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Original Article

In vitro and *in silico* anti-oxidant, cytotoxicity and biological activities of *Ficus benghalensis* and *Duranta repens*

Pukar Khanal *, Basanagouda M. Patil *

Department of Pharmacology and Toxicology, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research (KAHER), Belagavi 590010, India

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ABSTRACT

Objective: To report *in vitro* anti-oxidant activity and cytotoxicity of hydroalcoholic extract of *Ficus benghalensis* (bark) and *Duranta repens* (whole plant), and present the probable biological spectrum of major anti-oxidants from both plants.

Methods: The coarse powder of both plants was first extracted with 70% ethanol (maceration) followed by 99% ethanol (Soxhlet-extraction). Anti-oxidant activity of the extracts was evaluated using DPPH, H_2O_2, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), NO scavenging assay, total antioxidant capacity, cupric reducing antioxidant capacity (CUPRAC), and metal chelating assay. Cytotoxicity of both extracts was evaluated using MTT assay in both tumor and normal cell lines i.e. Chinese hamster ovary cells (CHO) and A549 cells. Biological activity of individual anti-oxidants from both medicinal plants was identified using prediction of activity spectra for substances and a docking study was performed by using autodock4.0.

Results: Hydroalcoholic extract of *F. benghalensis* and *D. repens* showed the highest free radical scavenging (ABTS) and chelating capacity respectively. Both extracts showed minimum cytotoxicity in normal cell lines compared to tumor cell lines. Computer imitation hits reflected the multiple biological activities agreeing with the folk use and some scientific reports. Further, we found the binding affinity of predicted anti-oxidant compounds with multiple protein molecules involved in oxidative stress.

Conclusion: The present study reports the probable anti-oxidant mechanism for two folk agents and also presents probable pharmacological activities via computer simulations.

1. Introduction

Traditional medicinal plants have high medicinal values due to preventive and curative properties in various diseases (Desai et al., 2008). Extensive research has been made for identifying the secondary metabolites from traditional folk medicines which exert the beneficial effects on human health. The majorities of the secondary metabolites (phytoconstituents) from folk medicines are anti-oxidants and may possess some pharmacological activities that could add beneficial effects to human health in the management of pathogenic conditions (Saso & Firuzi, 2014).

Ficus benghalensis L. belonging to family Moraceae is commonly recognized as "*banyan*", which is an Indian native tree and has been extensively investigated for managing endocrine (diabetes), respiratory, stress, and gastrointestinal disorders (Singh et al., 2009; Taur et al., 2007). *Duranta repens* L. belonging to family Verbenaceae is a flowering shrub native from Mexico to South America, which is widely grown throughout the world including South Asia. Ethinopharmacologically, the plant is used by people in the South-Eastern part of Nigeria to treat abscesses, malaria and as a vermifuge (Udobi et al., 2018). Similarly, multiple alpha-glucosidase inhibitors (Iqbal et al., 2004; Khanal & Patil, 2020), anti-plasmodial (Ijaz, Ahmad, Ahmad, ulHaq, & Wang, 2010), mosquitocidal (Udobi et al., 2018), and cytotoxic (Nikkon et al., 2008) compounds have been reported from different parts of *D. repens*.

Moreover, *F. benghalensis* and *D. repens* possess anti-oxidant properties; *F. benghalensis* has been reported to increase the anti-oxidant biomarkers i.e. catalase, reduced glutathione, and superoxide dismutase levels in pathogenic conditions (Shukla et al., 2004) and *D. repens* for its potential free radical scavenging capacity (Shahat et al., 2005). Further, it is to be understood that, activation of oxidative stress is a complex system (involves multiple proteins/pathways and varies based on the disease pathogenesis) and identification of probable anti-oxidant mechanism is quite

* Corresponding authors.
E-mail addresses: pukarkhanal58@gmail.com (P. Khanal), drbmpatil@klepharm.edu (B.M. Patil).

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complicated. However, simultaneous utilization of both computer simulations and wet lab experiments could solve these difficulties.

The present study reports the probable anti-oxidant mechanism of two folk medicines i.e. *F. benghalensis* and *D. repens* using both *in silico* and *in vitro* approach. Since these plants have diverse medicinal values, we aimed to investigate the anti-oxidant efficacy of hydroalcoholic extract of *F. benghalensis* (bark) and *D. repens* (whole plant) in various *in vitro* models, cytotoxicity potency and also predicted the probable biological activity of anti-oxidants from both sources.

2. Materials and methods

2.1. Plant collection and authentication

Wild grown *D. repens* (whole plant) and *F. benghalensis* (barks) were collected from local areas of Belagavi, India. The collected plants were authenticated by a botanist in the Indian Council of Medical Research-National Institute of Traditional Medicine (ICMR-NITM), Belagavi, and the herbarium was deposited for the same (accession number: RMRC-1405 and 1406) for future reference.

