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Phytochemical profiling and antioxidant activities of bodhi tree leaf extract (*Ficus religiosa* L.) on nitric oxide and catalase

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Abstract: Metabolic syndrome is on the verge of becoming a global epidemic. It is a complex, chronic illness that has epidemic proportions in most industrialized nations. This study examines the antioxidant potential of a sacred plant, the bodhi tree (*Ficus religiosa*). The capability of *F. religiosa* to suppress nitric oxide (NO) production and enhance catalase activity was determined using colorimetry methods. Additionally, liquid chromatography-mass spectroscopy was used to determine the phytochemical profile of the plant. The contribution of detected compounds was predicted using activity spectra for biologically active substances online. This study revealed the activity of *F. religiosa* to suppress NO production with IC_{50} value was 63.57µg/mL. The activity of *F. religiosa* to increase the catalase activity did not follow the increase of extract concentration, in contrast with the NO test. The concentration with optimum activity was 10 µg/mL. The extract can increase catalase activity by 45.45% in this concentration. The presence of corchorifatty acid F, D-(-)-Quinic acid, 2,5-dihydroxybenzaldehyde and gentisic acid, confirmed by LC-MS/MS, might contribute to the antioxidant activity of *F. religiosa*. In summary, the antioxidant activity of *F. religiosa* was mediated by NO production suppression and catalase activity enhancement.

Keywords: Antioxidants, catalase, *Ficus religiosa*, nitric oxide, plant extract.

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INTRODUCTION

Various plants have been used to maintain body health. However, many plants have no solid scientific data to support further use and development, such as the Bodhi tree (*Ficus religiosa* L.). Bodhi has been used as a medicine for over 4000 years and is included in the Rasayana group based on Ayurveda (Sharma, 2006). Further, Bodhi is a tree of life. It is sacred by Hindus and Buddhists worldwide, with various ecological and medical benefits.

Bodhi is traditionally used for rejuvenation, according to Ayurveda (Sharma, 2006). It is associated with its antioxidant function in the modern context. Antioxidants help us to withstand many oxidative stress-related diseases such as cancer, metabolic syndrome, atherosclerosis, malaria, Alzheimer's disease, rheumatoid arthritis, neurodegenerative diseases and preeclampsia (Kıran *et al*., 2023).

Several researchers tested the antioxidant activity of bodhi plants. Root extract at a dose level of 500mg/kg shows significant antioxidant activity in carbon tetrachloride-induced liver injury in mice. The extract has remarkable antioxidant activity. It has shown decreased lipid per oxidation levels and increased catalase (CAT), glutathione per oxidase, glutathione reductase, glutathione S-transferase and super oxide dismutase levels (Gupta *et al*., 2011). The bark of this plant demonstrated antioxidant activity in tests with cell cultures of keratinocytes and mice induced with type 2 diabetes (Dixit *et al*., 2016; Kirana *et al*., 2009). The antioxidant activity of the bark is also confirmed in a study conducted by (Shahid *et al*., 2021; Shankar *et al*., 2021). The bodhi fruit has also been proven to have antioxidant activity (Gupta *et al*., 2011). Besides root and bark, the plant's leaf has also been tested as an antioxidant and demonstrated a 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (Krishanti *et al*., 2010).

Leaves, as the most significant part of the plant, have insufficient scientific data regarding the mechanism of their antioxidant activity even though it was proven to have flavonoid, the well known antioxidant compounds. It differs from other Ficus species in which leaves have been used extensively and known for their many biological activities. They are *Ficus arnottiana, Ficus carica, Ficus lyrate, Ficus Tsiela, Ficus sycomorus, Ficus thonningii, and Ficus lutea* (Nawaz *et al*., 2019). No research has been conducted to determine the phytochemical profile of *F. religiosa* leaves related to its antioxidant potential. Therefore, this study aimed to confirm and further explore the potential antioxidant activity of the bodhi plant.

