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ORIGINAL ARTICLE

Inhibition of Cytochrome P450 (CYP3A4) Activity by Extracts from 57 Plants Used in Traditional Chinese Medicine (TCM)

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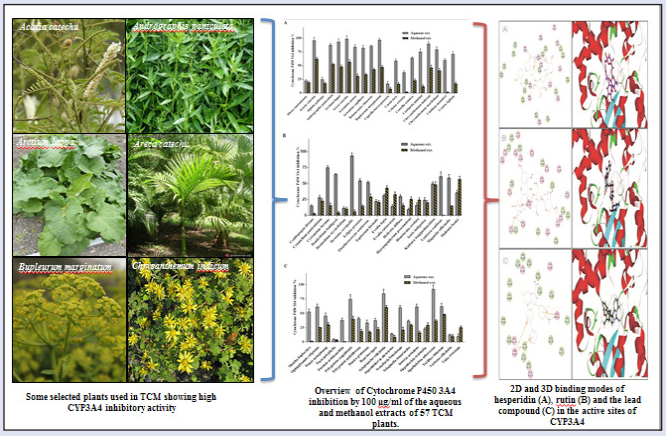
ABSTRACT

Background: Herbal medicine is widely used all over the world for treating various health disorders. It is employed either alone or in combination with synthetic drugs or plants to be more effective. **Objective**: The assessment of the effect of both water and methanol extracts of 57 widely used plants from Traditional Chinese Medicine (TCM) against the main phase I metabolizing enzyme CYP3A4 *in vitro* for the first time. **Materials and Methods:** The inhibition of cytochrome P450 activity was evaluated using a luminescence assay. The principal component analysis (PCA) was used to correlate the inhibitory activity with the main secondary metabolites present in the plant extracts. Molecular modeling studies on CYP3A4 (PDB ID 4NY4) were carried out with 38 major compounds present in the most active plant extracts to validate the observed inhibitory effect. **Results:** Aqueous extracts of *Acacia catechu*, *Andrographis paniculata, Arctium lappa, Areca catechu, Bupleurum marginatum, Chrysanthemum indicum, Dysosma versipellis,* and *Spatholobus suberectus* inhibited CYP3A4 is more than 85% (at a dose of 100 µg/mL). The corresponding methanol extracts of *A. catechu, A. paniculata, A. catechu*, *Mahonia bealei*, and *Sanguisorba officinalis* inhibited the enzyme by more than 50%. Molecular modeling studies revealed that two polyphenols, namely hesperidin and rutin, revealed the highest fitting scores in the active sites of the CYP3A4 with binding energies equal to -74.09 and -71.34 kcal/mol, respectively. **Conclusion:** These results provide evidence that many TCM plants can inhibit CYP3A4, which might cause a potential interference with the metabolism of other concomitantly administered herbs or drugs.

Key words: Cytochrome P450, herbal–drug interaction, principal component analysis (PCA), Traditional Chinese Medicine (TCM), virtual screening

SUMMARY

- In this study, the inhibitory activity of the aqueous and methanol extracts of 57 widely used plants from Traditional Chinese Medicine (TCM) against the main phase I metabolizing enzyme CYP3A4 was tested *in vitro* for the first time.
- Aqueous extracts of *Acacia catechu, Andrographis paniculata, Arctium lappa, Areca catechu, Bupleurum marginatum, Dysosma versipellis*, and *Spatholobus suberectus* inhibited CYP3A4 by more than 85% (at a dose of 100 µg/mL).
- The activity could be attributed to the presence of polyphenolics as revealed from the multivariate chemometric analysis and molecular modeling study.
- These results provide evidence that many TCM plants can inhibit CYP3A4, which might cause a potential interference with the metabolism of other concomitantly administered herbs or drugs.



Abbreviation used: CHARMm: Chemistry at HARvard Macromolecular Mechanics, CYP: Cytochrome P450, DMSO: Dimethyl Sulfoxide, PCA: Principal Component Analysis, PDB: Protein Data Bank, TCM: Traditional Chinese Medicine

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INTRODUCTION

Phytomedicine is increasingly receiving attention from both the public and healthcare professionals.[1] However, possible interaction(s) between herbal preparations and other concomitantly administered synthetic medications may cause very serious medical problems.[2-5] Pharmacokinetic interactions between medicinally active plants and other synthetic drugs may cause a change in liberation, absorption, distribution, metabolism, excretion, or toxicity of a respective drug. Apart from that, plant extracts may cause many pharmacodynamic interactions with the receptors and enzymes leading to enhanced or attenuated pharmacological action of therapeutics, which might cause unwanted effects.[6,7] Therefore, understanding the ability of medicinal plants to modulate the metabolizing enzymes is really crucial for a responsible treatment of patients.[8]

The cytochrome P450 (CYP) enzymes are the main players in Phase I metabolism and also involved in the oxidation and elimination of a wide

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array of xenobiotics (such as drugs and toxins). Drug metabolism involves around 15 different CYP isoforms in which CYP 1A2, 2C9, 2C19, 2D6, 2E1, and 3A4 are the most abundant.[9] CYP3A4 acts on lipophilic substrates and metabolizes about 50% of the drugs in the liver[10] whereas CYP2D6 exhibits a preference for positively charged molecules, usually containing a basic nitrogen. On the other hand, CYP2C9 metabolizes weakly anionic molecules, while CYP1A2 acts on polyaromatic hydrocarbons, and CYP2E1 metabolizes small relatively soluble organic compounds.[11]

CYP3A4 is the most abundant isoform of the human CYP system accounting for approximately 28% of the whole enzyme system.[12,13] The CYP3A4 enzyme usually introduces a hydroxyl or epoxy group to many lipophilic substrates. Then, the hydroxylated xenobiotics become conjugated with glucuronic acid, sulfate, or amino acids. In turn, they become eliminated via the kidney and urine.[14]

A number of therapeutic agents, including natural products, are the main substrates of CYP3A4. Among them, quinidine, vinblastine, ergotamine, berberine, and colchicine are the most known[15] while nifedipine and diazepam figure as common synthetic substrates.[16] Furthermore, CYP3A4 activity can be inhibited by many plant extracts.[17,18] Grapefruit with the furanocoumarin derivatives and flavonoids[19] and kava-kava with its kavalactones[20] are prominent examples for herbal drugs that can cause clinically important modulation in CYP3A4 activity. On the other hand, prolonged use of *Hypericum* extracts, containing hypericin, results in an induction of the enzyme activity.[21-23]

Because the plants used in Traditional Chinese Medicine (TCM) are diverse, our knowledge about the interactions between these herbal drugs and the CYP system is rather limited.[1,24] Therefore, in this communication, we investigated the potential inhibition of CYP3A4 by 57 widely used TCM plants to explore the relevance of this activity with regard to adverse effects. Moreover, statistical analysis using principal component analysis (PCA) was applied to correlate the inhibitory activity with the main compounds present in the plant extracts. Additionally, molecular docking was carried out with 38 major secondary metabolites found in the bioactive plant extracts to validate the inhibition results.