2.2. Preparation of hydroalcoholic extract

The collected plants were washed under running water, shade dried and turned into a coarse powder. The hydroalcoholic extract of both plants was prepared as explained by Cos et al. (2006). Briefly, the coarse powder was macerated with 70% ethanol with occasional shaking for 7 d followed by filtration; marc was then subjected for soxhlet extraction using 95% ethanol. Later, both the filtrates were combined, concentrated under rotatory evaporator (IKA RV 10), and reduced pressure which yield 10.92% and 38.52% for *F. benghalensis* and *D. repens* respectively.

2.3. Qualitative phytochemical analysis

Hydroalcoholic extract of *F. benghalensis* and *D. repens* were screened for the presence of secondary metabolites such as steroids, polyphenols, triterpenes, saponins, flavonoids and alkaloids as described by previous method (Njoku et al., 2011).

2.4. Total polyphenol and flavonoid content

Total polyphenol and flavonoid content of both extracts was quantified using the spectrophotometer method as explained by Ainsworth and Gillespie (2007) and Chandra et al. (2014) respectively to estimate gallic acid and quercetin equivalent in triplicates.

2.5. In vitro anti-oxidant activity

2.5.1. Free radical scavenging assay

Hydroalcoholic extracts of *F. benghalensis* and *D. repens* were tested for DPPH and ABTS free radical scavenging capacity as explained by Choi et al. (2002) and Re et al. (1999), respectively. Suitable controls were taken without test samples. All the experiments were performed in triplicates and IC_{50} was calculated.

2.5.2. Hydrogen peroxide scavenging assay

Fenton reaction system (Fe^{3+} ascorbate EDTA H_2O_2) was used to assess the ability of hydroalcoholic extracts to scavenge hydroxyl radicals (Kumari, Deori, Elancheran, Kotoky, & Devi, 2016a) using ascorbic acid as a standard. Appropriate controls were taken without containing test samples and standards. Experiments were performed in triplicates and IC_{50} was calculated.

2.5.3. Total anti-oxidant capacity

The ability of phosphomolybdenum complex formation by the hydroalcoholic extract was determined by incubating extract with ammonium molybdate in a suitable medium for 90 min and quantifying the absorbance at 695 nm. Appropriate controls were taken without the test sample, ascorbic acid was taken as standard, and percent of the anti-oxidant effect was evaluated in triplicate (Prieto et al., 1999).

2.5.4. Nitric oxide scavenging capacity

Nitric oxide released from the sodium nitroprusside and its scavenging capacity by extracts was measured according to the previously described method (Ebrahimzadeh et al., 2008). Gallic acid was taken as standard and the experiments were performed in triplicate and IC_{50} was calculated.

2.5.5. CUPRAC (Cu^{2+} to Cu^{+} reducing Assay)

Reducing power of both extracts was determined as explained by Köse et al. (2015). Ascorbic acid was used as a reference and the absorbance was measured as 450 nm against blank reagent. An experiment was performed in triplicate and the percentage reduction capacity was calculated.

2.5.6. Metal chelating assay

The metal chelating capacity of both extracts was performed as described from the previous method (Kumari et al., 2016b). Ethylenediaminetetraacetic acid was used as a standard. The absorbance was measured at 562 nm and the percentage of chelating activity was calculated in triplicate.

2.6. In vitro cytotoxicity

Chinese hamster ovary (CHO) and adenocarcinomic human alveolar basal epithelial cells (A549) were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in culture flasks (Tarsons India Pvt. Ltd, Kolkata, India) in Dulbecco's Modified Eagle Medium (DMEM) supplemented with penicillin (100 IU/mL), amphotericin B (5 mg/mL), 10% inactivated fetal bovine serum (FBS), and streptomycin (100 mg/mL) in a humidified atmosphere of 5% CO_2 at 37 °C. Cytotoxicity of the hydroalcoholic extract of *F. benghalensis* and *D. repens* was evaluated using *in vitro* (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983). Briefly, the trypsinized cell monolayers were washed with culture medium and incubated for 24 h in 96-well microtiter plates. The cells were then treated with different concentrations of hydroalcoholic extract, incubated for 48 h under a humidified environment (37 °C and 5% CO_2). After incubation, 10 μL of MTT (5 mg/mL in phosphate buffer saline) was added to each well and incubated again for 4 h. The absorbance was measured using a microtiter plate reader at a wavelength of 570 nm.

2.7. In silico biological spectrum and cytotoxicity profile of anti-oxidant compounds

The phytoconstituents present in the *F. benghalensis* (bark) and *D. repens* (whole plant) were mined using Chemical Entities of Biological Interest (ChEBI) (https://www.ebi.ac.uk/chebi/) and PhytoChemical Interactions (PCIDB) (https://www.genome.jp/db/pcidb) databases and published literatures. The compounds predicted for anti-oxidant capacity were filtered at the pharmacological activity (*Pa*) > 0.5 and their biological spectrum were predicted using *Pa* > 0.9 using PASS ONLINE (Filimonov et al., 2014). Similarly, the cytotoxicity of the compounds was predicted using CLC-Pred (Lagunin et al., 2018) at the *Pa* > pharmacological inactivity (Pi).

