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ORIGINAL RESEARCH ARTICLE

Biological activity spectra of the main phytochemicals of *Silybum marianum* L. Gaertn. by *in silico* study

RANJINI R*

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ABSTRACT

The main objective of bioinformatics is to augment the perception of biological data. Bioinformatics gains information from computer analysis of biological practices. Biologically active principles have both pharmaceutical and adverse effects on the organisms. PASS (Prediction of Activity Spectra for Substances) software is used to estimate the general efficacy and safety of the phytochemicals. PASS simultaneously predicts several hundreds of biological activities of natural and synthetic chemical compounds. The average precision of prediction is about 90%. The extract from the seed of Silybum marianum contains silymarin, which is a complex mixture of polyphenolic molecules, including seven closely related flavonolignans, namely, silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, silydianin and one flavonoid namely taxifolin. Silymarin has been used to treat various hepatic diseases, including chronic and acute liver diseases in canines and felines. It is used as a nutritional supplement to treat liver diseases and toxicities and prevent certain cancers in companion animals. In the present study, the main phytochemicals reported from Silybum marianum L. Gaertn. were subjected to in-silico evaluation using PASS software. The methods, biological activity spectra and significance of the in-silico study are discussed.

INTRODUCTION

Silybum marianum L. Gaertn. is a medicinal plant used to remedy various ailments in man and animals over the years. *S. marianum* (Milk thistle) is a tall Asteraceae family herb with large, prickly white veined green leaves. The flowers are reddish-purple and end in sharp spines. Silymarin, which is the active component of this herb, is mainly used as a hepatoprotective, anti-inflammatory, anti-cancer, antihypertensive, hypocholesterolaemic, anti-diabetic, and antioxidant (Gazak, 2007). Extract of the seeds is used as a demulcent, anti-haemorrhagic, galactagogue agent that induces milk secretion and is used in the treatment of uterine disorders (Anestis *et al*., 2011). Bioinformatics is a multidisciplinary field that develops techniques, databases and software tools for understanding biological statistics. Computational techniques developed by experts in bioinformatics have been helpful to scientists and researchers in other fields (Lagunin *et al*., 2003). Prediction of Activity Spectra for Substances (PASS) is a web-based application that predicts the biological activity spectrum of a compound based on its structure, functional groups and molecular geometry. Biological activities depend on the structure and physico-chemical properties of any compound. Biologically active compound reveals a widespread spectrum of different activities, including pharmacological effects, mechanism of action, interaction with metabolic enzymes and adverse or toxic effects. In the present study, the main

*Madras Veterinary College, Vepery, Chennai 600007, Tamil Nadu, India; Corresponding Author: Email: ranjinir1999@gmail.com
Doi: https://doi.org/10.62029/jmaps.v45i1.ranjini

phytochemicals reported from *Silybum marianum* L. Gaertn. were subjected to *in-silico* evaluation using PASS software.

Traditional milk thistle extract is made from the seeds, which contain approximately 65–85% silymarin (a flavonolignan complex) and 15–35% fatty acids, including linoleic acid. Silymarin is a complex mixture of polyphenolic molecules, including seven closely related flavonolignans, specifically silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, silydianin and one flavonoid namely taxifolin (Kroll *et al.*, 2007). Silibinin, a semipurified fraction of silymarin, is primarily a mixture of two diastereoisomers, silybin A and silybin B, in equal proportion (Hogan *et al*., 2007). Milk thistle-based supplements have been measured to have the highest mycotoxin concentrations of up to 35 mg/kg when compared to various plant-based dietary supplements (Veprikova *et al*., 2015). Because of nitrate content, the plant is toxic to cattle and sheep. Potassium nitrate, when eaten by ruminants, the bacteria in the animal's stomach cracks the chemical down, producing nitrite ions. Nitrite ions combine with haemoglobins to produce methemoglobins, blocking oxygen transport. The result is a category of oxygen deprivation (Ramakrishnan *et al*., 2009). The protective effects of this herbal medicine, *Silybum marianum,* have been proven in the treatment of lung diseases, prostate diseases, nephrotoxicity, hepatotoxicity, viral hepatitis, cancer, *in vitro* fertilisation, mycotoxicity, neurotoxicity, depression, and polycystic ovary. Silymarin can safeguard the brain, liver, kidney and heart against ischemia-reperfusion injury due to its antioxidant activity and radical scavenging. The potential known mechanisms of action of silymarin protection are obstruction and adjustment of cell transporters, p-glycoprotein, estrogenic and nuclear receptors (WenWu *et al*., 2009). Additionally, silymarin reverses antibiotic resistance, has anti-inflammatory effects, defensive effects on erythrocyte lysis and cisplatin-induced acute nephrotoxicity. Silymarin has also inhibited apoptosis and follicular development in patients undergoing *in vitro* fertilisation (Karimi *et al*., 2011).