MATERIALS AND METHODS

Plant materials

TCM plants were purchased from Chinese markets. Their identity was ascertained in our laboratory through DNA barcoding technique.[25] Voucher specimens are stored at the Department of Biology, Institute of Pharmacy and Molecular Biotechnology, Heidelberg University under the accession numbers P6835-P6919.

Preparation of the plant extracts

One hundred gram of dried plants were grounded to a fine powder. Plant powders were refluxed with 1 L of either methanol or deionized, distilled water (analytical grade) for 4 h. The methanol (MeOH) extracts were dried over anhydrous Na_2SO_4 and evaporated till dryness under vacuum at 45°C, whereas the water (H_2O) extracts were evaporated directly under the same conditions until dryness. Then, they were lyophilized overnight to ensure optimum dryness. The extracts are kept in tight sealed vials at -20°C away from light until use.

CYP3A4 activity

The CYP3A4 assay kit (P450-Glo™, Promega®, Mannheim, Germany) was used to determine the potential inhibition of recombinant human CYP3A4 enzyme by different plant extracts according to the manufacturer protocol.[26] Briefly, the samples were prepared using dimethyl sulfoxide (DMSO) to give final concentrations of 100, 200, and 500, or 1000 µg/mL where DMSO did not exceed 1% of the solutions. Equal volumes (12.5 µL) of each tested sample and the reaction mixture containing 5 mM luciferin-6'-benzyl ether (CYP3A4 specific substrate) in 100 mM phosphate buffer (pH 7.4) and the enzyme (1 pmol/µL) were incubated at 25°C for 10 min. Then, 25 µL of NADPH regeneration system containing 26 mM NADP+, 66 mM glucose-6-phosphate, 66 mM $MgCl_2$, and 40 U/mL glucose-6-phosphate dehydrogenase in 5 mM citrate buffer (pH 5.5) in 1 M phosphate buffer was added and left for 30 min to activate the enzyme. A luciferin detecting reagent (50 µL) was added to stop the CYP3A4 enzyme activity. The luminescence was detected after 20 min using a Tecan™ SafireII Reader (Tecan™, Crailsheim, Germany). The effects of different extracts were evaluated in triplicate relative to blank control containing 1% DMSO. Ketoconazole (10 µM) was used as a positive control.

Molecular modeling studies

In silico molecular modeling of the major compounds present in the bioactive extracts was carried out using Discovery Studio 2.5 (Accelrys® Inc., San Diego, CA, USA) using C-Docker protocol applying both pH-based as well as rule based ionization methods to simulate the physiological conditions and to evaluate the influence of ionization of various ionizable groups on the behavioral interaction of the secondary metabolites at the active site of the enzyme. The x-ray crystal structure of CYP3A4 (PDB ID 4NY4, 2.95 Å) co-crystalized with its lead compound (L), (8R)-3,3-difluoro-8-[4-fluoro-3-(pyridine-3-yl)phenyl]-8-(4-methoxy-3-methylphenyl)-2,3,4,8-tetrahydroimidazo[1,5-a] pyrimidine- 6-amine), was obtained from the protein data bank (www.pdb.org). The standard protein preparation protocol was applied to construct the structure of the enzymes. This was briefly done by the addition of hydrogen atoms to the enzyme and cleansing of all undesirable interactions. Then, the binding site was determined by detection of the binding mode of bioactive conformation of the lead compound (L) with CYP3A4.[27] The structures of the selected compounds were docked inside the binding site after applying CHARMm as the force field and the binding energies and modes for the selected docking poses were determined as described before.[28]

Statistical analysis

Data were presented as means ± mean standard deviation. One-way analysis of variance (ANOVA) with Tukey's *post hoc* test was used to identify statistically significant ($P < 0.05$) differences between the groups. Analyses were performed with Prism 5.0 software (Graph Pad®, San Diego, USA). Chemometric analysis of the data was performed using unsupervised pattern recognition techniques applying PCA, where a matrix of the total number of samples (57 samples) multiplied by the different tested doses for both water and alcoholic extracts (six variables) was constructed. PCA was performed by Unscrambler® 9.7 (CAMO, AS, Norway).

RESULTS

All TCM extracts inhibited CYP3A4 activity to some degree [Table 1]. Generally, the H_2O extracts were more potent than the MeOH extracts, and their corresponding activities were positively correlated as shown in Figure 1 a-c. Therefore, multivariate analysis was applied to statistically evaluate the significance of inhibition of the CYP enzyme. Moreover, clustering the plants samples based on their activity and their chemical profiling was established. The PCA score plot [Figure 2] resulted in two orthogonal PCs, which explained about 89% of the variance in 180-dimensional space using only the first two components (the first PC accounts for 65% of the total variance followed by the second PC with 24%). PCA score plot classified the samples into five main clusters according to their similarity of cytochrome P450 inhibition utilizing

Table 1: Inhibition of CYP3A4 activity by aqueous and alcoholic extracts from 57 TCM plants