2.8. In silico docking study

We targeted five different enzymes that are primarily involved in the production of reactive oxygen species (ROS) i.e. lipoxygenase (PDB:1N8Q), cytochrome P450 (PDB:1OG5), myeloperoxidase (PDB:1DNU), xanthine oxidase (PDB:3NRZ), and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (PDB:2CDU); retrieved from RCSB Protein Data Bank (https://www.rcsb.org/). The molecules were in complex with water molecules and other heteroatoms; removed using Discovery studio 2016 (Dassault Systèmes BIOVIA, 2016). Ligands were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/), drawn in ChemSketch (https://www.acdlabs.com/resources/freeware/chemsketch/) if not available in PubChem, and minimized using mmff94force field (Halgren, 1996); converted into .pdbqt. MolSoft(http://www.molsoft.com/mprop/) was used to predict the druglikeness of individual compounds; top three compounds scoring highest druglikeness hit i.e. 7-*O*-α-*D*-glucopyranosyl-3,5-dihydroxy-3′-(4′′-acetoxyl-3′′-methylbutyl)-6,4′-dimethoxyflavone, 3,7-dihydroxy-2-[4-hydroxy-3-(4-hydroxy-3-methylbutyl)phenyl]-5,6-dimethoxy-4H-1-benzopyran-4-one, and naringenin from *D. repens* and 3-*O*-*trans*-*p*-coumaroyltormentic acid, mucusisoflavone C, and wighteone from *F. benghalensis* were chosen for docking studies using autodock4 (Morris et al., 2009). After docking, the pose scoring the lowest binding energy was chosen to visualize the ligand–protein interaction using Discovery studio 2016.

3. Results

3.1. Preliminary phytochemical investigation, total polyphenol and flavonoid content

A preliminary phytochemical investigation identified the presence of steroids, polyphenols, triterpenes, alkaloids, saponins, and flavonoids in *D. repens* whereas flavonoids, polyphenols, triterpenes, and steroids were present in *F. benghalensis*. Total polyphenol content was (23.2 ± 0.6) μg/mL and (52.06 ± 2.19) μg/mL in *F. benghalensis* and *D. repens*, respectively. Similarly, total flavonoid content was (100.24 ± 4.21) μg/mL and (122.36 ± 1.94) μg/mL in *F. benghalensis* and *D. repens*, respectively.

3.2. In vitro anti-oxidant activity

Among seven different anti-oxidant activities, the hydroalcoholic extract of *F. benghalensis* was identified to possess the highest ABTS scavenging capacity of (45.73 ± 1.17) μg/mL compared with gallic acid of (30.75 ± 1.6) μg/mL; whereas hydroalcoholic extract of *D. repens* showed the highest metal chelating capacity of (41.21 ± 0.69) μg/mL compared with EDTA of (23.12 ± 2). Both extracts also possessed other anti-oxidant properties like scavenging of DPPH, NO, H_2O_2 free radical, and reducing power assay. However, the IC_{50} values in other models were comparatively higher; IC_{50} of both test agents on each *in vitro* anti-oxidant model is summarized in Table 1.

3.3. In vitro cytotoxicity

The hydroalcoholic extract of *F. benghalensis* and *D. repens* showed higher cytotoxicity in A549 cell lines i.e. (193.78 ± 6.58) μg/mL and (104.02 ± 4.97) μg/mL respectively compared to CHO. Further, both hydroalcoholic extracts were significantly non-cytotoxic ($P < 0.001$) to CHO cell lines compared to A549 cell lines. The IC_{50} of tested extracts with each cell line is summarized in Table 2.

3.4. In silico biological spectrum and cytotoxicity profile of anti-oxidant compounds

We identified twenty-one different phytoconstituents from *F. benghalensis* and thirty-eight from *D. repens* using multiple open-source databases. Among them, twelve compounds from *F. benghalensis* and twenty-one compounds *D. repens* were identified for keyword "anti-oxidant" at *P*a > 0.5. These compounds were further assessed for their potency in other biological activates in which the majority of the phytoconstituents from *F. benghalensis* were histidine kinase inhibitors and hepatoprotectant from *D. repens*. Additionally, phytoconstituents from *D. repens* were also predicted as an immunostimulant, hepatoprotective, apoptosis agonist, anti-ulcerative, chemopreventive, wound healer, TP53 and ICAM1 expression enhancer and as the substrate for multiple isoenzymes like CYP1A1, CYP1A2, CYP1B, and CYP2C12. Likewise, phytoconstituents from *F. benghalensis* were also predicted as the inhibitor of multiple like aldehyde oxidase, chalcone isomerase, chlordecone reductase, glycerol dehydrogenase, MAP kinase, and monophenol monooxygenase and substrate of UTG1A10, UTG1A6, and UTG1A9 (Fig. 1). Similarly, the compounds were predicted to be more cytotoxic in cancer cell lines compared to normal. The cluster of Pa *vs.* Pi of multiple phytoconstituents from both plants in cancer and normal cell lines is represented in Fig. 2.