MATERIALS AND METHODS

Any compound's predicted biological activity profile can be obtained by its structural formula. Thus, prediction is also achievable for every virtual structure designed in a computer but not yet synthesised. To access the PASS-Online (Prediction of Activity Spectra for Substances) service, prior registration is mandatory, which is free. One should agree with the terms and conditions for usage of that service. The activity spectrum of any compound may be obtained by drawing the molecule's structure using Marvin sketch, Chem sketch, Smile format or through MolFile. Prediction is based on analysing structure activity-relationships for more than 3,00,000 biologically active substances, including drugs, drug candidates, leads and toxic compounds (Parasuraman, 2011). In the present study, the biological activity spectra of eleven different phytochemicals of *Silybum marianum,* namely, apigenin, dehydrosilybin, isosilybin, isosilychristin, kaempferol, naringin, quercetin, silybin, silychristin, silydianin and taxifolin were obtained by *in silico* study using PASS software.

Flow sheet of instructions to use PASS

Enter PASS website www.pharmaexpert.ru

↓

PASS Homepage is opened

↓

Click Registration

↓

Fill up the registration form and click submit.

↓

PASS Online Homepage is opened

(www.pharmaexpert.ru / passonline/predict.php)

↓

Enter the User name and password and click ok.

↓

Click Predict New compound.

↓

New applet software is opened.

↓

Draw the structure of the compound using Marvin's sketch and click predict

↓

Activities of the compound will be displayed.

The structure of a molecule may be drawn using Chemsketch. The molecule's structure may also be saved in chem sketch or MDL Mol files and directly uploaded to the PASS prediction website, which predicts the biological activity spectra of the molecules. The structure can be drawn directly in PASS prediction software using a JAVA applet that uses a 2D chemical sketch–drawing program, Marvin Sketch (Figs. 1 & 2). The biological spectra of the phytochemicals are presented with pa (probable activity) and pi (probable inactivity). The activity's efficacy and toxicity value are also provided (Fig. 3). Biological activity results from a chemical compound's interaction with a biological entity. In preclinical testing, the biological entity is represented by the experimental models (*in vitro*) and experimental animals (*in vivo*). In clinical study, it is human.

RESULTS AND DISCUSSION

Biologically active compound reveals a broad spectrum of different activities. Some are valuable in treating specific diseases, but others cause different inhibitive or toxic effects. The entire complex of activities caused by any compound in biological entities is called the "biological activity spectrum of the substance". PASS software predicts the biological activity spectrum based on the structure of any compound. The spectrum is presented as a list of activities with appropriate Pa and Pi, sorted in descending order of the distinction (Pa-Pi)>0. Pa and Pi are the probability estimates for the phytochemical to be active and inactive for each type of action from the biological activity spectrum (Fig. 3). Their values vary from 0.000 to 1.000. It is logical that only those types of activities Pa > Pi may be revealed by the compound and are placed into the biological activity spectrum. If Pa > 0.7, the compound is very likely to reveal this activity in experiments, but the chance of being the analogue of the well-known pharmaceutical agents for this compound is also high. If 0.5 < Pa < 0.7, the compound will likely reveal this activity in *in vitro* experiments. However, this probability is less, and the compound is not so similar to the recognised pharmaceutical agents. If Pa < 0.5, the compound is unlikely to expose this activity in experiments, but if this activity is confirmed, the compound might be a new chemical entity (Sadym *et al*., 2003).

