INT. J. BIOL. BIOTECH., 9 (3): 257-262, 2012.

LIGAND-BASED SCREENING OF CHEMICAL CONSTITUENTS OF *GLYCYRRHIZA GLABRA* IN SEARCH OF INHIBITORS OF XANTHINE OXIDASE

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ABSTRACT

The ligand-based screening of the chemical compounds found in *Glycyrrhiza glabra* has been performed by Random forest modeling to search the inhibitors of xanthine oxidase, which has been implicated in oxidative injury to tissue and in many other diseases. The hits obtained by RF modeling are mostly flavonoids with Kumatakenin, Isoquercitrin and astragalin among the top of the list with 0.97, 0.96 and 0.95 RF score, and thus potentially be inhibitors of enzyme xanthine oxidase.

Key words: Xanthine oxidase, Random Forest Modeling, *Glycyrrhiza glabra*

INTRODUCTION

The enzyme xanthine oxidase (EC 1.1.1.204) catalyses the oxidation of hypoxanthine and xanthine to uric acid with reduction of NAD to NADH, which play a crucial role in gout (Tsutomu *et al*., 1991). The reoxidation of xanthine oxidase, produces hydrogen peroxide and superoxide radical where molecular oxygen acts as electron acceptor (Fridovich, 1970). Thus, xanthine oxidase could be an important biological source of superoxide radicals, which can cause oxidative stress and thus, involved in many pathological processes such as inflammation, atherosclerosis, cancer and aging (Halliwell *et al.*, 1992).

Gout is a disease caused due to the accumulation of uric acid in joints and kidneys which results in acute arthritis and uric acid nephrolithiasis. The use of xanthine oxidase inhibitors such as allopurinol, is one therapeutic approach for gout (Elion *et al.*, 1966; Emmerson, 1996), which blocks the synthesis of uric acid from purines. However, use of allopurinol result in a number of adverse side effects (Wallach, 1998), which can sometimes be life threatening (Bouloc *et al.*, 1996; Kumar *et al.*, 1996; Umpie´rrez *et al.*, 1998; Yale *et al.*, 1996).

In addition to gout, inhibition of xanthine oxidase can also be used for the treatment of hepatitis and brain tumor because increased serum xanthine oxidase levels are found in hepatitis and hepatotoxicity (Bowman and Rand, 1980; Kokoglu *et al.*, 1990) as well as in brain tumors (Kokoglu *et al.*, 1990).

Thus there is a need to develop inhibitors of xanthine oxidase and a potential source of such compounds could be the medicinal plants which can be used to treat gouty arthritis.

The stem of *Glycyrrhiza glabra*, belonging to the family of Leguminosae, has been used as a traditional herb in China and Indonesia to treat throat inflammation and Hepatitis (Usia *et al.*, 2006; Sener and Bingol, 1988).

In the work reported here, ligand-based virtual screening of the chemical constituents of *Glycyrrhiza glabra* by Random Forest modelling has been performed in a search for potential inhibitors of xanthine oxidase.

Random Forest (RF) methodology, one of the best known forms of multiple decision tress, uses a small set of active compounds to search a much larger data set for new candidates.

The main object of a decision tree is to find a distinction on the basis of a set of molecular descriptors that will identify molecular features shared by different subsets of known active compounds against a particular target and accordingly filter out compounds within the dataset in which these combinations of molecular properties are lacking. RF is among the top performers of the many other available machine learning (mathematical) and traditional statistical tools which are used in the drug discovery and development process, such as, Partial Least Squares (PLS), (Sheridan *et al*., 1994) *k*-Nearest Neighbors (*k*-NN), (Kauffman and Jurs, 2001) Multiple Linear Regression (MLR), (Kauffman and Jurs, 2001) Linear Discriminant Analysis (LDA), (Bakken and Jurs, 2000) and Support Vector Machines (SVM) (Doniger *et al*., 2002) and thus used here in this study.

MATERIALS AND METHODS

Two data sets were compiled; the first, test dataset, contains information on phytochemical compounds with known activity against xanthine oxidase enzyme (Table 1); the second, target dataset, provides details of known chemical constituents of *Glycyrrhiza glabra*. Both the two datasets were compiled from Duke's Phytochemical and

Ethnobotanical Database (http://www.ars-grin.gov/duke) and the NCBI PubChem database (Chen *et al.*, 2009; http://pubchem.ncbi.nlm.nih.gov).