3.5. In silico molecular docking

3-*O*-*trans*-*p*-coumaroyltormentic acid from *F. benghalensis* was predicted to have the highest binding affinity with human myeloperoxidase and mucusisoflavone C, wighteone with NADPH oxidase. Similarly, naringenin from *D. repens* was predicted to have the highest binding affinity with NADPH oxidase and cytochrome P450 (CYP450), 3,7-dihydroxy-2-[4-hydroxy-3-(4-hydroxy-3-methylbutyl)phenyl]-5,6-dimethoxy-4*H*-1-benzopyran-4-one with NADPH oxidase and 7-*O*-α-*D*-glucopyranosyl-3,5-dihydroxy-3′-(4′′-acetoxyl-3′′-methylbutyl)-6,4′-dimethoxyflavone with CYP450. The binding affinity of an individual compound with each target was summarized in Table 3 (*F. benghalensis*) and Table 4 (*D. repens*). The interaction of 3-*O*-*trans*-*p*-coumaroyltormentic acid with myeloperoxidase (binding energy: −10.9 kcal/mol via one hydro-

Table 1
In-vitro anti-oxidant activity of hydroalcoholic extract of *F. benghalensis* and *D. repens*.

In vitro anti-oxidant activities	IC_{50} of extracts/(μg·mL^{-1})		$IC_{50}$of reference compounds/(μg·mL^{-1})		
	F. benghalensis (bark)	*D. repens* (whole plant)	Gallic acid	EDTA	Ascorbic acid
DPPH scavenging assay	73.99 ± 2.22	70.90 ± 2.13	–	–	25.88 ± 4.847
Hydrogen peroxide (H_2O_2) scavenging assay	50.67 ± 1.77	47.71 ± 0.71	–	–	47.71 ± 0.71
Nitric oxide (NO) scavenging assay	69.02 ± 2.57	67.85 ± 4.25	55.66 ± 0.64	–	–
Total anti-oxidant capacity (TAC)	51.45 ± 1.23	53.86 ± 2.421	–	–	38.71 ± 2.54
CUPRAC (Cu^{2+} to Cu^{+} reducing assay)	55.51 ± 0.54	54.52 ± 2.93	–	–	38.02 ± 2.25
Metal chelating assay	55.95 ± 0.92	41.21 ± 0.69	–	23.12 ± 2.16	–
ABTS scavenging assay	45.73 ± 1.17	53.72 ± 2.13	30.75 ± 1.637	–	–

Table 2
Cytotoxicity of hydroalcoholic extract of *F. benghalensis* and *D. repens* in cell lines.

Cell lines	Test agents	$IC_{50}/(\mu g \cdot mL^{-1})$
CHO	*F. benghalensis*	257.47 ± 3.60
	D. repens	226.37 ± 15.19
A549	*F. benghalensis*	193.78 ± 6.58
	D. repens	104.02 ± 4.97

gen bond interaction with ALA389) and 7-*O*-α-*D*-glucopyranosyl-3,5-dihydroxy-3′-(4′′-acetoxyl-3′′-methylbutyl)-6,4′-dimethoxyflavone with CYP450 (binding energy: −9.4 kcal/mol via five hydrogen bonds with TRP120, ARG43, THR301, and GLY296); represented in Fig. 3.

4. Discussion

The present study investigated the antioxidant and cytotoxic effects of hydroalcoholic extract of *F. benghalensis* (bark) and *D. repens* (whole plant) via *in silico* and *in vitro* approach. Further, probable biological activities of anti-oxidant phytoconstituents were also predicted. Data mining using multiple open-source databases and published literature reflected the presence of multiple phytoconstituents under the phytochemistry of polyphenols and flavonoids in both plants which were also quantified via total polyphenol and flavonoid content in the hydroalcoholic extract. These polyphenols and flavonoids are potent anti-oxidants (Pandey & Rizvi, 2009; Pietta, 2000) which could possess a beneficial effect via the neutralization of ROS and transition metal ions in the pathogenesis of multiple human diseases. Researchers have investigated the multiple extract(s)/isolated compound(s) of *F. benghalensis* (Kundap, Jaiswal, Sarawade, Williams, & Shaikh, 2017; Panday & Rauniar, 2016, 2016b) and *D. repens* (Agawane et al., 2019; Nikkon et al., 2008) primarily associated with the activation of ROS system leading to further complications.

Additionally, due to the high content of polyphenols and flavonoids and their anti-oxidant properties, *F. benghalensis* and *D. repens* possess medicinal values which need to be investigated for their multiple pharmacological properties including their cytotoxic potential. In the present study, the cytotoxic potential of anti-oxidant compounds from both plants was predicted to be more in tumor cell lines compared to normal; also demonstrated via *in vitro* MTT assay using both cancer and normal cell lines. The rapid activation of the ROS system was reported in cancer pathogenesis (Liou & Storz, 2010) which could be neutralized via selected folk medicinal plants. In the present study, we found both agents to possess more cytotoxic effect in A549 cells compared to CHO. This means phytoconstituents present in both extracts may have more tendency to inhibit the growth of tumor cells compared to normal cells. Since the IC_{50} of both extracts are comparatively lower in tumor cells than normal, they may constitute some phytoconstituents which could possess more binding affinity or modulate protein/pathway(s) involved in tumor pathogenesis but not in normal cells; needs to be further investigated.