Biological Activity Spectra of Phytochemicals of *Silybum marianum*

1. **Apigenin**

Pa	Pi	Activity
0,973	0,001	Chlordecone reductase inhibitor
0,967	0,002	Membrane integrity agonist
0,946	0,002	Membrane permeability inhibitor
0,946	0,004	CYP2C12 substrate
0,942	0,002	2-Dehydropantoate 2-reductase inhibitor
0,941	0,002	Kinase inhibitor
0,936	0,001	Aryl-alcohol dehydrogenase (NADP+) inhibitor
0,937	0,003	Aldehyde oxidase inhibitor
0,931	0,001	P-benzoquinone reductase (NADPH) inhibitor
0,931	0,003	Anaphylatoxin receptor antagonist
0,924	0,002	Peroxidase inhibitor
0,921	0,002	Antimutagenic
0,918	0,002	Histidine kinase inhibitor
0,914	0,002	NADPH-ferrihemoprotein reductase inhibitor
0,912	0,001	Quercetin 2,3-dioxygenase inhibitor
0,911	0,005	HIF1A expression inhibitor
0,907	0,002	CYP1A inducer
0,906	0,003	UGT1A6 substrate
0,902	0,005	Ubiquinol-cytochrome-c reductase inhibitor

2. Dehydrosilybin

Pa	Pi	Activity
0,991	0,001	TP53 expression enhancer
0,971	0,001	Free radical scavenger
0,968	0,001	Hemostatic
0,969	0,002	Membrane integrity agonist
0,935	0,004	HIF1A expression inhibitor
0,932	0,002	Hepatoprotectant
0,929	0,003	Chlordecone reductase inhibitor
0,914	0,002	Monophenol monooxygenase inhibitor
0,903	0,001	NOS2 expression inhibitor
0,900	0,002	Anticarcinogenic

3. Isosilybin

Pa	Pi	Activity
0,956	0,001	Free radical scavenger
0,957	0,003	Membrane integrity agonist
0,939	0,002	Hepatoprotectant
0,936	0,002	APOA1 expression enhancer
0,914	0,005	TP53 expression enhancer
0,909	0,003	Lipid peroxidase inhibitor
0,859	0,003	Antioxidant
0,857	0,008	HIF1A expression inhibitor
0,844	0,003	HMOX1 expression enhancer