This study selected the leaves as samples. This laboratory experimental study began with preparing the bodhi plant

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extract and conducting phytochemical analysis using the liquid chromatography-mass spectroscopy (LC-MS/MS) method. The prediction of activity spectra for biologically active substances (PASS) online was used to predict the contribution of detected compounds to the antioxidant activity of the plant. Then, the research continues by testing the inhibitory activity of nitric oxide (NO) and CAT *in vitro* as a representative antioxidant test. Nitric oxide (NO) plays a vital role in the control of numerous physiological processes. Excessive synthesis of nitric oxide (NO) can cause harm to tissues, hence it is important to suppress its production. On the other hand, catalase is an enzyme with antioxidant properties, therefore it is beneficial to enhance its activity.

MATERIALS AND METHODS

Materials

Leaves of the bodhi plant were collected from March to April 2022. This study included other materials such as water, ethanol 96%, vitamin C (Sigma, purity >99%), sodium nitroprusside (Loba Chemie, purity 99%), water for injection (WFI) (Sigma), phosphate buffer (Sigma), Greiss reagent (Sigma), potassium phosphate (Merck), hydrogen peroxide (Merck, purity 50%), CAT enzyme (Worthington, ≥40,000 units permg protein, 10mL), UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS Thermo Scientific, Accucore C18, 100 × 2.1 mm, 1.5µm (Thermo Scientific), formic acid, acetonitrile and methanol. The software includes Marvin Sketch, Chem Draw, and PASS Online (http://www.way2drug.com/PASSOnline/predict.php). Database sources used are Chem Spider and Pubchem (https://pubchem.ncbi.nlm.nih.gov/).

Extract preparations

Fine powder of bodhi leaf simplicia (*Ficus religiosa* L.) at 500 g was macerated by pouring the powder into a glass jar, adding 96% ethanol solvent in a 1: 2 w/v for 24 h. Then, it was filtered and squeezed and the dregs were added to 96% ethanol again until submerged. Soaking and filtering were performed for 3 × 24h (3 days with three repetitions or solvent changes). Then, the macerate or filtrate obtained was collected and evaporated at low pressure with a water bath with a temperature of 40ºC. Results were obtained in the form of concentrated ethanol extract. Furthermore, the yield of bodhi leaf extract (*Ficus religiosa* L.) was calculated.

Screening of natural compounds by LC-MS/MS

Extract at 0.5g was put in a 10-mL flask and added with methanol. The sample was sonicated for 30 min and then filtered using a 0.2 µm polytetrafluoroethylene membrane filter. The filtered sample was injected into the UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS (Thermo Scientific). The UHPLC analysis is equipped with a *HESI*-MS/MS with positive ionization mode. The sample was analyzed using Accucore C18, 100 × 2.1 mm, 1.5µm (Thermo Scientific), at a column temperature of 30ºC, with an eluent of H_2O + 0.1% formic acid (A) dan acetonitril + 0.1% formic acid (B) with gradient 0-1 min (5% B), 1-25 min (5%-95% B), 25-28 min (95% B) and 28-30 min (5% B). The eluent was adjusted at a flow rate of 0.2 ml/min, injection volume of 2.0µl and mass range of 200-1500 m/z. Parameters HESI source were sheath gas flow rate of 15, aux gas flow rate of 3, spray voltage of 3.80, sweep gas flow rate of 0, capillary temperature of 320ºC, aux gas heater temperature of 0ºC, S-lens RF level of 50.

Biological activity prediction

A web server from PASS Online (http://way2drug.com/passonline/index.php) was used to predict the biological activity of the compounds found through LC-MS/MS analysis. The substance's SMILES or mol file was entered to forecast the biological activities. The ability to operate as an antioxidant or to reduce oxidative stress was related to its activity as a free radical scavenger, NF-E2-related factor two stimulant, CAT stimulant, NO scavenger and NO antagonist (Rocha *et al*., 2022; RUMENGAN *et al*., 2021; Voronova *et al*., 2022).

Computer software called PASS is used to forecast various pharmacological actions for other compounds, such as phytoconstituents (Khurana *et al*., 2011). This spectrum's prediction by PASS is based on an investigation of the structural activity link between the training sets of over 205,000 chemicals (Goel *et al*., 2011). The potential of the bioactive compounds was predicted and screened using this service, which offered over 4000 different types of biological activity for drug discovery (Lagunin *et al*., 2000).