Scientific name (Family)*	H_2O extract			MeOH extract		
	1000 µg/ml	200 µg/ml	100 µg/ml	500 µg/ml	200 µg/ml	100 µg/ml
Abrus cantoniensis (Fabaceae) (AC1)	90.70 ± 8.38	61.35 ± 5.47	22.40 ± 2.65	83.55 ± 3.05	35.02 ± 3.19	19.29 ± 2.78
Acacia catechu (Fabaceae) (AC2)	100.19 ± 10.31	99.25 ± 8.02	96.01 ± 5.92	93.14 ± 7.41	71.55 ± 4.27	62.34 ± 3.40
Alpinia galanga (Zingiberaceae) (AG)	84.21± 4.78	57.34 ± 3.97	24.92 ± 4.19	35.96 ± 5.92	31.37 ± 2.08	17.65 ± 2.56
Andrographis paniculata (Acanthaceae) (AP)	100.10 ± 8.18	92.43 ± 6.40	87.57 ± 3.62	89.83 ± 7.53	69.03 ± 4.02	52.53 ± 2.18
Arctium lappa (Asteraceae) (AL)	99.14 ± 5.21	96.48 ± 5.69	93.47 ± 5.93	85.32 ± 7.73	48.46 ± 3.53	47.92 ± 3.66
Areca catechu (Arecaceae) (AC3)	100.68 ± 9.53	99.74 ± 5.75	98.94 ± 6.24	92.88 ± 6.91	71.48 ± 5.99	56.94 ± 4.35
Artemisia annua (Asteraceae) (AA)	100.44 ± 11.66	96.11 ± 7.31	83.96 ± 4.62	76.99 ± 5.52	35.30 ± 4.12	31.01 ± 3.98
Artemisia capillaris (Asteraceae) (AC4)	99.42 ± 9.70	89.94 ± 8.37	82.36 ± 5.40	86.21 ± 8.94	58.07 ± 3.29	33.95 ± 2.27
Belamcanda chinensis (Iridaceae) (BC)	99.58 ± 9.82	90.76 ± 1.42	85.90 ± 2.05	71.96 ± 4.87	61.07 ± 4.75	42.99 ± 2.55
Bupleurum marginatum (Apiaceae) (BM)	100.36 ± 8.26	98.85 ± 5.85	96.98 ± 3.14	85.37 ± 5.30	73.84 ± 4.01	47.21 ± 3.15
Capsella bursa-pastoris (Brassicaceae) (CB)	98.59 ± 4.24	67.70 ± 4.00	17.13 ± 4.64	85.14 ± 8.83	20.03 ± 3.91	7.35 ± 3.22
Cassia tora (Fabaceae) (CT1)	94.95 ± 7.17	74.52 ± 5.75	58.72 ± 3.70	25.58 ± 3.29	25.81 ± 4.08	16.38 ± 3.08
Celosia cristata (Amaranthaceae) (CC)	47.13 ± 6.42	44.44 ± 6.80	37.38 ± 3.99	25.42 ± 5.26	2.75 ± 1.48	1.79 ± 1.02
Centella asiatica (Apiaceae) (CA)	98.74 ± 8.38	80.92 ± 6.62	64.16 ± 2.40	80.39 ± 5.45	45.74 ± 2.03	22.89 ± 3.22
Centipeda minima (Asteraceae) (CM1)	100.83 ± 6.24	83.40 ± 5.83	75.33 ± 5.71	51.61 ± 4.71	23.46 ± 4.16	12.14 ± 3.05
Chrysanthemum indicum (Asteraceae) (CI)	99.07 ± 9.45	91.34 ± 3.93	89.88 ± 3.91	76.85 ± 4.94	57.49 ± 5.28	46.18 ± 4.99
Chrysanthemum morifolium (Asteraceae) (CM2)	99.65 ± 10.23	87.67 ± 5.56	79.67 ± 5.06	79.25 ± 6.11	58.16 ± 5.45	41.00 ± 4.26
Cnidium monnieri (Apiaceae) (CM3)	99.96 ± 8.12	81.95 ± 8.24	59.79 ± 3.51	83.23 ± 5.96	23.53 ± 3.11	1.51 ± 1.46
Croton tiglium (Euphorbiaceae) (CT2)	99.03 ± 10.88	86.25 ± 7.62	71.14 ± 4.64	36.58 ± 4.70	19.14 ± 3.06	16.86 ± 3.14
Cymbopogon distans (Poaceae) (CD)	94.43 ± 7.56	69.19 ± 6.20	14.72 ± 2.21	13.01 ± 3.78	4.27 ± 2.10	2.71 ± 1.53
Cynanchum paniculatum (Asclepidaceae) (CP)	86.27 ± 8.58	47.55 ± 4.64	27.95 ± 3.90	34.37 ± 4.59	28.70 ± 3.19	22.62 ± 3.62
Cyrtomium fortune (Dryopteridaceae) (CF)	98.81 ± 7.26	97.15 ± 5.42	74.91 ± 3.39	70.31 ± 6.65	27.72 ± 3.73	15.47 ± 4.11
Dendrobium loddigesii (Orchidaceae) (DL)	97.02 ± 4.68	71.48 ± 5.59	64.47 ± 1.40	32.05 ± 4.91	10.39 ± 2.67	4.44 ± 2.37
Desmodium styracifolium (Fabaceae) (DS)	69.16 ± 6.46	42.27 ± 3.37	11.16 ± 2.78	54.70 ± 3.89	20.04 ± 4.61	10.22 ± 3.12
Dysosma versipellis (Berberidaceae) (DV)	98.11 ± 5.85	95.49 ± 7.61	93.49 ± 4.60	19.92 ± 4.77	13.04 ± 2.82	5.97 ± 2.88
Eclipta prostata (Asteraceae) (EP)	96.93 ± 5.01	70.07 ± 7.86	54.43 ± 3.51	40.96 ± 5.61	18.89 ± 2.69	14.49 ± 2.02
Eleutherococcus senticosus (Araliaceae) (ES)	91.61 ± 7.71	72.71 ± 5.68	51.73 ± 2.30	62.99 ± 7.29	34.77 ± 3.90	29.03 ± 4.51
Equisetum hiemale (Equisetaceae) (EH)	73.77 ± 8.74	16.75 ± 6.85	21.99 ± 3.11	65.56 ± 6.31	30.23 ± 3.27	20.49 ± 3.09