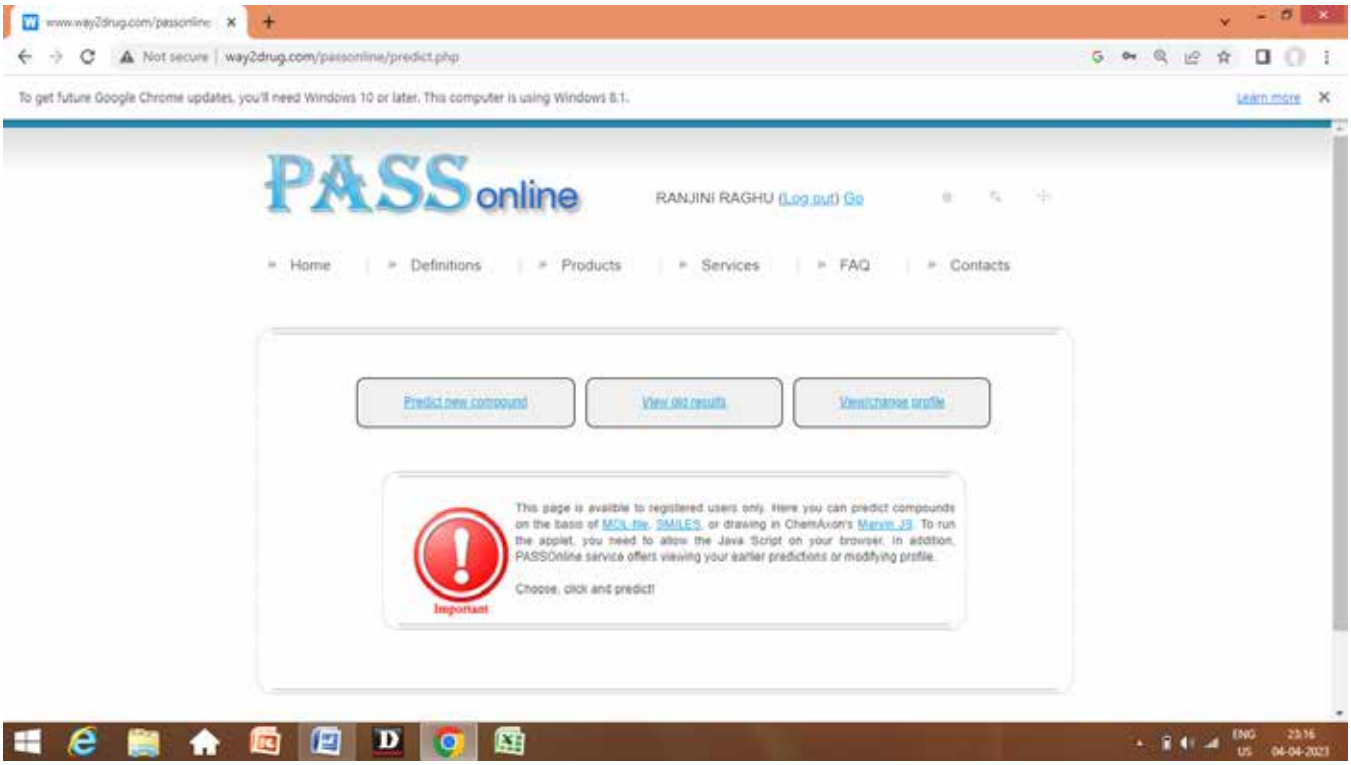

Figure 1: PASS-Homepage

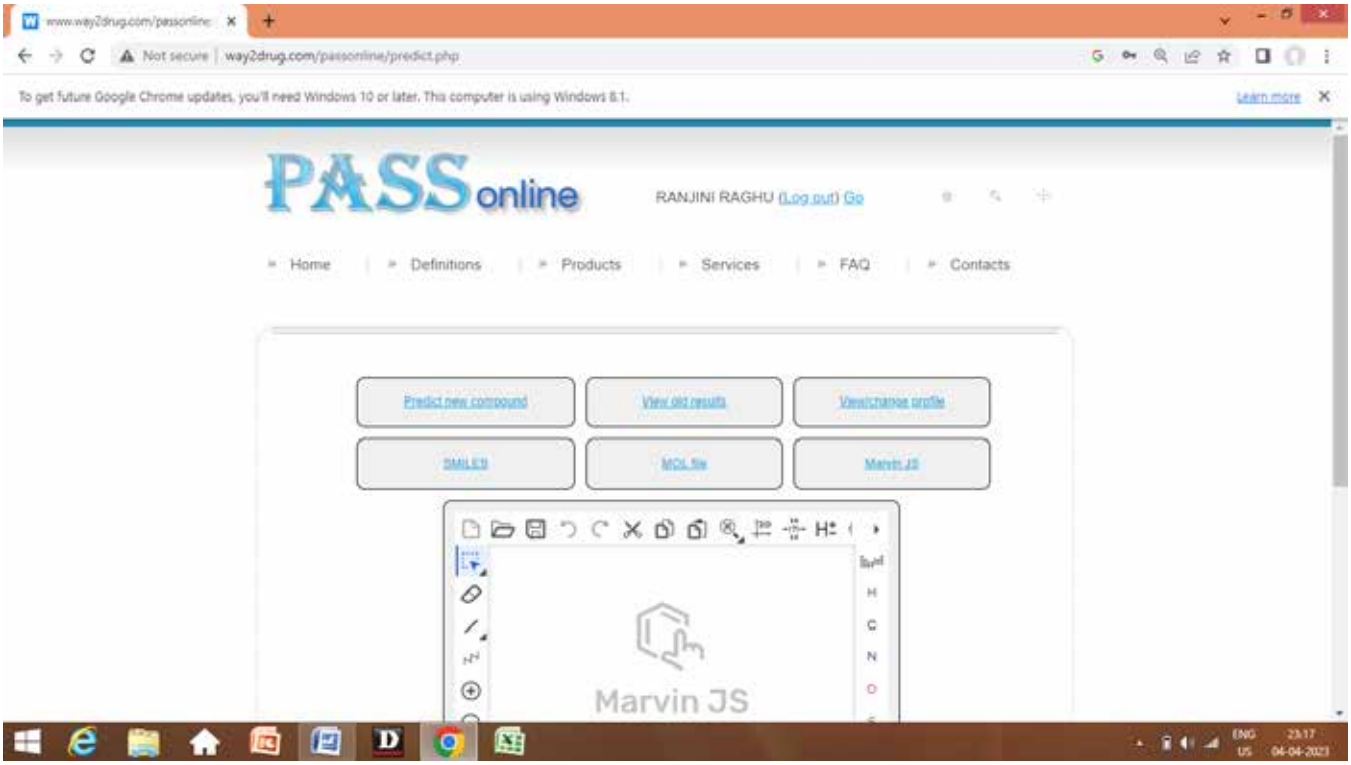

Figure 2: PASS-Marvin Sketch

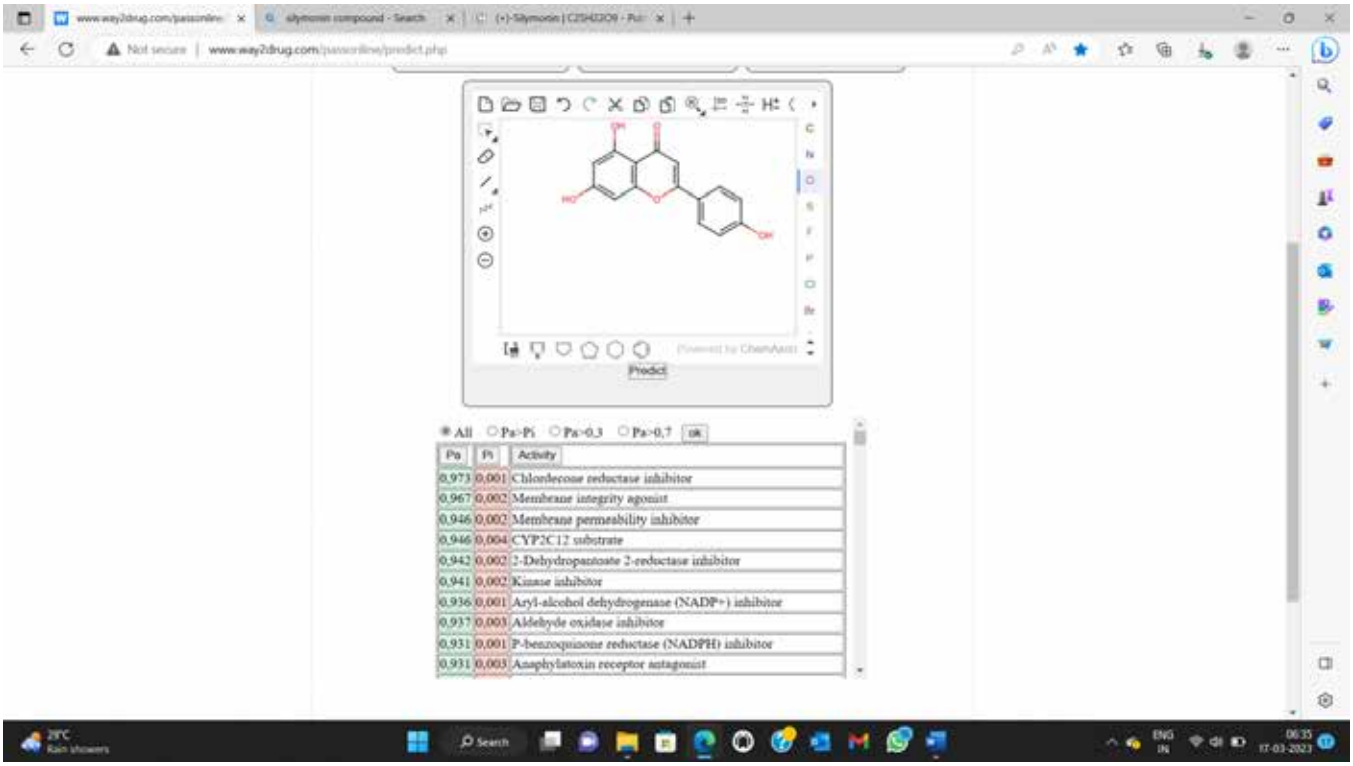

Figure 3: Biological Activity Spectrum of Apigenin – PASS

0,828	0,001	Skin whitener
0,817	0,003	Monophenol monooxygenase inhibitor
0,809	0,006	CYP1A substrate
0,800	0,005	CYP1A1 substrate

4. Isosilychristin

Pa	Pi	Activity
0,938	0,004	TP53 expression enhancer
0,934	0,002	Antioxidant
0,926	0,002	APOA1 expression enhancer
0,888	0,002	Free radical scavenger
0,885	0,007	HIF1A expression inhibitor
0,891	0,013	Membrane integrity agonist
0,868	0,003	Hepatoprotectant
0,856	0,003	Chemopreventive
0,849	0,004	CYP1A1 substrate
0,815	0,005	UDP-glucuronosyltransferase substrate
0,809	0,006	CYP1A substrate

5. Kaempferol

Pa	Pi	Activity
0,983	0,001	Chlordecone reductase inhibitor
0,974	0,002	Membrane integrity agonist
0,969	0,002	HIF1A expression inhibitor