In the studies reported here, Random Forest (RF) methodology (as implemented within Statistica version 9.1, Statsoft Ltd., Bedford, UK) was used to search for compounds from *Glycyrrhiza glabra* that might be active against xanthine oxidase.

Three different RF models were generated for this purpose i.e Constitutional, Radial Distribution Function (RDF) (Hemmer *et al*., 1999) and Three dimensional (3D) MoRSE (Schuur, 1996), with each one using a different set of molecular descriptors. There were 32 xanthine oxidase active compounds used in the training set together with 184 compounds found in *Glycyrrhiza glabra*.

Three sets molecular descriptors were calculated for each entry in both the data sets using DRAGON Professional version 5.5 (2007), Talete SRL, Italy.

RESULTS AND DISCUSSION

The list of compounds from different herbs that have been shown to inhibit the enzyme xanthine oxidase is presented in Table 1. The compounds from the target dataset predicted to be active against xanthine oxidase by three of the RF models; Constitutional, RDF and 3D-MoRSE and the average of the three RF scores are presented in Table 2.

Compounds were classified as active if they achieved a score of 0.50 or above in all three RF models. They were thus classified as active by ≥50% votes from the full ensemble of trees in each RF model.

During this study, Kumatakenin, Isoquercitrin, Astragalin and Prunetin (Fig. 1) are the compounds predicted to be most active against xanthine oxidase with the RF score 0.97, 0.96 and 0.95 respectively (Table 2), and thus could be the potent inhibitors of the enzyme xanthine oxidase.