The probability of activity (Pa) and probability of inactivity (Pi) is used to estimate a compound's projected activity spectrum (Goel *et al*., 2011). Compounds with a Pa value greater than a Pi value were the only ingredients thought to be possible for a certain pharmacological activity (Goel *et al*., 2011; Khurana *et al*., 2011). The accuracy of the forecast findings increases with increasing Pa values (Lagunin *et al*., 2000).

The Pa value is calculated using the following criteria: (1) Pa >0.7, the tested compound has a form and activity similar to the drug compound; (2) Pa = 0.5-0.7, the tested compound has different forms and is less likely to exhibit drug-like activity and (3) Pa <0.5, the tested compounds may not exhibit drug-like activity. However, the test substance might be a brand-new chemical molecule if laboratory testing shows the presence of this action (Lagunin *et al*., 2000).

Antioxidant activity

Nitric oxide scavenging activity

The scavenging capacity of the extracts and ascorbic acid, utilized as the standard, is the NO scavenging test foundation. NO was produced when sodium nitroprusside

(SNP) spontaneously broke down in a 0.02-M phosphate buffer (pH of 7.4). The Griess reaction was used to determine nitrite ion production after NO reacts with oxygen. The reaction mixture at 1 mL containing 10 mM SNP and various extract amounts (10, 20, 40, 50, 60, 80, 100µg/mL) were incubated at 37ºC for 1h. Griess reagent at 0.5mL was added to an aliquot of 0.5mL and homogenized. The chromophore's absorbance was determined at 540 nm. The experiments were conducted three times in triplicate. The percentage of nitrite generated by SNP alone was used to express the results.

CAT -like activity

CAT-like activity, which is the ability of the extract to break down hydrogen peroxide (H_2O_2) added to the incubation medium, was assessed as previously mentioned (Quintans-Júnior *et al*., 2013). H_2O_2 was briefly diluted to a final concentration of 0.059 mM in 0.02 M phosphate buffer (pH 7.0). Extract of 1.9 mL in various concentrations was put into micro plate wells with a 1mL solution. The CAT enzyme of 0.1mL was then introduced. The mixture's absorbance was measured in a spectrophotometric plate reader at 240 nm after 5 min of incubation at 37ºC. Incubation was continued for 4 min before the mixture's absorbance was measured for the second time. The CAT-like activity was observed based on the H_2O_2 decomposition rate. Experiments were run in triplicate three times each. The percentage of the rate of H_2O_2 breakdown was used to express the data.

STATISTICAL ANALYSIS

GraphPad Prism 8.3.3 (GraphPad Software Inc., Sandiego, CA, USA) was used for data analysis. The Kruskal-Wallis test was employed to further investigate not normally distributed data after a conventional one-way analysis of variance. Sample values were deemed significant at *p*-values of <0.05. All values were expressed as mean ± standard deviation.

RESULTS

Screening of natural compounds by LC-MS/MS

The untargeted screening and identification of chemical compounds from *F. religiosa* extract were conducted through LC-MS/MS**.** table 1 shows the identified peaks in positive modes.

A literature study confirmed that 4 of the 30 compounds with the highest peak have antioxidant activity, including D-(-)-Quinic acid, corchorifatty acid F, 2,5-Dihydroxybenzaldehyde, and gentisic acid. The structure of the compounds is presented below (fig. 1).

Biological activity prediction

Twelve compounds with the biggest area sample according to LC-MS/MS analysis were analyzed in this test, including corchorifatty acid F, D-(-)-Quinic acid, (-)-pinellic acid, A-12(13)-EpODE, 16-Hydroxyh-exadecanoic acid, 13(S)-HOTrE, Mitoxantrone, (±) 13-HODE, 10,16-Dihydroxyhexadecanoic acid, (-)-pinellic acid, 2,5-Dihydroxybenzaldehyde, gentisic acid, and D-(+)-Malic acid. Only D-(-)-Quinic acid has related activity (antioxidant, Pa > 0.830) in Pa of >0.7.

Further analysis was made of 4 compounds with scientific activity data, including D-(-)-Quinic acid, corchorifatty acid F, 2,5-Dihydroxybenzaldehyde, and gentisic acid. The activity spectra prediction results are presented in table 2.