0,965	0,001	2-Dehydropantoate 2-reductase inhibitor
0,961	0,001	Aryl-alcohol dehydrogenase (NADP+) inhibitor
0,959	0,001	Kinase inhibitor
0,959	0,001	P-benzoquinone reductase (NADPH) inhibitor
0,957	0,002	Membrane permeability inhibitor
0,956	0,001	Peroxidase inhibitor
0,951	0,001	Quercetin 2,3-dioxygenase inhibitor

6. Naringin

Pa	Pi	Activity
0,959	0,001	Chemopreventive
0,930	0,002	Anticarcinogenic
0,915	0,002	Hepatoprotectant
0,914	0,008	CDP-glycerol glycerophosphotransferase in ibitor
0,913	0,008	Membrane integrity agonist
0,902	0,002	Proliferative diseases treatment
0,901	0,002	Free radical scavenger

7. Quercetin

Pa	Pi	Activity
0,973	0,002	Membrane integrity agonist
0,969	0,002	HIF1A expression inhibitor
0,962	0,001	Peroxidase inhibitor
0,957	0,002	HMOX1 expression enhancer
0,951	0,001	CYP1A inducer
0,944	0,002	UGT1A6 substrate
0,945	0,004	CYP1A substrate

0,940	0,001	Antimutagenic
0,940	0,003	CYP1A1 substrate
0,939	0,002	UGT1A10 substrate