Evodia lepta (Rutaceae) (EL)	91.49 ± 4.34	50.15 ± 6.45	31.27 ± 2.48	70.33 ± 6.25	52.48 ± 3.32	42.95 ± 3.82
Evodia rutaecarpa (Rutaceae) (ER)	87.83 ± 5.18	56.70 ± 5.88	14.27 ± 3.07	63.82 ± 5.28	40.18 ± 5.76	32.85 ± 4.25
Hedyotis diffusa (Rubiaceae) (HD)	91.29 ± 3.45	69.61 ± 6.06	29.66 ± 2.88	46.04 ± 5.39	36.80 ± 4.08	15.56 ± 3.13
Harpagophytum procumbens (Pedaliaceae) (HP)	80.58 ± 7.31	28.37 ± 8.50	9.29 ± 2.34	23.83 ± 4.41	21.56 ± 3.22	25.49 ± 3.91
Houttuynia cordata (Saururaceae) (HC)	60.29 ± 4.80	24.79 ± 4.85	15.76 ± 3.94	61.79 ± 4.35	28.07 ± 4.58	24.26 ± 3.69
Isatis indigotica (Brassicaceae) (II)	58.38 ± 8.56	30.02 ± 6.40	23.96 ± 4.41	60.93 ± 3.51	29.35 ± 2.01	19.99 ± 3.21
Kadsura longipedunculata (Schisandraceae) (KL)	96.56 ± 7.88	69.51 ± 6.91	49.55 ± 3.16	82.12 ± 6.09	59.23 ± 4.21	48.30 ± 4.70
Lonicera confusa (Caprifoliaceae) (LC)	95.77 ± 9.12	79.62 ± 9.86	60.91 ± 7.46	37.31 ± 3.34	17.98 ± 1.09	0.44 ± 0.27
Magnolia officinalis (Magnoliaceae) (MO)	88.79 ± 7.45	74.96 ± 6.43	58.68 ± 5.96	50.51 ± 3.88	29.25 ± 3.15	14.85 ± 0.62
Mahonia bealei (Berberidaceae) (MB)	69.56 ± 12.05	50.33 ± 4.22	35.54 ± 4.31	90.04 ± 8.24	72.43 ± 5.32	56.72 ± 4.93
Mentha haplocalyx (Lamiaceae) (MH)	96.56 ± 2.88	76.20 ± 6.26	52.15 ± 4.91	38.13 ± 3.93	17.27 ± 1.12	1.19 ± 0.37
Ophioglossum vulgatum (Ophioglossaceae) (OV)	96.71 ± 6.15	60.72 ± 7.62	60.86 ± 5.09	82.64 ± 3.09	37.85 ± 2.23	24.91 ± 0.54
Panax notoginseng (Araliaceae) (PN)	94.37 ± 2.37	67.68 ± 2.19	44.91 ± 5.41	37.71 ± 4.06	35.97 ± 3.20	29.83 ± 3.86
Paris polyphylla (Melanthiaceae) (PP)	70.76 ± 5.63	19.45 ± 4.37	4.08 ± 2.19	42.72 ± 2.55	14.41 ± 3.11	2.92 ± 1.18
Patrinia scabiosaefolia (Valerianaceae) (PS)	99.77 ± 4.01	69.41 ± 3.22	37.78 ± 3.69	10.78 ± 4.19	0.36 ± 0.65	0.61 ± 0.04
Polygonum cuspidatum (Polygonaceae) (PC)	95.94 ± 5.28	85.73 ± 4.08	74.20 ± 8.05	85.61 ± 6.04	62.24 ± 2.78	39.81 ± 4.68
Polygonum multiflorum (Polygonaceae) (PM)	94.22 ± 7.66	79.15 ± 5.98	40.41 ± 2.49	80.72 ± 5.07	33.25 ± 1.21	18.02 ± 3.41
Punica granatum (Rosaceae) (PG)	97.91 ± 9.43	68.52 ± 3.42	33.48 ± 4.75	72.82 ± 2.36	29.88 ± 4.88	16.76 ± 2.18
Rosa laevigata (Rosaceae) (RL)	96.12 ± 7.48	82.05 ± 4.92	37.37 ± 3.53	40.90 ± 2.10	38.36 ± 7.29	21.52 ± 3.73
Sanguisorba officinalis (Rosaceae) (SO)	97.99 ± 11.76	91.30 ± 7.19	83.94 ± 7.95	89.67 ± 4.14	73.83 ± 3.18	59.96 ± 3.52
Saposhnikovia divaricata (Apiaceae) (SD)	86.58 ± 9.84	47.51 ± 3.84	13.69 ± 1.51	34.72 ± 5.02	17.89 ± 3.67	8.21 ± 3.14
Scutellaria baicalensis (Lamiaceae) (SB)	95.61 ± 8.58	84.42 ± 5.99	59.43 ± 3.90	54.01 ± 3.09	30.39 ± 4.92	20.55 ± 4.91
Selaginella tamariscina (Selaginellaceae) (ST)	86.92 ± 11.38	51.42 ± 2.60	36.23 ± 2.13	41.58 ± 5.06	28.29 ± 3.27	28.89 ± 1.93
Senecio scandens (Asteraceae) (SS1)	94.42 ± 9.60	77.90 ± 1.01	61.07 ± 4.98	56.31 ± 3.66	24.58 ± 4.63	15.80 ± 3.14
Siegesbeckia orientalis (Asteraceae) (SO)	64.03 ± 8.55	35.13 ± 4.62	22.79 ± 2.17	65.09 ± 2.31	30.37 ± 2.52	28.76 ± 4.82
Spatholobus suberectus (Fabaceae) (SS2)	97.29 ± 12.98	95.76 ± 4.46	91.50 ± 7.65	78.95 ± 6.09	50.55 ± 3.90	36.42 ± 2.13
Taxillus chinensis (Loranthaceae) (TC)	99.58 ± 10.64	79.40 ± 5.12	61.50 ± 5.69	64.60 ± 2.16	42.54 ± 4.04	47.41 ± 1.07
Verbena officinalis (Verbenaceae) (VO)	88.05 ± 9.19	26.46 ± 3.82	11.85 ± 2.10	39.13 ± 2.39	25.00 ± 1.24	9.54 ± 3.26
Viola yezoensis (Violaceae) (VY)	37.28 ± 7.18	21.92 ± 8.04	9.29 ± 4.67	71.90 ± 2.21	36.62 ± 1.38	24.97 ± 2.75