Data shows that all of the predicted compounds can act as antioxidants. The compounds supported the activity of extract to inhibit the NO production. CAT activity was stimulated, especially by 2,5-Dihydroxybenzaldehyde and gentisic acid. Additionally, the antioxidant capacity of *F. religiosa* was predicted to be mediated through NF-E2-related factor 2 (NRF2) stimulation. NRF2 is the primary regulator of cellular antioxidant defenses. Thus, its activation or stimulation may boost antioxidant defenses (Oksanen *et al*., 2020).

Antioxidant activity

NO scavenging activity

The current investigation revealed that *F. religiosa* extracts successfully suppressed NO generation. The extract demonstrated a dose-dependent NO inhibition activity in comparison to controls (fig. 2). The IC_{50} value was 63.5682 µg/mL using linear regression plot equation, $y = 0.5762x + 13.372$ with R^2 0.9912.

CAT-Like Activity

The CAT-like assay demonstrated that extract could increase CAT activity. This activity keeps growing over time (fig. 3). However, the increase in extract concentration does not increase the activity. Extract with a concentration of 10µg/mL demonstrated the best action (fig. 4). The activity was comparable with the activity of vitamin C at 2 µg/mL as a control.

DISCUSSION

Phytochemical screening using LC-MS/MS analysis demonstrated the presence of several compounds with antioxidant activity. The method also used to determine potential antioxidant compound from leaves of other plant species (Sukardi *et al*., 2022). One of them is D-(-)-Quinic acid. The activity supported its capability to reverse memory loss (Liu *et al*., 2020). Thus, it can nutritionally support nicotinamide and tryptophan synthesis in the gastrointestinal (GI) tract, which enhances DNA repair and inhibits nuclear factor kappa B via increased tryptophan and nicotinamide production (Lund and Leanderson, 2009).

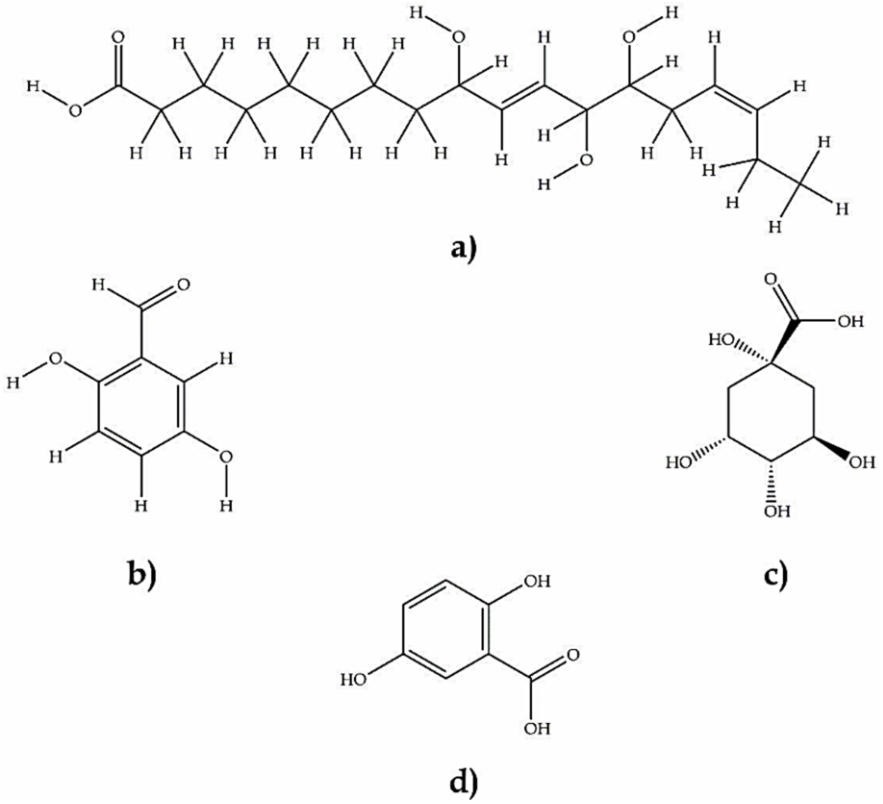
Table 1: Phyto-compounds detected in *F. religiosa* extract using LC-MS/MS analysis