0,938	0,003	Membrane permeability inhibitor
0,934	0,001	Quercetin 2,3-dioxygenase inhibitor
0,933	0,001	MAP kinase stimulant
0,931	0,002	UGT1A9 substrate
0,930	0,001	CYP1A1 inducer
0,930	0,003	CYP1A inhibitor
0,928	0,002	NADPH oxidase inhibitor
0,924	0,001	Beta-carotene 15,15'-monooxygenase inhibitor
0,920	0,001	Chalcone isomerase inhibitor
0,924	0,007	CYP2C12 substrate
0,909	0,001	CYP1A1 inhibitor
0,909	0,003	CYP1A2 inhibitor
0,910	0,004	CYP1A2 substrate

8. Silybin

Pa	Pi	Activity
0,956	0,001	Free radical scavenger
0,957	0,003	Membrane integrity agonist
0,939	0,002	Hepatoprotectant
0,936	0,002	APOA1 expression enhancer
0,914	0,005	TP53 expression enhancer
0,909	0,003	Lipid peroxidase inhibitor
0,859	0,003	Antioxidant
0,857	0,008	HIF1A expression inhibitor
0,844	0,003	HMOX1 expression enhancer
0,828	0,001	Skin whitener
0,817	0,003	Monophenol monooxygenase inhibitor
0,809	0,006	CYP1A substrate
0,800	0,005	CYP1A1 substrate