*The code assigned for each plant on the chemometric analysis. Data are presented as mean ± SD of three replicate.

Table 2: Major secondary metabolites present in the plants producing an inhibition of the CYP 3A4 activity at 100 µg/mL higher than 50%

Name/Family	Code/ Part used	Compounds	Ref.
Acacia catechu (Fabaceae)	P6836/Resin	**Monomeric and polymeric flavonoid derivatives:** catechin, epicatechin, epicatechin gallate, procatechinic acid, quercetin and kaempferol.	[38]
Andrographis paniculata (Acanthaceae)	P6838/ Herb	**Diterpenolactones:** andrographolide, 14-deoxy-11-dehydroandrographolide, 14-deoxy-11-oxoandrographolide, 5-hydroxy-7,8,2',3'-tetramethoxyflavone, neoandrographolide, paniculide-A, paniculide-B and paniculide-C.	[39, 40]
Arctium lappa (Asteraceae)	P6839/Seeds	**Lignans:** Lappaol F, diarctigenin and arctigenin	[41]
Areca catechu (Arecaceae)	P6840/ Seeds	**Alkaloids:** arecoline, arecaidine, arecolidine, guvacine, guvacoline, isoguvacine, norarecaidine and norarecoline.	[42]
Artemisia annua (Asteraceae)	P6841/ Herb	**Sesquiterpene lactone:** Artemisinin, deoxyartemisinin, artemisinic acid, arteannuin-B, stigmasterol, friedelin, friedelan-3 beta-ol, artemetin, and quercetagetin 6,7,3',4'-tetramethyl ether.	[43, 44]
Artemisia capillaris (Asteraceae)	P6842/ Herb	**Flavone:** 5, 2',4'-trihydroxy 6,7,5'-trimethoxyflavone. **Phenylalkynes**: capillaridins A-H, capillin, capillene and *O*-methoxycapillene, 6'-*O*-caffeoyl-*p*-hydroxyacetophenone-4-*O*-*β*-D-glucopyranoside and 6-amino-9-[1-(3,4-dihydroxyphenyl) ethyl]-9*H*-purine. **Coumarins:** coumarin, scopoletin	[45, 46]
Belamcanda chinensis (Iridaceae)	P6843/Rhizome	**Phenolic compounds:** Belalloside A, belalloside B, belamphenone, resveratrol, iriflophenone, irisflorentin, tectorigenin, irilin D, tectoridin, iristectorin A, iristectorin B, hispiduloside, androsin, irigenin, iridin, and jaceoside,	[47]
Bupleurum marginatum (Apiaceae)	P6845/ Herb	**Flavonoids and steroids:** Rutin, isoquercetrin, isorhamnetin, quercetin, *β*-sitosterol, α-spinasterol, daucosterol, α-spinasterol glucoside.	[34]
Cassia tora (Fabaceae)	P6847/ Seeds	**Anthraquinones and anthraquinones derivatives:** Chrysophanol, naphtho-α–pyrone-toralactune, physcion, rubrofusarin, chrysophonic acid -9 –anthrone, dianthrone glycosides (sennoside A, B).	[48, 49]
Centella asiatica (Apiaceae)	P6849/ Herb	**Triterpenes**: madecassic acid, brahmic acid and asiatic acid. Triterpenoid ether glycosides: madecassoside, asiaticoside, brahmoside and brahminoside.	[50]
Centipeda minima (Asteraceae)	P6850/ Herb	**Sesquiterpene lactones**: 6-*O*-methylacrylylplenolin, 6-*O*-isobutyroylplenolin, and 6-*O*-angeloylplenolin, senecioylplenolin, aurantiamide acetate, tetrahydrohelenalin. **Flavonoids:** quercetin-3-methyl ether, quercetin-3,3'-dimethyl-ether, quercetin-3,7,3'-trimethyl-ether, quercetin-3,7,3',4'-tetramethyl-ether and α-cyperone.	[51]
Chrysanthemum indicum (Asteraceae)	P6851/ Flowers	**Flavonoids**: (2*S*)-eriodictyol 7-*O*-*β* -D-glucopyranosiduronic acid, (2*R*)-eriodictyol 7-*O*-*β* -D-glucopyranosiduronic acid, (2*S*,3*S*)-1-phenyl-2,3-butanediol 3-*O*-*β*-D-glucopyranoside, apigenin 7-*O*-*β* -D-glucopyranoside, diosmetin 7-*O*- *β* -D-glucopyranoside, quercetin 3,7-di-*O*-*β*-D-glucopyranoside,, luteolin 7-*O*- *β* -D-glucopyranoside, luteolin 7-*O*-*β*-D-glucopyranosiduronic acid, acacetin 7-*O*-α-L-rhamnopyranosyl)-*β*-D-glucopyranoside and eupatilin.	[52]
Chrysanthemum morifolium (Asteraceae)	P6852/ Flowers	**Triterpene alcohol:** pentacyclic triterpene diols and triols: faradiol, heliantriol B, heliantriol C, 22-alpha-methoxyfaradiol, arnidiol, and faradiol alpha-epoxide, maniladiol, erythrodiol, longispinogenin, coflodiol, heliantriol A, brein and uvaol, calenduladiol and heliantriol B. **Phenolic compounds:** luteolin-7- *O*-β-glucoside, quercitrin. chlorogenic acid, 3,5-*O*-caffeoylquinic acid, galuteolin, acacetin-7-*O*-β-d-glucopyranoside, acacetin-7-*O*-α-l-rhamopyranoside, eriodictyol, eriodictyol 7-*O*-glucuronide, arabinogalactan, vitexin-2-O-rhamnoside, quercetin-3-galactoside, luteolin-7-*O*-β- glucuronide, diosmetin, acacetin, and apigenin. **Sesquiterpenes:** chrysanthediol A, chrysanthediacetate B and chrysanthediacetate C and *β*-dictyopterol, chrysandiol, chrysartemins A and B, and chlorochrymorin.	[53, 54]
Cnidium monnieri (Apiaceae)	P6854/ Seeds	**Bitter principles:** imperatorin, xanthotoxin, isopimpinellin, bergapten, osthole, and daucosterol	[55]
Croton tiglium (Euphorbiaceae)	P6856/ Seeds	**Phorbol esters of the tigliane type:** 12-*O*-tetradecanoylphorbol-13-acetate, 13-*O*-acetylphorbol-20-linoleate, 13-*O*-tigloylphorbol-20-linoleate, 12-*O*acetylphorbol-13-tigliate, 12-*O*-decanoylphorbol-13-(2-methylbutyrate), 12-*O*-tigloylphorbol-13-(2-methylbutirate) and 12-*O*-acetylphorbol-13-decanoate, and 12-*O*-(2-methylbutiroyl)-phorbol-13-decanoate.	[56]
Cyrtomium fortune (Dryopteridaceae)	P6859/ Rhizome	**Phenolic compounds:** 6'-methylglucuronide-5-hydroxy-chromone, ethyl α-D-glactopyranoside, neoechinulin A, 9,12,13-trihydroxyoctadeca-10(*E*),15(*Z*)-dienoic acid and phellopterin.	[57]
Dendrobium loddigesii (Orchidaceae)	P6860/ Herb	**Alkaloids:** dendrobine, nobiline, shihunidine, shihunine, moscatilin. **Phenolic compounds:** Loddigesiinols G–J, crepidatuol B.	[58]
Dysosma versipellis (Berberidaceae)	P6862/ Rhizome	**Lignans:** podophyllotoxin **Flavonoids:** quercetin, maohuoside B, epimedin A,	[59]
Eclipta prostrata (Asteraceae)	P6863/Herb	**Triterpenes, terthiophene, coumarins and isoflavone derivatives** 5-Hydroxymethyl-(2,2':5',2")-terthienyl tiglate, 5-hydroxymethyl-(2,2':5',2")-terthienyl agelate, 5-hydroxymethyl-(2,2':5',2")-terthienyl acetate, ecliptal, orobol, wedelolactone, demethylwedelolactone, isodemethylwedelolactone, alpha-formylterthienyl, strychnolactone, *β*-sitosterol, nonacosanol, stearic acid, lacceroic acid, 3,4-dihydoxy benzoic acid, eclalbasaponins II, I, III, XI and XII.	[60, 61]