No.	Name	Formula	Annot. DeltaMass [ppm]	Calc. MW	RT [min]	# Chem Spider Results	Mz Cloud Best Match	Sample Area
1	Corchorifatty acid F	C18 H32 O5	0.05	328.225	12.368	4	91.2	9.98E+08
2	D-(-)-Quinic acid	C7 H12 O6	-3.04	192.0628	1.09	9	98.5	6.25E+08
3	(±)13-HODE	C18 H32 O3	-0.08	296.2351	20.027	14	91.5	1.98E+08
4	2,5-Dihydroxybenzaldehyde	C7 H6 O3	-6.21	138.0308	5.363	0	98.9	1.65E+08
5	Gentisic acid	C7 H6 O4	-4.85	154.0259	3.799	10	93.8	1.64E+08
6	D-(+)-Malic acid	C4 H6 O5	-6.56	134.0206	1.11	0	97.8	1.52E+08
7	5-Methoxysalicylic acid	C8 H8 O4	-4.05	168.0416	8.051	27	93	1.49E+08
8	Salicylic acid	C7 H6 O3	-6.21	138.0308	9.937	0	98.6	1.08E+08
9	(±)9-HpODE	C18 H32 O4	0.87	312.2303	17.856	18	91.2	73857133
10	2-(Acetylamino)hexanoic acid	C8 H15 N O3	-3.53	173.1046	7.041	8	94.2	52962893
11	Citric acid	C6 H8 O7	-1.73	192.0267	1.45	16	92.6	52692671
12	4-Hydroxycoumarin	C9 H6 O3	-4.54	162.031	8.39	7	95.8	48702783
13	Benzoic acid	C7 H6 O2	-7.69	122.0358	6.891	0	97.6	41585374
14	2-(Acetylamino)hexanoic acid	C8 H15 N O3	-3.53	173.1046	6.814	8	95.6	41503420
15	4-Oxoproline	C5 H7 N O3	-7.15	129.0417	1.126	0	97.1	32419826
16	Chlorogenic acid	C16 H18 O9	0.67	354.0953	6	10	98.3	30628394
17	(±)13-HODE	C18 H32 O3	-0.08	296.2351	22.98	14	92.1	30395917
18	4-Nitrophenol	C6 H5 N O3	-5.91	139.0261	10.189	0	90.7	26000519

Table 2: Antioxidant activities prediction using PASS online

No.	Compounds	Pa	Pi	Biological Activity
1	Corchorifatty acid F	0.427	0.010	Antioxidant
		0.270	0.018	Nitric oxide antagonist
		0.237	0.026	Nitric oxide scavenger
2	D-(-)-Quinic acid	0.830	0.003	Antioxidant
		0.541	0.008	Free radical scavenger
		0.300	0.007	Nitric oxide scavenger
		0.326	0.043	NF-E2-related factor 2 stimulant
		0.200	0.038	Nitric oxide antagonist
3	2,5-Dihydroxybenzaldehyde	0.486	0.011	Free radical scavenger
		0.457	0.010	NF-E2-related factor 2 stimulant
		0.382	0.014	Antioxidant
		0.331	0.004	Nitric oxide scavenger
		0.243	0.024	Nitric oxide antagonist
		0.171	0.076	Catalase stimulant
4	Gentisic acid	0.576	0.007	Free radical scavenger
		0.502	0.006	NF-E2-related factor 2 stimulant
		0.406	0.012	Antioxidant
		0.348	0.004	Nitric oxide scavenger
		0.264	0.019	Nitric oxide antagonist
		0.181	0.058	Catalase stimulant

Pa = (probability “to be active”), Pi = (probability “to be inactive”)



a) Corchorifatty acid F; b) 2,5-dihydroxy benzaldehyde; c) D-(-)-Quinic acid; d) gentisic acid.

Fig. 1: Chemical Compounds Detected in *Ficus religiosa* Extract by LC-MS/MS.