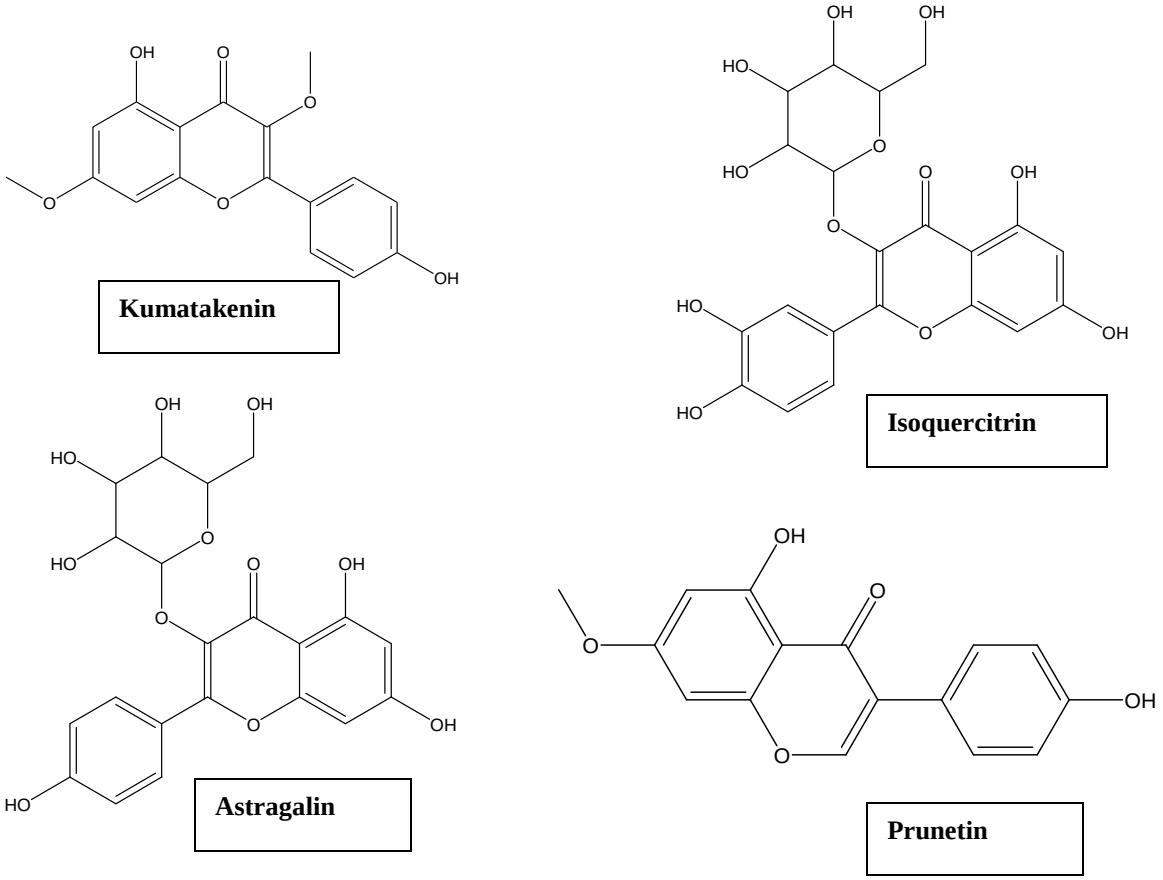

Fig. 1. Structures of the top four hits obtained by RF modelling

Table 1. List of known active compounds against xanthine oxidase in the test dataset.

S.No.	Compound	S.No.	Compound
1	Allicin	17	Glycyrrhisoflavanone
2	Apigenin	18	Glycyrrhisoflavone
3	Baicalein	19	Hyperoside
4	Caffeic acid	20	Isohamnetin
5	Casuarinin	21	Isoterchebin
6	Catechin	22	Licochalcone-A
7	Corilagin	23	Licochalcone-B
8	Ellagic acid	24	Licocoumarone
9	Epicatechin	25	Luteolin
10	Epicatechin Gallate	26	Methyl Gallate
11	Epigallocatechin	27	Morin
12	Esculetin	28	Nepetin
13	Flavone	29	Osthol
14	Fraxetin	30	Penta-o-Galloyl-Beta-D-Glucose
15	Gallic acid	31	Procyanidin
16	Geraniin	32	Quercetin

Table 2. List of compounds in the target dataset found in *Glycyrrhiza glabra*.

1	1-acetylpyrrole	47	Ethyl Linolenate	93	Kaempferol	139	Octacosan
2	1-Methyl-2-Formyl-Pyrrole	48	Ethyl Palmitate	94	Kumatakenin	140	Octadecane
3	1-Pentadecanol	49	Ethyl Phenol	95	Lavandvlol	141	Octadecanal
4	18-Alpha Glycyrrhetinic acid	50	EthylPhenylacetate	96	Licochalcone-A	142	Octanoic acid
5	18-Alpha Glycyrrhizinic acid	51	Eugenol	97	Licochalcone-B	143	Onocerin
6	18-Beta Glycyrrhetinic acid	52	Fenchone	98	Licocoumarone	144	Ononin
7	2-Acetyl-5-Methyl-Furan	53	Ferulic acid	99	Licoflavanone	145	Oxalic acid
8	2-Acetyl- Furran	54	Formononetin	100	Licoflavonol	146	P-Cymene
9	2-Acetyl Pyrrole	55	Furfural	101	Licoisofalvanone	147	P-Hydroxybenzoic acid
10	2-Butyl-2-Octenal	56	Furfural alcohol	102	Licoisoflavone-A	148	Paeonol
11	Acetic acid	57	Furfural butyrate	103	Licoisoflavone-B	149	Pentanoic acid
12	Acetoin	58	Furfural formate	104	Licoricidin	150	Phaseollinisoflavan
13	Acetol	59	Furfural propionate	105	Licoricone	151	Phenethyl Alcohol
14	Acetophenone	60	Galangin	106	Licuraside	152	Phenyl Acetaldehyde
15	Alpha Terpineol	61	Gamma butyrolactone	107	Ligustrazine	153	Phenyl Propionic acid
16	Anethole	62	Gamma heptalactone	108	Linalol	154	Pinocembrin
17	Apigenin	63	Gamma hexalactone	109	Linalool-A-Oxide	155	Prunetin
18	Apioglycyrrhizin	64	Gamma nonalactone	110	Linalool-B-Oxide	156	Pseudoionone
19	Araboglycyrrhizin	65	Gamma octalactone	110	Linoleic Acid Ethyl Ester	157	Pyrazole
20	Astragalin	66	Genistein	112	Linolenic Acid Ethyl Ester	158	Quercetin
21	Benzaldehyde	67	Geraniol	113	Liquiritigenin	159	Riboflavin
22	Benzoic acid	68	Glabranin	114	Liquiritin	160	Salicylic acid
23	Benzyl Alcohol	69	Glabrene	115	Liquoric Acid	161	Schaftoside
24	Bergapten	70	Glabridin	116	Malic Acid	162	Sinapic acid