Eleutherococcus senticosus (Araliaceae)	P6919/Roots	**Glycans:** Eleutherans A, B, C, D, E, F, and G, eleutheroside C **Lignans and their glycosides:** sesamine, eleutheroside D **Triterpene saponins:** eleutheroside I, K, L, and M **Steroid glycosides:** eleutheroside A **Hydroxycoumarins:** isofraxidin **Phenylacrylic acid derivatives:** eleutheroside B, E and E1. Sinapaldehyde glucoside, coniferaldehyde glucoside, coniferin, 1,5-di-*O*-caffeoylquinic acid, 3',5'-*O*-dicaffeoylquinic acid, 4',5'-*O*-dicaffeoylquinic acid eleutheroside E2, isomaltol 3-*O*-α-glucopyranoside.	[62]
Lonicera confusa (Caprifoliaceae)	P6880/ Wood	**Flavonoids:** Rutin, quercetin, luteolin -7-O-*β*-D-galactoside, lonicerin, tricin, tricin-7-O-β-D-glucopyranoside, chrysoeirol-7-O-neohesperidoside, and tricin-7-O-neohesperidoside. β-sitosterol and tetratriacontane **Phenolic acids:** Chlorogenic acid, 3,5-dicaffeoylquinic acid and caffeic acid.	[63]
Magnolia officinalis (Magnoliaceae)	P6882/ Bark	**Tannins and flavonoids:** gallic acid, sennosides A and B, hesperidin, naringin, rutin, syringin, magnolol and honokiol.	[64]
Mahonia bealei (Berberidaceae)	P6883/ Wood	**Alkaloids:** Berberine and other isoquinoline alkaloids	[65]
Mentha haplocalyx (Lamiaceae)	P6884/ Herb	**Miscellaneous compounds:** Triterpene saponins, flavonoids, rutin, tannins, monoterpenes (menthol). benzoic acid, trans-cinnamic acid, *β*-sitosterol, ursolic acid and daucosterol.	[66, 67]
Ophioglossum vulgatum (Ophioglossaceae)	P6885/ Herb	**Flavonoids:** Quercetin-3-O-[(6-caffeoyl)-β-glucopyranosyl(1→3) α-rhamnopyranoside]-7-O-α-rhamnopyranoside, kaempferol-3-O-[(6-caffeoyl)-β-glucopyranosyl (1→3)-α-rhamnopyranoside]-7-O-α-rhamnopyranoside, quercetin-3-O-methyl ether, 3-O-methylquercetin-7-O-diglycoside 4'-O-glycoside and ophioglonin	[68]
Polygonum cuspidatum (Polygonaceae)	P6894/ Rhizome	**Anthraquinones:** piceid, anthraglycoside A, anthraglycoside B, emodin, physcion, rhein, and chrysophanol. **Stilbenes:** resveratrol and polydatin.	[69, 70]
Sanguisorba officinalis (Rosaceae)	P6901/ Rhizome	**Hydrolysable and condensed tannins mostly derivatives of gallic and ellagic acids:** methyl 4-*O*-β-D-glucopyranosy-5-hydroxy-3-methoxylbenzoate, 3,3',4'-tri-*O*-methylellagic acid, fisetinidol-(4α-8)-catechin, and (+)-catechin. **Flavonoids:** rutoside, quercetin and kaempferol.	[71]
Scutellaria baicalensis (Lamiaceae)	P6903/Wood	**Flavonoids, iridoid glucosides:** baicalein, wogonin and oroxylin.	[72]
Senecio scandens (Asteraceae)	P6905/ Herb	**Pyrrolizidine alkaloids, terpenoids:** lupenone, oleanane, β-sitosterol, daucosterol, adonifoline, phydroxy benzeneacetic acid, 2-(1,4-dihydroxy-cyclohexanyl) -acetic acid, hyperoside, linarin.	[73]
Spatholobus suberectus (Fabaceae)	P6907/Bark	**Flavonoids:** 3',4',7-trihydroxyflavone, eriodictyol, plathymenin, dihydroquercetin, butin, neoisoliquiritigenin, dihydrokaempferol, liquiritigenin, and 6-methoxyeriodictyol. Ononin, pruneitin, gallocatechin, catechin, epicatechin, syringic acid, vanillic acid and daucosterol.	[74]
Taxillus chinensis (Loranthaceae)	P6909/Herb	**Flavonoids:** avicularin, quercetin, hyperin, d-catechol and quercitrin	[75]

all the tested doses as different variables. Cluster I included samples were the strongest CYP inhibitors. This cluster comprises *Acacia catechu*, *Andrographis paniculata*, *Arctium lappa*, *Artemisia annua*, *Artemisia capillaris*, *Belamcanda chinensis*, *Bupleurum marginatum*, *Chrysanthemum indicum*, *Chrysanthemum morifolium*, *Polygonum cuspidatum*, *Sanguisorba officinalis*, and *Spatholobus suberectus*. These samples mostly clustered in the right quadrant. Cluster II was also found in the same right quadrant exhibiting a moderate CYP inhibition (both the water and alcoholic extracts). The left side of the PCA plot contained all samples with an inhibition lower than approximately 50% at the lowest tested dose. They were subdivided into three main clusters (cluster III, IV, and V).

From the results displayed in Table 1 and Table 2, and the multivariate analysis, it is obvious that the majority of secondary metabolites reported in both chemical abstracts and Medline for the corresponding bioactive extracts are phenolic secondary metabolites [Figure 3]. These compounds were docked on CYP3A4 to validate the observed biological activity [Table 3]. The results revealed that two flavonoids namely hesperidin and rutin were the most potent CYP3A4 inhibitors as evidenced from their high fitting scores and consequently, higher stability within the active sites as compared with the lead compound. The binding energies were -74.09, -71.34, and -47.08 kcal/mol for hesperidin, rutin, and original lead compound (L) for the pH-based ionization mode, respectively. We should mention that the binding mode of the lead compound L revealed formation of one hydrogen or ionic bond with the residue Arg 212 of the CYP3A4, in addition to the formation of three π bonds, two of them with the amino acid residue Arg 105 while the third with Arg 212. Whereas for hesperidin six hydrogen or ionic bonds and a π bond were detected, two hydrogen bonds and a π bond are formed with the residue Arg 105, one hydrogen bond with each of Arg 375, Asn 441, Cys 442, and Pro 434. Moreover, rutin forms nine hydrogen or ionic bonds, three of them with the residue Arg 105, two with Glu 374, and a hydrogen bond with each of Arg 375, Asn 441, Gly 481, and Ile 443 in addition to the formation of a π bond with the amino acid residue Arg 105 [Figure 4]. Thus, the formation of extra hydrogen bonds or ionic bonds (if the phenolic OH-groups is dissociated) with the amino acid residues at the active sites of the enzyme is responsible for the comparative firm binding of the two flavonoids with respect to the lead compound. This was revealed from the results of the molecular docking using the rule-based ionization mode [Table 3] that examine the influence of ionization of various functional groups on its interaction at the binding site.