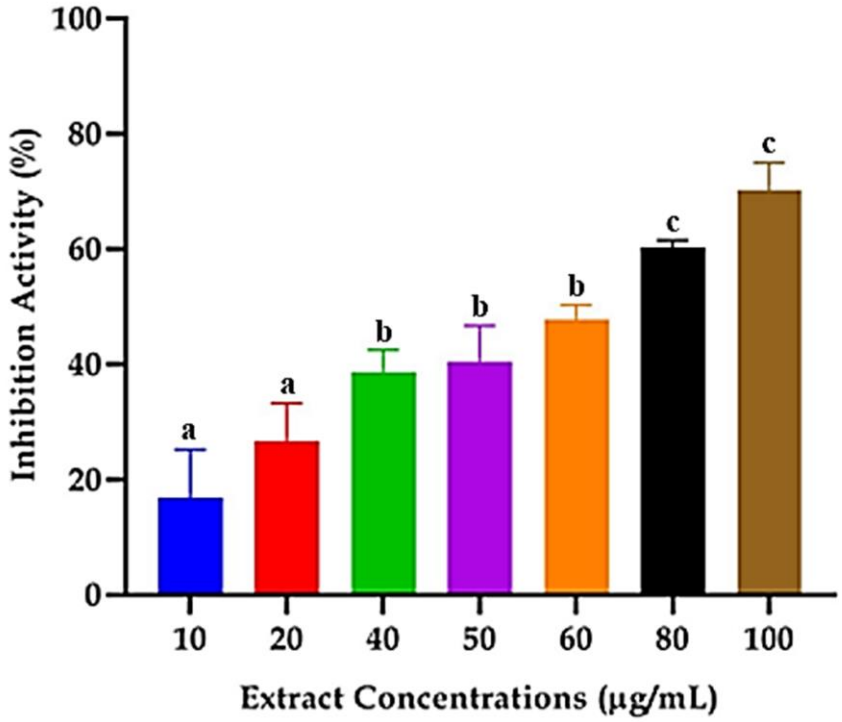


Fig. 2: Nitric oxide (NO) scavenging assay. Bars represent mean ± SD values. The significance was showing with different superscript ($p<0.001$, 1-way ANOVA followed by Tukey's post-hoc test).

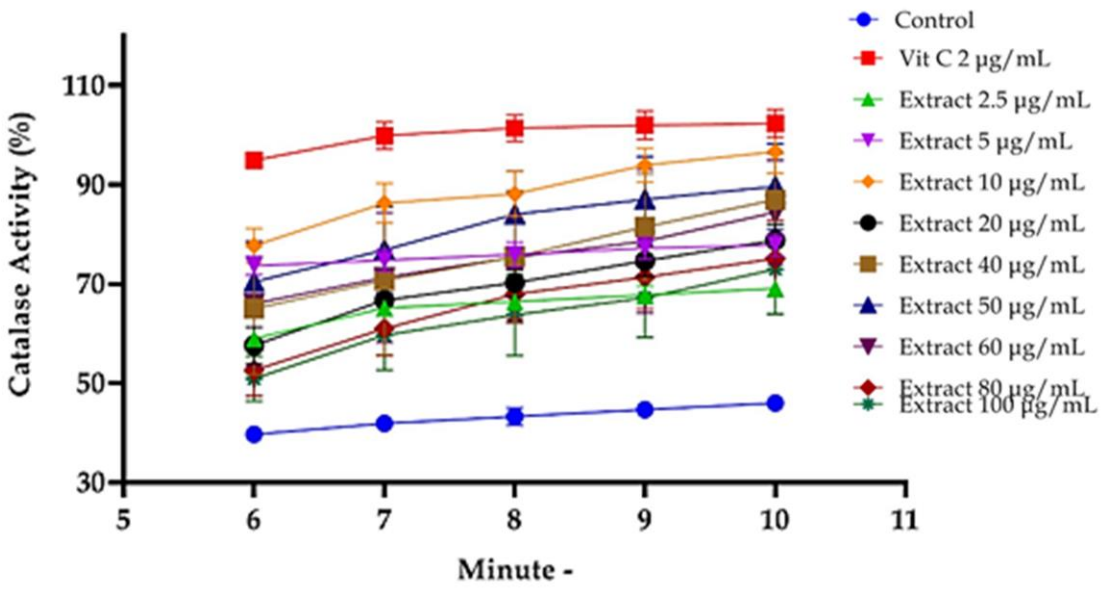


Fig. 3: Catalase-like (CAT-like) activity in every minute. Line represent mean ± SD values