Cont'd. table 2

25	Beta Amyrin	71	Glabrol	117	Maltol	163	Soyasaponin-1
26	Beta Carotene	72	Glabrone	118	Mannitol	164	Soyasaponin-II
27	Beta Sitosterol	73	Glycyrol	119	Methyl ethyl ketone	165	Stigmasterol
28	Betaine	74	Glycyrram	120	Methyl hexadecanoate	166	Terpinen-4-ol
29	Betulic Acid	75	Glycyrrhetic acid	121	Methyl hexanoate	167	Tetracosan-1-ol
30	Butanoic acid	76	Glycyrrhetol	122	Methyl salicylate	168	Tetracosane
31	Butyl Phthalate	77	Glycyrrhisoflavanone	123	Myrtenal	169	Tetradecanoic acid
32	Butyric Anhydride	78	Glycyrhisoflavone	124	N-Methyl-2-Pyrrolidone	170	Tetramethyl Pyrazine
33	Camphor	79	Glycyrrhizin	125	N-Nonacosane	171	Thiamin
34	Caproic acid	80	Glyzaglabrin	126	N-Tetradecane	172	Thujone
35	Carvacrol	81	Guaiacol	127	Naringenin	173	Thymol
36	Cumic Alcohol	82	Hederasaponin-C	128	Neoliquiritin	174	Tiglaldehyde
37	Decane	83	Herniarin	129	Neoisoliquiritin	175	Tricosane
38	Decanoic acid	84	Hispaglabridin-A	130	Niacin	176	Tridecane
39	Difurfuryl ether	85	Hispaglabridin-B	131	Nonadecane	177	Tridecanoic acid
40	Docosane	86	Isoglycyrol	132	Nonanoic acid	178	Trimethyl Pyrazine
41	Dodecane	87	Isolicoflavonol	133	O-Acetyl Salicylic acid	179	Umbelliferone
42	Dodecanoic	88	Isoliquiritigenin	134	O-Cresol	180	Undecane
43	Echinatin	89	Isoliquiritin	135	O-Methoxy Phenol	181	Undecanoic acid
44	Eicosane	90	Isomucronulatol	136	O-Tolunitrile	182	Uralsaponin-B
45	Estragole	91	Isoquercitrin	137	Oct-1-en-3-ol	183	Vitexin
46	Ethyl Linoleate	92	Isoschaftoside	138	Octacosan-1-ol	184	Xanthotoxin

Table 3. Compounds predicted to be active against xanthine oxidase by RF modeling.

IHD compounds	Predicted Activity			
	Constitutional	RDF	3DMoRES	Average
Kumatakenin	1.00	0.95	0.97	0.97
Isoquercitrin	0.99	0.95	0.94	0.96
Astragalin	1.00	0.90	0.96	0.95
Prunetin	0.99	0.89	0.94	0.94
Formononetin	0.99	0.79	0.97	0.92
Ononin	0.99	0.79	0.97	0.92
Isolicoflavonol	0.93	0.86	0.94	0.91
Kaempferol	0.95	0.85	0.93	0.91
Licoflavonol	0.93	0.84	0.96	0.91
Licoisoflavone-A	0.93	0.84	0.96	0.91
Isoliquiritin	0.97	0.83	0.92	0.91
Glycyrhisoflavone	0.93	0.87	0.91	0.90
Neoisoliquiritin	0.97	0.85	0.89	0.90
Vitexin	1.00	0.82	0.89	0.90
Liquiritin	0.91	0.83	0.95	0.90
Licoflavanone	0.90	0.85	0.92	0.89
Echinatin	0.93	0.83	0.88	0.88
Genistein	0.86	0.85	0.93	0.88
Neoliquiritin	0.91	0.82	0.90	0.88
Licochalcone-B	0.96	0.79	0.87	0.87