Biologically, oxidative stress occurs due to the prolonged activation of the ROS system and transition metal ions which alters the task of homeostatic proteins (Chen et al., 2018); is one prime basis for the pathogenesis of diseases and associated complications. Structurally, the polyphenols and flavonoids contain the high amount of hydrogen donating capacity which can stabilize and delocalize the unpaired electrons, by forming the hydrogen bond with free radicals and terminating the Fenton reaction. The free radicals like $H^{\bullet}$, $OH^{\bullet}$, $Cl^{\bullet}$, $ClO^{\bullet}$, $NO^{\bullet}$, $NO_2^{\bullet}$ $O_2^{\bullet}$, $ROO^{\bullet}$, $LOO^{\bullet}$ are produced in the body due to the breakage of chemical bond (primarily covalent bond) keeping one electron, a redox reaction in which cleavage of a radical produces another radical (Pham-Huy et al., 2008) and production of transition metals due to Fenton reaction.

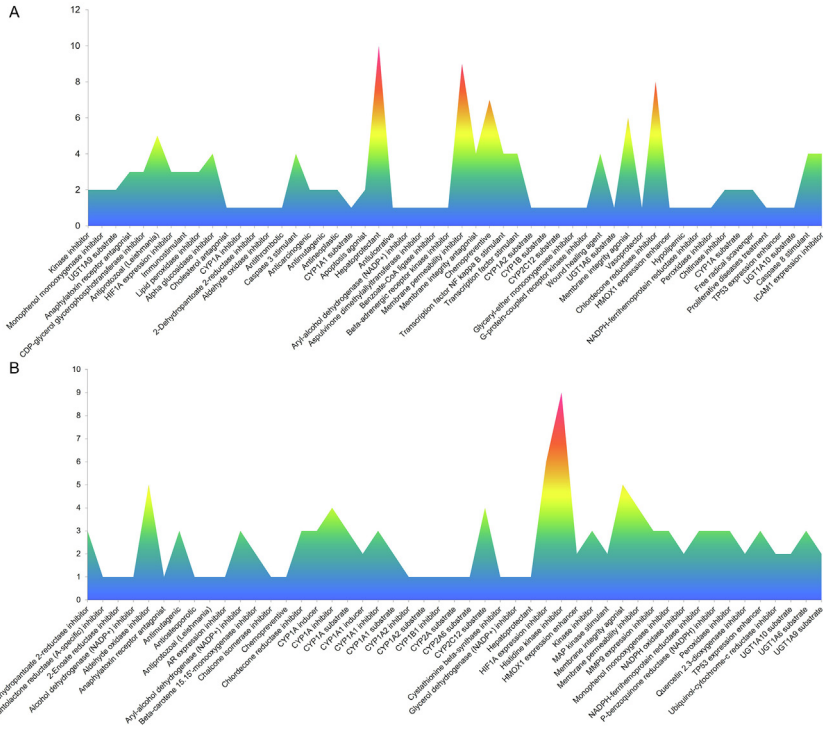

Fig. 1. Biological spectrum of phytoconstituents from *D. repens* (A) and *F. benghalensis* (B).