Table 3: *In silico* molecular modeling of some selected major compounds from our TCM plants on CYP3A4 applying both pH and rule based ionization modes

Compound	Binding energy (Kcal/mol)	
	pH-based	Rule-based
Hesperidin	-74.09	-63.90
Rutin	-71.34	-71.34
Asiaticoside	-70.07	-87.72
12-*O*-tetradecanoylphorbol-13-acetate	-69.48	-71.60
Lonicerin	-69.14	-69.14
Fisetinidol-(4α-8)-catechin	-63.92	-66.76
Belalloside A	-58.33	-58.33
Loddigesiinol G	-57.45	-59.62
Luteolin-7-O-*β*-glucoside	-53.00	-59.18
Podophyllotoxin	-49.46	-49.47
Piceid	-49.40	-49.40
Linarin	-48.90	-45.91
Arctigenin	-48.27	-46.51
Avicularin	-47.80	-48.43
(Ligand: L)	-47.08	-46.51
Neoisoliquiritigenin	-47.07	-48.96
Heliantriol	-44.34	-44.33
Oleanolic acid	-43.18	-51.73
Andrographolide	-43.06	-40.68
Capillaridin A	-42.90	-42.91
Ophioglonin	-41.86	-41.86
Eleutheroside B	-41.64	-43.27
Ursolic acid	-41.60	-52.43
Catechin	-39.38	-41.85
6-*O*-Angeloylplenolin	-39.26	-39.26
Berberine	-38.04	-38.04
Quercetin	-37.89	-37.89
3,3',4'-tri-*O*-methylellagic acid	-37.60	-37.60
Wedelolactone	-36.20	-31.83
Neoechinulin A	-33.60	-33.60
Physcione	-32.93	-32.93
Baicalein	-31.79	-31.79
Chrysophanol	-29.12	-29.12
Artemisinin	-29.11	-29.11
Dendrobine	-28.72	-26.65
Scopoletin	-26.14	-26.13
Arecoline	-24.90	-24.89
Chrysanthediol B	-23.63	-23.63

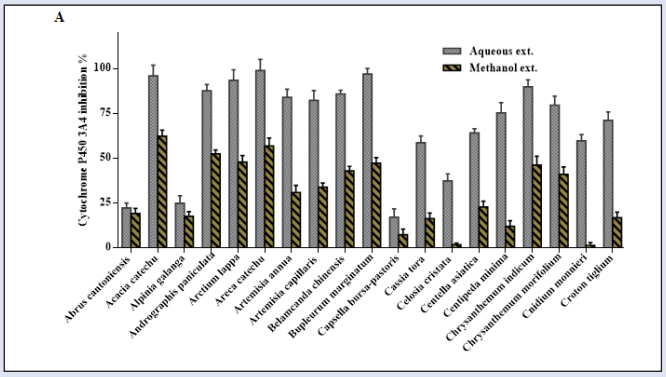

Figure 1 (a): Overview of Cytochrome P450 3A4 inhibition by 100 µg/mL of the aqueous and methanol extracts of 57 TCM plants.

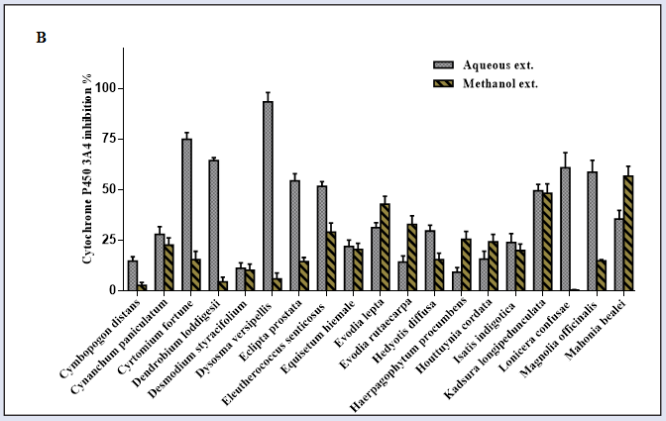

Figure 1 (b): Overview of Cytochrome P450 3A4 inhibition by 100 µg/mL of the aqueous and methanol extracts of 57 TCM plants.

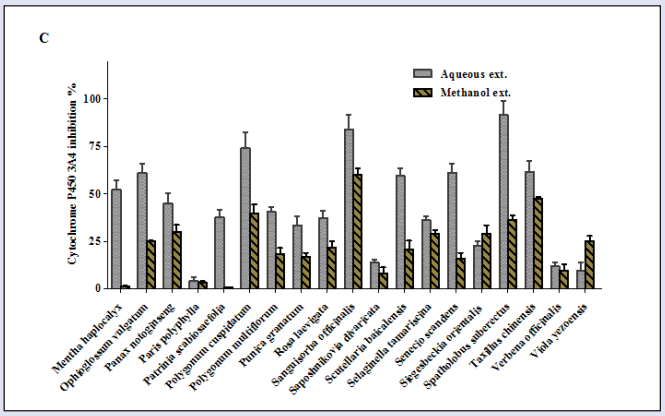

Figure 1 (c): Overview of Cytochrome P450 3A4 inhibition by 100 µg/mL of the aqueous and methanol extracts of 57 TCM plants.

DISCUSSION

The notable CYP3A4 inhibitory activity of many of the tested plants could be interpreted in virtue of their secondary metabolites mainly because of polyphenolic class of compounds. One of the underlying causes of this potent efficacy could be attributed to the formation of hydrogen and ionic bonds at the active sites of an enzyme due to the existence of several reactive phenolic OH groups. The phenolic hydroxyl groups can partly dissociate under physiological conditions resulting in O^- ions interacting with positively charged amino groups, such as in arginine, lysine, and histidine. The charged and polar polyphenols interact with proteins by forming ionic bonds in addition to hydrogen bonds with several amino acids at the active site which might lead to enzyme inhibition and loss of function.[29]

In the same context, extracts from the heartwood of *A. catechu* (Fabaceae) exhibited a potent CYP3A4 inhibition that could be attributed to the high contents of catechins and epicatechins (≈50% of the content) that were previously reported to inhibit CYP3A4.[30,31] Additionally, the activity of *A. catechu* (Arecaceae) could be ascribed to their contents of tannins with their polyphenolic structures especially arecatannin A_1–A_3 which are abundant in both extracts; as polyphenols they are able to bind the enzyme directly. Moreover, the alkaloid content with mainly arecoline (in the methanol extract) could contribute to the overall activity although this action has not been described for CYP3A4, but arecoline significantly inhibits other forms of cytochrome especially CYP1A1.[32]

Besides, a plethora of secondary metabolites with exocyclic methylene groups as sesquiterpene lactones or reactive double or triple bonds can covalently bind to proteins with SH groups. These protein modification