Cont'd table 3

IHD compounds	Predicted Activity			
	Constitutional	RDF	3DMoRES	Average
Naringenin	0.85	0.84	0.90	0.86
Quercetin	0.87	0.88	0.84	0.86
Galangin	0.86	0.76	0.91	0.84
Glyzaglabrin	0.92	0.83	0.78	0.84
Apigenin	0.86	0.74	0.90	0.83
Isoliquiritigenin	0.85	0.71	0.89	0.82
Glabrone	0.85	0.87	0.72	0.81
Licoisoflavone-B	0.90	0.82	0.71	0.81
Glycyrrhisoflavanone	0.88	0.73	0.80	0.80
Licoisofalvanone	0.89	0.79	0.72	0.80
Licoricone	0.82	0.76	0.82	0.80
Licuraside	0.93	0.63	0.83	0.80
Riboflavin	0.68	0.79	0.91	0.79
Glabranin	0.71	0.77	0.83	0.77
Liquiritigenin	0.69	0.71	0.86	0.75
Licoricidin	0.74	0.80	0.69	0.74
Schaftoside	0.96	0.57	0.66	0.73
Glycyrol	0.71	0.76	0.70	0.72
Glabrene	0.80	0.81	0.54	0.72
Pinocembrin	0.69	0.63	0.82	0.71
Glabrol	0.68	0.80	0.61	0.70
Isoschaftoside	0.96	0.56	0.57	0.70
Licocoumarone	0.67	0.73	0.64	0.68
Licochalcone-A	0.67	0.77	0.59	0.68
Isomucronulatol	0.70	0.76	0.53	0.66
Herniarin	0.74	0.61	0.62	0.66
Hispaglabridin-A	0.68	0.74	0.55	0.66
Glabridin	0.70	0.72	0.54	0.65
Isoglycyrol	0.66	0.75	0.53	0.65

Most of the hits obtained by RF modeling and predicted to be active against xanthine oxidase are flavonoids (Table 3). These Flavonoids have many biological and pharmacological activities e.g. antibacterial, antiviral and antioxidant, and are also the inhibitors of several enzymes (Vanden Berghe *et al.*, 1993; Bors *et al.*, 1990). It has been reported that flavonoids not only inhibit xanthine oxidase (Masayoshi et al, 1985) but also have superoxide scavenging activities (Sichel *et al.*, 1991; Hu *et al.*, 1995).

Therefore, flavonoids could be a promising remedy for human gout and ischemia by decreasing both uric acid and superoxide concentrations in human tissues (Cotelle *et al*., 1992).

REFERENCES

Bakken, G.A., and P.C. Jurs (2000)**.** Classification of multidrug-resistance reversal agents using structure-based descriptors and linear discriminant analysis. *J. Med. Chem.*, 43, 4534-4541.

Bors, W., W. Heller, C. Michel and M. Saran (1990). In: *Methods in Enzymology* (Packer, L., Glazer, A. N., Eds.); Academic Press: New York, Vol. 186, 343-355.

Bouloc A., P. Reygagne, P. Lecoz and L. Dubertret (1996) Perforating foot ulceration with allopurinol therapy. *Clin. Exp. Dermatol*., 21: 351–352.

Bowman W. C. and M.J. Rand (1980). *Textbook of Pharma-cology*, 2nd Edn, pp. 2.43–2.44, 21.3, Blackwell, Sydney.

Chen, B., D. Wild and R. Guha (2009). Pub Chem as a Source of Polypharmacology**.** *J. Chem. Inf. Model*., 49: 2044–2055.

Cotelle, N., J.L. Bernier, J.P. Henichart, J.P. Catteau, E. Gaydou and J.C. Wallet (1992). Scavenger and antioxidant properties of ten synthetic flavones. *Free Rad. Biol. Med*., 13: 211-219.

Doniger, S., T. Hofmann and J. Yeh (2002). Predicting CNS permeability of drug molecules: comparison of neural network and support vector machine algorithms. *J. Comput. Biol*., 9: 849-864.

Elion G. B., A. Kovensky and G. H. Hitchings (1966). Metabolic studies of allopurinol, an inhibitor of xanthine oxidase. *Biochem. Pharmacol.,* 15: 863 – 880

Emmerson, B.T. (1996). The management of gout. *N. Engl. J. Med*.,334: 445–451.

