [6,7,8].

Phytochemicals from various parts of the plant such as leaves, flowers, seeds, barks and roots can be isolated. The isolation is primarily done by extraction process, whereby the constituents of the plants are removed using a solvent. The primary way of extraction of organic molecules of interest to biologists and medicinal investigators involve breaking open the cells. Cell disruption is carried out in a variety of ways. The method depends on type of tissue used. A wide variety of secondary metabolite, such as Alkaloids, Tannins, Glycosides, Flavonoids, and proteins are present in such plant extracts. To screen the presence of these components different qualitative chemical tests are performed. The main characteristic of an antioxidant is its ability to trap free radicals .These free radicals may oxidise nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids polyphenols and flavonoids scavenge free radicals such as peroxide, hyperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.

Materials And Methods

Sample Collection

The fruit samples *Helicteres Isora* were carefully picked from different areas in local Chennai (Tamilnadu, India) and were ensured to be free from any pathogenic contamination. The collected fruit samples were dried for several days at 37^0c. The dried fruits were then ground in a grinding mortar made for laboratory. The fruit samples of *Helicteres Isora* was collected and were dried under shade. The dried materials of fruit were powdered and then extracted with three solvents. The samples are filtered through watermann paper. The different qualitative phytochemical tests were performed for the fruit extracts. The samples were found to contain most phytochemical components [9,10].

Extraction of plant material powder by maceration method using organic solvents was done by mixing 5g of powdered plant material with 20ml of ethanol. The mixtures were kept for 24 hours in tightly sealed vessels at 55^0c in shaker incubator, protected from sunlight, and mixed several times with a sterile glass rod. This mixture is filtered through watermann paper and the residue, if necessary can be further extracted. Then extracts are scrapped out from the container and stored at 22^0c in desiccators until further use. The same procedure was followed for the other organic solvent as methanol instead of ethanol [10,11].

Antioxidant activity assays

The free radical scavenging activity was measured in vitro by1,1-diphenyl-2-picrylhydrazyl (DPPH) in 100% Ethanol was prepared and 1ml of this solution was added to 1ml of the dried plant extract dissolved in ethanol 1mg/ml. The blank was prepared by adding 1ml ethanol and 1ml DPPH solution alone to the test tube. The optical density (OD) of

the solution was the read at 517nm in UV spectrophotometer for every 15 seconds [12,13]. The radical scavenging activities of the extracts were represented as the percentage inhibition of DPPH radicals.

Total antioxidant capacities of the sample assessed by Ferric Reducing Ability of Plasma (FRAP) assays [14]. The antioxidant activity values similarly varied with the DPPH and FRAP assays. The method described measures the ferric reducing bumin, solid (bovine serum albumin, fraction V, ability of plasma (FRAP). At low pH, when a ferric- Sigma), bilirubin calibrator solution (Sigma), and Trotripyridyltriazine (FeIII-TPTZ) complex is reduced to lox (Aldrich Chemical Co., U.S.A.) were used to pretheferrous (FeII) form, an intense blue color with an pare aqueous antioxidant solutions. DL-a-Tocopherol absorption maximum at 593 nm develops. Significant and positive linear correlations were found between total antioxidant capacities and phenolic contents (R = 0.89–0.97), indicating that phenolics were the dominant antioxidant constituents in the tested medicinal plants. Preliminary identification of the major phenolic compounds from 83 selected medicinal plants by reversed-phase HPLC revealed phenolic acids, tannins, flavonoids, curcuminoids, coumarins, lignans, and quinines.

RESULT AND DISCUSSION

The Methanol extract of the fruit sample-1 showed the maximum radical scavenging activity. Aqueous extract showed the moderate activity. The least scavenging activity was shown by Ethanol extract. It was found that the radical scavenging activity reduced by time. Preliminary identification of the major phenolic compounds by reversed-phase HPLC revealed phenolic acids, tannins, flavonoids, curcuminoids, coumarins, lignans, and quinines. The complete qualitative phytochemical screening of the fruit samples is shown in the table-1. The antioxidant activity values similarly varied with the DPPH and FRAP assays (Table 2 & 3). Significant and positive linear correlations were found between total antioxidant capacities and phenolic contents (R = 0.89–0.97), indicating that phenolics were the dominant antioxidant constituents in the tested medicinal plants.