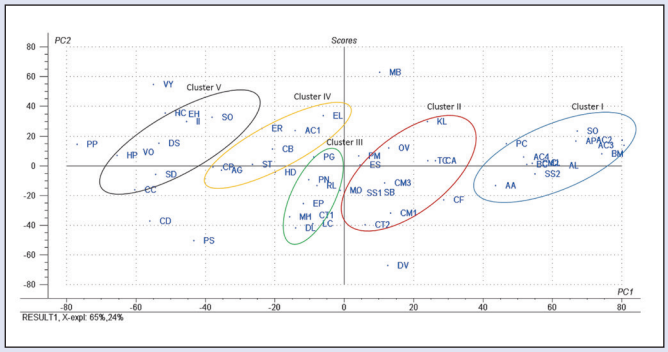

Figure 2: PCA score plot of the aqueous and methanol extracts of 57 tested plants; the codes used are those listed in [Table 1]

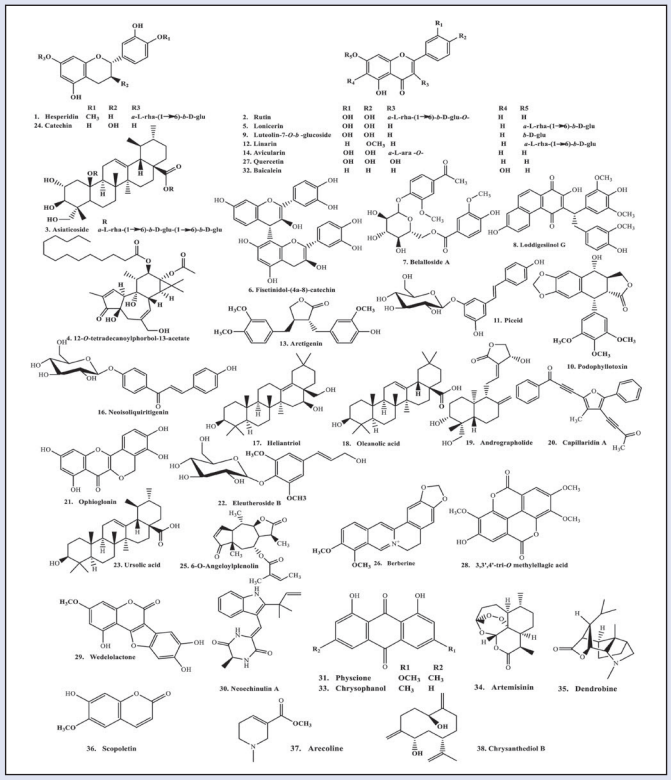

Figure 3: Representative structures of the major CYP 3A4 inhibitors.

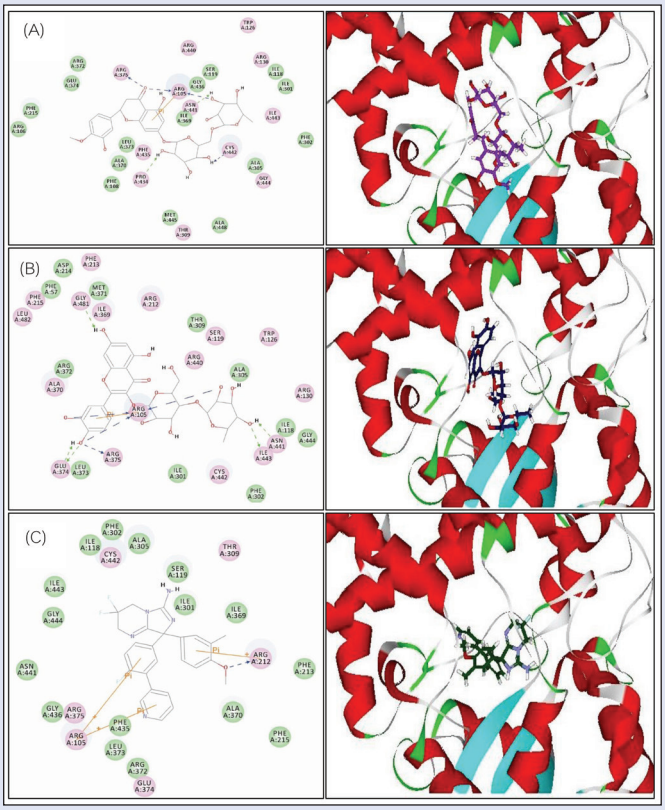

Figure 4: 2D and 3D binding modes of hesperidin (A), rutin (B), and the lead compound (C) in the active sites of CYP3A4 using pH-based ionization mode.

greatly affect the three dimensional structure of proteins with inhibition of their activity and function.[29] *A. paniculata* and *C. morifolium* showed notable inhibition to CYP3A4 that could be due to the presence of certain sesquiterpenes with exocyclic methylene groups as andrographolide and chrysanthediol B, respectively that exerted reasonable fitting scores in the docking experiment with binding energy equals to -43.06 for the first and -23.63 kcal/mol for the latter in addition to the presence of several phenolic compounds in the tested extracts.

The inhibitory potency of *A. lappa* (Asteraceae) is apparently due to its content of lignans, sesquiterpenes, polyacetylenes, and sulfur containing compounds, which are known inhibitors of many cytochromes.[33] The difference in *B. marginatum* extracts activity could be attributed to the presence of large quantities of flavonoids, mainly rutin, isoquercetrin, quercetin, and isorhamnetin.[34] However, the high solubility of these flavonoids, mainly rutin, in water explains the higher activity of water rather than the alcoholic extracts. Besides, the presence of lignans with a methylenedioxy group in an aromatic ring which are known CYP inhibitors[29] and saikosaponins, which showed a significant inhibition of both CYP1A2 and CYP3A4 may have significantly boosted the efficacy.[35]

An additional representative empathizing on the difference between the potency of water and methanol extracts is *M. bealei* (Berberidaceae) where the alcoholic extract exerted higher inhibitory activity than the aqueous one. This notable activity could be explained in view of its richness of alkaloids as berberine which carries a methlenedioxy group-a known inhibitor of CYP1A1 and a substrate for the CYP2C9 and CYP2D6.[36,37] However, the presence of few polyhydroxylated flavonoids could explain the moderate activity of the aqueous extract.

CONCLUSIONS

In this study, the effects of 57 widely used TCM plants on the key metabolizing enzyme CYP3A4 were assessed to understand the potential adverse effects that might occur by concomitant administration of many herbal preparations with other drugs. In addition, plant secondary metabolites can also inhibit or stimulate the expression of CYP genes. Since the aqueous extracts of many medicinal plants inhibit the activity of cytochrome p450 enzymes *in vitro*, as a consequence many adverse interactions can be expected. Furthermore, *in vitro* and *in vivo* studies are required on other cytochrome P450 isoforms to help patients who rely on phytomedicine beside other western medicine in treatment avoiding hazards that might be serious.

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Conflicts of interest

There are no conflicts of interest

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