Fridovich, I. (1970). A mechanism for the pro- duction of ethylene from methional. The generation of the hydroxyl radical by xanthine oxidase. *J. Biol. Chem*., 245, 4053-4057.

Halliwell, B., J. M. C. Gutteridge and C. E. Cross (1992). Free radicals, antioxidants, and human disease: where are we now? *J. Lab. Clin. Med.*, 119: 598-620.

Hemmer, M.C., V. Steinhauer and J. Gasteiger (1999). Deriving the 3D structure of organic molecules from their infrared spectra. *Vibrational Spectroscopy*, 19: 151-164.

Hu, J. P., M. Calomme, D. Lasure, T. De Bruyne, L. Pieters, A. Vlietinck and D. A. Vanden Berghe (1995). Structure-activity relationship of flavonoids with superoxide scavenging activity. *Biol. Trace Elem. Res*., 47: 327-331.

Kauffman, G.W. and P.C. Jurs (2001). QSAR and *k*-nearest neighbor classification analysis of selective cyclooxygenase-2 inhibitors using topologically based numerical descriptors. *J. Chem. Inf. Comput. Sci.*, 41: 1553-1560.

Kokoglu, E., A. Belce E. Ozyurt and Z. Tepeler (1990). Xanthine oxidase levels in human brain tumors. *Cancer Lett*., 50: 179–181.

Kumar, A., N. Edward, M.I. White, P.W. Johnston and G.R. Catto (1996). Allopurinol, erythema multiforme, and renal insufficiency. *BMJ*., 312: 173–174.

Masayoshi, I., M. Ayako, M. Yoshiko, T. Nahoko and F. Michi (1985). Inhibition of xanthine oxidase by flavonoids. *Agric. Biol. Chem*., 49: 2173-2176.

Schuur, J.H., P. Selzer and Gasteiger (1996). The coding of the three-dimensional structure of molecules by molecular transforms and its application to structure-spectra correlations and studies of biological activity. *J. Chem. Inf. Comput. Sci*.**,** 36*:* 334-344.

Sener, B and F. Bingol (1988). Screening of Natural Sources for Antiinflammatory Activity. *Int. J. Crude Drug Res*., 26 (4): 197-207.

Sheridan, R.P., R.B. Nachbar and B.L. Bush (1994). Extending the trend vector: the trend matrix and sample-based partial least squares. *J. Comput. Aided Mol. Des.*, 8: 323-340.

Sichel, G., C. Corsaro, M. Scalia, A.J. Di Bilio and R.P. Bonomo (1991). In vitro scavenger activity of some flavonoids and melanins against O_2 . *Free Rad. Biol. Med*., 11: 1-8.

Tsutomu, H., Y. Taeko, Y. Rieko, I. Yukihiko, M. Muneto, Y. Kazufumi, A. Isao, N. Sansei, N. Tadataka, Y. Masao and O. Takuo (1991). Inhibitory effects of galloylated flavonoids on xanthine oxidase. *Planta Med*., 57: 83-84.

Umpie´rrez A., J. Cuesta-Herranz, M. De Las Heras, M. Lluch-Bernal, E. Figueredo and J. Sastre (1998). Successful desensitization of a fixed drug eruption caused by allopurinol. *J. Allergy Clin. Immunol.*, 101: 286 – 287.

Usia, T., H. Iwataa, A. Hiratsuka, T. Watabe, S. Kadota and Y. Tezuka (2006). CYP3A4 and CYP2D6 inhibitory activities of Indonesian medicinal plants. *Phytomedicine* 13: 67-73.

Vanden Berghe, D. A. R., A. Haemers and A.J. Vlietinck (1993). In: *Bioactive Natural Products: Detection, Isolation and Structural Determination* (Colegate, S. M., Molyneux, R. J., Eds.); CRC Press: London, Chapter 17, pp 405-440.

Wallach, S. L. (1998). The side effects of allopurinol. *Hosp. Pract*., 33: 22.

Yale S. H., E. S. Yale and D. S. Mann (1996). Fever, rash, and angioedema after a course of allopurinol. *Hosp. Pract*., 31: 92–94.

(*Accepted for publication August 2012*)