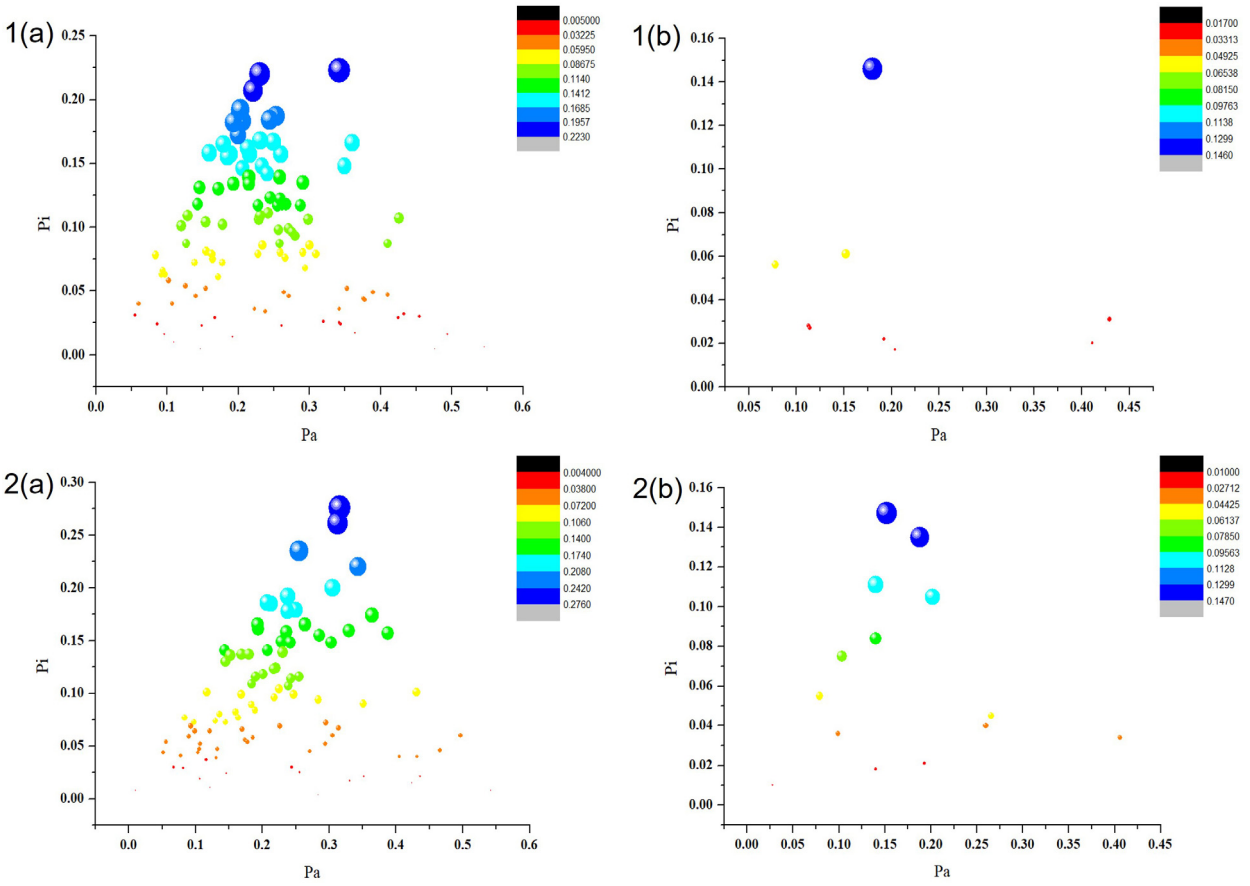

Fig. 2. Cytotoxicity of phytoconstituents from *F. benghalensis* (1) and *D. repens* (2) in cancer (a) and normal cell lines (b).

Table 3
Binding affinity of phytoconstituents from *F. benghalensis* with targets related to ROS system.

Phytoconstituents	Lipoxygenase (PDB:1N8Q)			Myeloperoxidase (PDB:1DNU)			Xanthine oxidase (PDB:3NRZ)			Cytochrome P450 (PDB:1OG5)			NAD(P)H oxidase (PDB:2CDU)		
	BE	NHB	HBR	BE	NHB	HBR	BE	NHB	HBR	BE	NHB	HBR	BE	NHB	HBR
3-*O-trans-p*-coumaroyltormentic acid	−9	3	ARG786, GLY265, ASN146	−10.9	1	ALA389	−8.4	2	GLN102, LYS95	−8.4	2	TYR42, ARG377	−9.8	1	GLU163
Mucusisoflavone C	−10.6	2	THR274, ASM556	−10.4	3	THR292, THR296, THR168	−7.7	3	ASN71, THR52, ASN146	−10.6	2	THR30, GLY296	−11.1	4	ASN343, SER328, SER326
wighteone	−2.6	2	THR274	−8.1	1	GLU245	−6.5	1	GLU89	−8.9	3	PHE47, LEU20, GLN214	−9.3	1	LYS134

Note:BE: Binding energy in kcal/mol, NHB: number of hydrogen bonds, HBR: hydrogen bond residues.

Similarly, our present study demonstrated the anti-oxidant efficacy of hydroalcoholic extract of *F. benghalensis* and *D. repens* to neutralize the free radicals and transition metal ions respectively.

Additionally, the modern experimental pharmacology utilizes *in silico* models to screen the phytoconstituents present in medicinal plants under investigation. Similarly, in the present study, multiple open source databases were utilized to mine the phytoconstituents present in respective plants; utilized the Prediction of Activity Spectra for Substances (PASS) to filter the compounds of anti-oxidant capacity at *P*a > 0.5 i.e. 50 percent and create the database of respected probable biological activities (*P*a > 0.9) for each anti-oxidant compounds. Prediction of biological activity was based on the structure–activity relationships (SAR) which included the training set of >205,000 compounds predicting >3750 biological activities (Filimonov et al., 2014). Interestingly, we found the prediction of anti-diabetic activity (α-glucosidase inhibitor) of *D. repens* which has been previously reported (Iqbal et al., 2004). Some of the predicted activities like hepatoprotective, lipid peroxidase inhibitor, and anti-carcinogenic could be further investigated using wet-lab protocols. Similarly, the anti-bacterial activity by inhibiting histidine kinase (Bem et al., 2014), hepatoprotective activity (Baheti & Goyal, 2011), peroxidase inhibition, and anti-protozoal effect of *F. benghalensis* were also predicted which needs to be further investigated.

Table 4
Binding affinity of phytoconstituents from *D. repens* with targets related to ROS system.