9. Silychristin

Pa	Pi	Activity
0,934	0,002	APOA1 expression enhancer
0,929	0,002	Free radical scavenger
0,931	0,004	TP53 expression enhancer
0,922	0,004	HIF1A expression inhibitor
0,916	0,007	Membrane integrity agonist
0,906	0,003	Antioxidant
0,890	0,003	UDP-glucuronosyltransferase substrate
0,846	0,003	HMOX1 expression enhancer
0,844	0,005	CYP1A substrate
0,819	0,004	UGT1A substrate
0,812	0,004	Chemopreventive
0,810	0,005	CYP1A1 substrate

10. Silydianin

Pa	Pi	Activity
0,825	0,002	Antihemorrhagic
0,798	0,012	Antineoplastic
0,775	0,014	TP53 expression enhancer
0,732	0,008	Trans-acenaphthene-1,2-diol dehydrogenase inhibitor
0,711	0,006	Chemopreventive
0,682	0,009	Histidine kinase inhibitor
0,655	0,009	Hepatoprotectant
0,698	0,055	Membrane integrity agonist
0,665	0,024	HIF1A expression inhibitor
0,645	0,008	APOA1 expression enhancer

11. Taxifolin

Pa	Pi	Activity
0,973	0,002	Membrane integrity agonist
0,969	0,002	HIF1A expression inhibitor
0,962	0,001	Peroxidase inhibitor
0,957	0,002	HMOX1 expression enhancer
0,951	0,001	CYP1A inducer
0,944	0,002	UGT1A6 substrate
0,945	0,004	CYP1A substrate
0,940	0,001	Antimutagenic
0,940	0,003	CYP1A1 substrate
0,939	0,002	UGT1A10 substrate
0,938	0,003	Membrane permeability inhibitor
0,934	0,001	Quercetin 2,3-dioxygenase inhibitor
0,933	0,001	MAP kinase stimulant
0,931	0,002	UGT1A9 substrate
0,930	0,001	CYP1A1 inducer
0,930	0,003	CYP1A inhibitor
0,928	0,002	NADPH oxidase inhibitor
0,924	0,001	Beta-carotene 15,15'-monooxygenase inhibitor
0,920	0,001	Chalcone isomerase inhibitor
0,924	0,007	CYP2C12 substrate
0,909	0,001	CYP1A1 inhibitor
0,909	0,003	CYP1A2 inhibitor
0,910	0,004	CYP1A2 substrate
0,898	0,001	2-Enoate reductase inhibitor
0,900	0,005	Ubiquinol-cytochrome-c reductase inhibitor

The result reveals that isosilybin, isosilychristin, silybin and silychristin are potent antioxidants. All the eleven phytochemicals of the present study, namely apigenin, dehydrosilybin, isosilybin, isosilychristin, kaempferol, naringin, quercetin, silybin, silychristin, silydianin and taxifolin act as membrane integrity agonist. HIF1A expression inhibitor activity occurs every day in the other ten phytochemicals of the present study except naringin. Dehydrosilybin, isosilybin, isosilychristin, silybin, silychristin and silydianin has the property of TP53 expression enhancer. Dehydrosilybin, isosilybin,