Table 1: Qualitative Phytochemical Screening of Fruit Extract

Phytochemical Compounds	Aqueous	Methanol	Ethanol
Molish	present	Present	present
Tannins	present	Present	present
Saponins	present	Present	present
Flavonoids	present	Absent	present
Alkaloids	present	Present	present
Anthocyanin & Betacyanin	Present	Present	present
Glycosides	present	Present	present
Proteins	present	Absent	absent

Table 2: DPPH Assay was used to measure free radical scavenging activity of fruit extract from H*elicteres Isora*

Extract	Blank	Sample after 5min	%Q	Sample after 10min	%Q	Sample after 15min	%Q	Sample after 20min	**%Q**
Aqueous	0	0.241	0.0301	0.200	0.025	0.132	0.0165	0.119	0.0148
Methanol	0	0.114	0.0009	0.112	0.0008	0.11	0.0009	0.117	0.0009
Ethanol	0	0.153	0.0012	0.149	0.0011	0.150	0.0012	0.151	0.0012

Table 3: FRAP Assay was used to determine antioxidant activity of fruit extract from H*elicteres Isora*

S.NO	SOLVENT	ABSORBANCE AT 593nm	FRAP VALUE ((µM/mg)
1	Sample1(Aqueous)	0.100	30
2	Sample2(Aqueous)	0.187	50
3	Sample1 (Methanol)	1.801	450
4	Sample2 (Methanol)	1.121	280
5	Sample1 (Ethanol)	1.446	350
6	Sample2 (Ethanol)	1.5	370

The aqueous extract of the bark has previously showed significant hypoglycaemic, lowering effect of hepatic enzymes [15], glycoprotein levels [16]. Saponin is the active compound identified from the fruit of *Helicteres isora* and is proved to have anti-diabetic activity [17]. In the present study we evaluated the interaction of saponin with the Anti-diabetic drug target glucokinase using Molecular docking studies. Glucokinase is an enzyme that facilitates phosphorylation of glucose to glucose-6-phosphate. Glucokinase occurs in cells in the liver, pancreas, gut, and brain of humans and most other vertebrates. In each of these organs it plays an important role in the regulation of carbohydrate metabolism by acting as a glucose sensor, triggering shifts in metabolism or cell function in response to rising or falling levels of glucose, such as occur after a meal or when fasting. Mutations of the gene for this enzyme can cause unusual forms of diabetes or hypoglycemia. Hence glucokinase has been considered as the drug target for treating diabetes.

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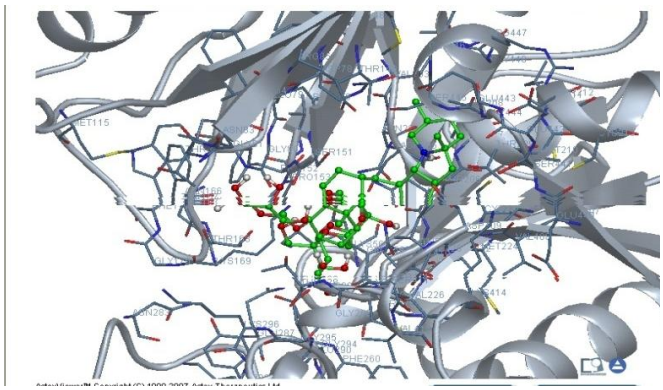

Figure 1: Saponin bound to the active site of Glucokinase

The three dimensional structure of glucokinase was downloaded from PDB database and was used for docking studies. Before docking partial atomic charges are applied to each atom of the ligand. We also distinguish between aliphatic and aromatic carbons: names for aromatic carbons start with 'A' instead of 'C'. AutoDock ligands are written in files with special keywords recognized by AutoDock. The root is a rigid set of atoms, while the branches are rotatable groups of atoms connected to the rigid root. The TORSDOF for a ligand is the total number of possible torsions in the ligand minus the number of torsions that only rotate hydrogens. TORSDOF is used in calculating the change in free energy caused by the loss of torsional degrees of freedom upon binding. After all the above conditions are set the ligand is saved in "pdbq" format. (Figure 1). The results of docking studies shows that saponin binds to glucokinase with an energy of -4.61kcal/mol, indicating that the active compound from the plant *Helecteres isora* is an efficient compound having strong anti-diabetic activity. The results of the present study can be used as an insight in determining all the active compounds from the various extracts of the fruit and studying its effect on diabetes using *insilico* and *invivo* approaches.

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