Phytoconstituents	Lipoxygenase (PDB:1N8Q)			Myeloperoxidase (PDB:1DNU)			Xanthine oxidase (PDB:3NRZ)			Cytochrome P450 (PDB:1OG5)			NAD(P)H oxidase (PDB:2CDU)		
	BE	NHB	HBR	BE	NHB	HBR	BE	NHB	HBR	BE	NHB	HBR	BE	NHB	HBR
Naringenin	−8.2	6	THR274, TYR 275, ASM 556, ARG 260, ALA 263	−8.3	0	–	−7.1	1	GLM144	−8.4	2	VAL113, SER365	−8.4	3	PRO 298, LYS 134, SER 41
3, 7-Dihydroxy-2-[4-hydroxy-3-(4-hydroxy-3-methylbutyl) phenyl]-5, 6-dimethoxy-4H-1-benzopyran-4-one	−8.1	3	VAL 588, TYR 512, ASP 428	−7.7	3	SER174, ASP 172	−7	5	TYR58, GLY12, THR86, TYR125	−8.2	2	ALA103, ASN217	−9	3	LYS 134, ASP 282, ALA 300
7-*O*-α-*D*-glucopyranosyl-3,5-dihydroxy-3′-(4′′-acetoxyl-3′′-methylbutyl)-6,4′-dimethoxyflavone	−7.9	2	LYS156, ASP190	−8.2	7	ARG23, SER174, THR16, PHE170, SER169, THR329	−7.2	7	SER69, SER123, ALA142, GLY145, GLM144	−9.4	5	TRP120, ARG43, THR301, GLY296	−9.1	2	THR9, ALA300

Note: BE: Binding energy in kcal/mol, NHB: number of hydrogen bonds, HBR: hydrogen bond residues.

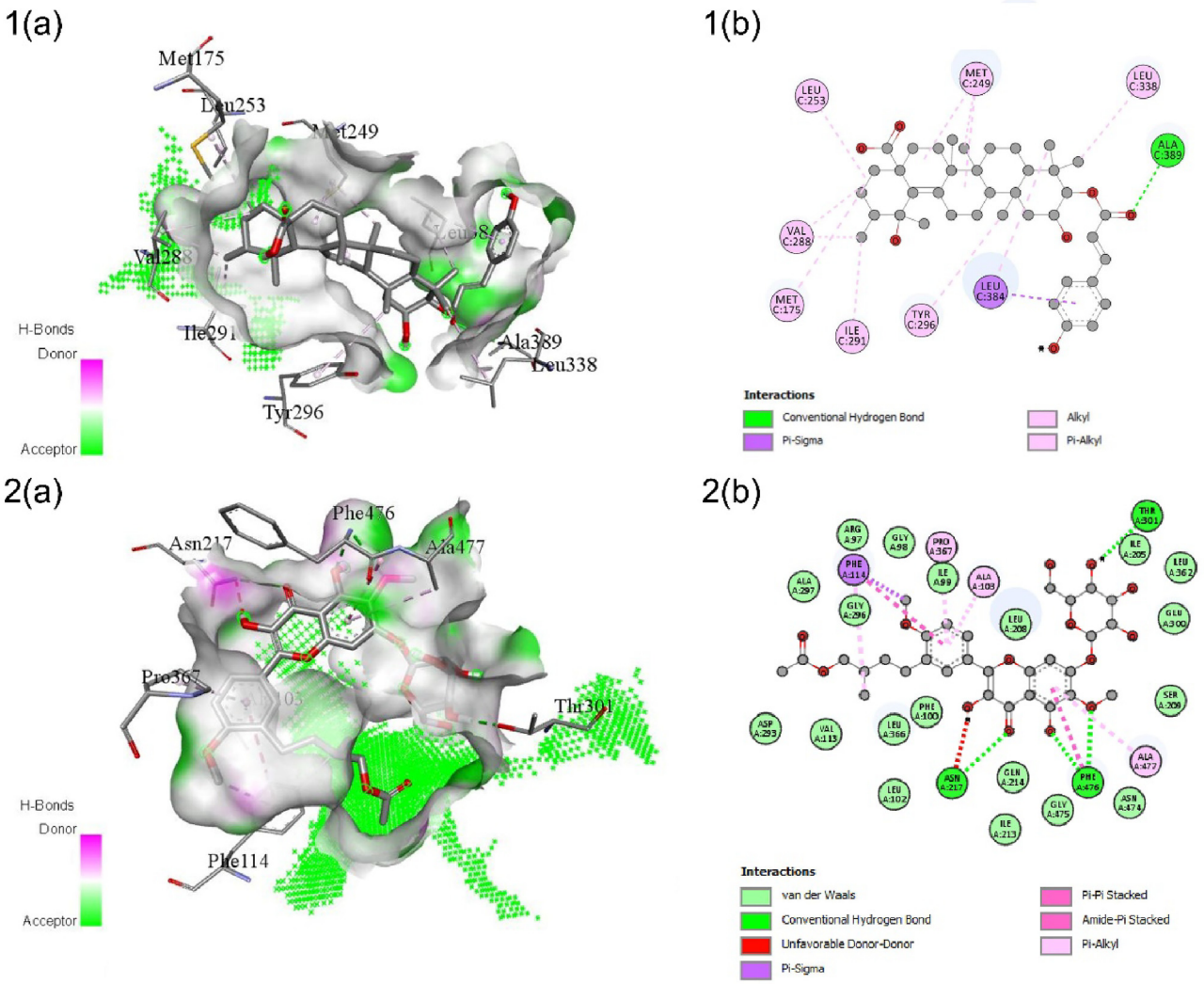

Fig. 3. 3D (a) and 2D (b) interaction of 3-*O*-*trans*-*p*-coumaroyltormentic acid with myeloperoxidase (1) and 7-*O*-α-*D*-glucopyranosyl-3,5-dihydroxy-3′-(4′′-acetoxyl-3′′-methylbutyl)-6,4′-dimethoxyflavone with CYP450 (2).