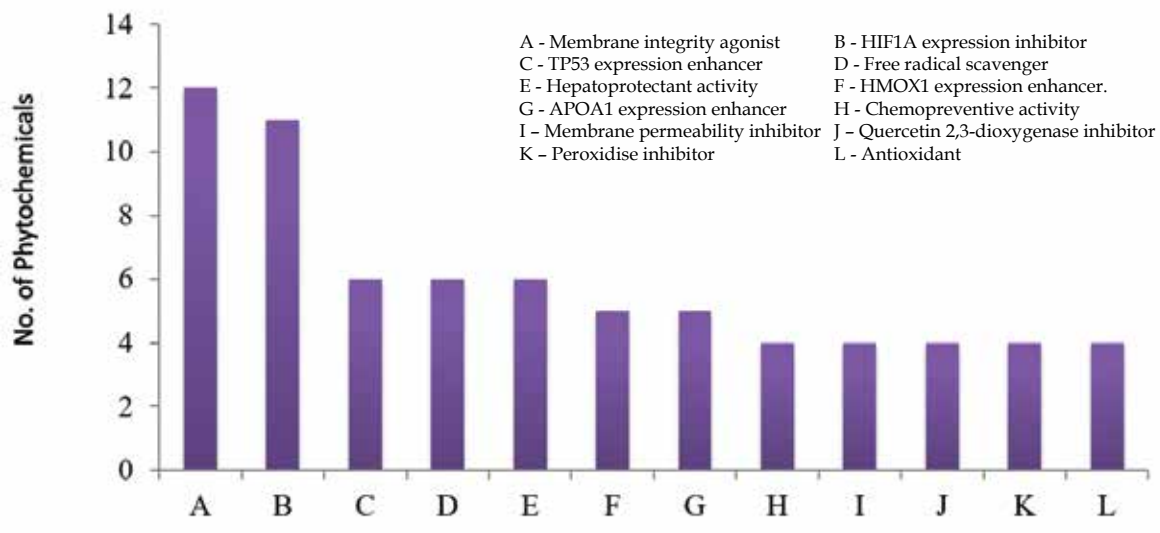

Figure 4: Phytochemicals with common activities

isosilychristin, silybin, silychristin and naringin function as free radical scavengers. Hepatoprotectant activity is expected in six different phytochemicals: dehydrosilybin, isosilybin, isosilychristin, silybin, naringin and silydianin. Isosilybin, quercetin, silybin, silychristin and taxifolin are HMOX1 expression enhancers. Isosilybin, isosilychristin, silybin, silychristin and silydianin possess APOA1 expression enhancer activity. Chemopreventive activity is common in four phytochemicals, namely, isosilychristin, silydianin, silychristin and naringin. Apigenin, kaempferol, quercetin and taxifolin function as membrane permeability inhibitors, Quercetin 2,3-dioxygenase inhibitor and peroxidase inhibitor (Fig. 4). In the biological activity spectra of the phytochemicals, the activities showing more than 90% efficiency are discussed. More other activities may be common in the eleven phytochemicals of the present study with less than 90% efficiency. The phytochemicals have synergy and polyvalent activity. In herbal medicine, synergy may lead to increased efficacy and attenuation of toxicity (William, 2005).

The findings of the biological activity spectra correlate with the *in vitro* and *in vivo* studies of the active principles reported in the literature. The significance of the present study is that the efficacy of the compounds' different activities and the negative impacts were obtained using the PASS algorithm, which may help design new drugs and identify the medicinal uses of the plant *Silybum marianum.* The phytochemicals can also correct the activities related to metabolism and disorder (Poroikov and Filimonov, 2002). *In vivo* and *in vitro* studies of phytochemicals' activities are expensive and consume more time (Anjali *et al*., 2001). *In silico* study, it is important because it is easy to perform, inexpensive, reliable, eco-friendly, accurate, and saves time.

CONCLUSION

The results of the *in-silico* study of phytochemicals activities are 90% reliable. After knowing the activities of the compounds, it may be confirmed by *in vitro in vivo* screening studies and clinical trials. Thus, bioinformatics is also helpful in conservation biology and protecting the environment by understanding the significance of medicinal plants, phytochemicals and their activities. New actions of the drug can be revealed through computer-aided prediction. Apart from the earlier literature, the present investigation discloses that apigenin, quercetin and taxifolin have antimutagenic activity with high efficacy. More drugs may be available for a particular disease in the medical field. One can select the best one with high efficiency and low toxicity by subjecting the structure of all the known compounds to PASS software. The spectra help select the suitable phytochemical as a drug for a specific ailment. One of the steps in drug designing is the knowledge to predict the bioactivities of the molecules. It begins with using *in silico*-screening rather than *in vitro*, *in vivo*, or *in situ*.

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