Further, *in silico* docking study was performed to predict the binding affinity of predicted anti-oxidants with multiple proteins involved in the induction of oxidative stress. Docking helps to identify the lead hit molecule which can be identified via three main points i.e. binding energy; represents binding affinity, number of hydrogen bond interactions and hydrogen bond residues (Khanal, Mandar, Magadum, Patil, & Hullatti, 2019a; Khanal, Mandar, Patil, & Hullatti, 2019b). To identify the lead anti-oxidant, the top three hits with highest druglikeness score from both plants were selected and docked against five free radical generators i.e. lipoxygenase, myeloperoxidase, xanthine oxidase, cytochrome P450, and NAD(P)H oxidase. Lipoxygenase oxygenates (O_2 insertion) polyunsaturated free fatty acid (such as linoleic and arachidonic acid) by redox reaction via switching the active site iron atom at Fe^{2+} to Fe^{3+} states to form lipid metabolites which contribute in the pathogenic conditions. This formation of "*oxygen centered fatty acid hydroperoxide radical*" is due to the insertion of O_2 at penta-dienyl radical (Wisastra & Dekker, 2014). Circulating neutrophils are the prime source of myeloperoxidase and possess an active role in the processing of inflammation and oxidative stress. Although, this enzyme kills microbes intracellularly, it also damages host tissue extracellularly (Tejaswi et al., 2017). Xanthine oxidase is an interconvertible form of xanthine dehydrogenase, which is the product of a single gene. Xanthine oxidase produces superoxide ($^{\bullet}O_2^-$) and uric acids by utilizing xanthine (substrate) and oxygen (cofactor) and is reported as the main source for oxygen radicals in epithelial, endothelial, connective and polymorphonuclear tissue cells. This enzyme plays a potential role in oxidative status, aldehyde detoxification, neutrophil mediation, and oxidative stress-mediated ischemic reperfusion (Chung et al., 1997). Similarly, CYP450 enzymes are involved in drug metabolism in the liver (Zanger & Schwab, 2013). During this process, if CYP450 fails to metabolize the substrate or any molecule then it activates the futile cycling process; elevates reactive oxygen species (Hrycay & Bandiera, 2015). Likewise, NADPH oxidase is identified to play a major role in the pathophysiology of inflammation, cell migration, apoptosis, endothelial dysfunction, diabetes, hypertension, hypertrophy, rarefaction, and angiogenesis (Paravicini & Touyz, 2008).

The present study reflects the binding affinity of the predicted anti-oxidants with the targets related to oxidative stress. Further, based on the binding affinity and number of hydrogen bond interactions, mucusisoflavone C from *F. benghalensis* was predicted to possess the potent anti-oxidant activity and may act as anaphylatoxin receptor antagonist and could be choice of a therapeutic agent in the complement system. Similarly, 7-*O*-α-*D*-glucopyrano syl-3,5-dihydroxy-3′-(4′′-acetoxyl-3′′-methylbutyl)-6,4′-dimethox yflavone from *D. repens* could be the choice of the lead molecule in oxidative stress associated pathogenesis. The compound was also predicted for a wide range of biological activities like chemopreventive, anticarcinogenic, hepatoprotectant, proliferative diseases treatment, membrane permeability inhibitor, lipid peroxidase inhibitor, and anaphylatoxin receptor antagonist at *P*a > 0.9 suggesting its utilization in the broad spectrum. Likewise, the predicted pathogenic conditions involve the strong correlation with the activation of the ROS system which could be one of the beneficial effects of anti-oxidant activity in managing such pathogenesis.

We believe the extract/fraction(s) should be the choice of testable agents rather than the individual compounds from medicinal plants to evaluate their safety and efficacy because extract(s)/fraction(s) are composed with multiple secondary metabolites; may act synergistically/additively to generate the pharmacological effect (Zhou et al., 2016) by targeting multiple proteins and regulating numerous pathways as we explained previously (Khanal & Patil, 2019, 2020; Khanal, Patil, Mandar, Dey, & Duyu, 2019c).

5. Conclusion

In conclusion, the present study evaluated two medicinal plants i.e. *F. benghalensis and D. repens* for their cytotoxic potential in normal and tumor cell lines, probable anti-oxidant mechanism, and predictable biological activities. Further, the present study predicted the lead hits as anti-oxidants from both medicinal plants. However, the *in silico* findings of the present study need to be validated by using well-established wet-lab protocols.

Authors contributions

Pukar Khanal experimented and drafted the manuscript. BM Patil designed the protocol and refined the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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