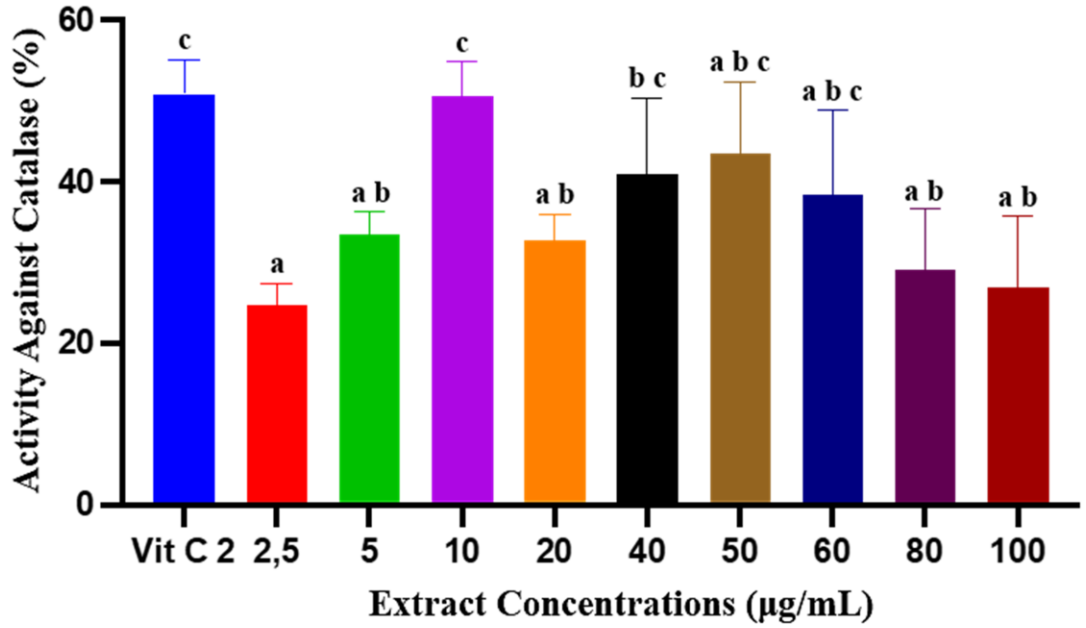


Fig. 4: Catalase-like (CAT-like) activity in minute 10. Bars represent mean ± SD values. The significance was showing with different superscript ($p<0.001$, 1-way ANOVA followed by Tukey's post-hoc test).

The other potential compound is corchorifatty acid F. It showed an inhibitory effect on NO production in mouse peritoneal macrophages (Yoshikawa *et al*., 1998). Then, the antioxidant activity of 2,5-Dihydroxybenzaldehyde has been proved (Estévez Brito *et al*., 2017). The antioxidant capacity of the compound as a phenolic derivative is intrinsically related to the hydroxyl group number in a molecule (Estévez Brito *et al*., 2017). Additionally, 2,5-Dihydroxybenzaldehyde has antioxidant activity against *Mycobacterium avium* subsp. *Paratuberculosis* (Nowotarska *et al*., 2014). Several in vitro tests have proved its antioxidant activity (Kheniche *et al*., 2022). Finally, gentisic acid has the most comprehensive scientific data as an antioxidant agent. Many studies have confirmed its antioxidant capacity. The presence of the phenoxyl group supported the antioxidant

properties of gentisid acid (Joshi *et al*., 2012). Its antioxidant capacity turned out to be pH-dependent, which is a better antioxidant at a pH of >4.5 (Trindade and Balter, 2020). Its ability to scavenge $DPPH^{\cdot}$ in methanol was improved by stoichiometric inhibition factor and higher solubility/polarity (Mardani-Ghahfarokhi and Farhoosh, 2020). It even optimized the antioxidant capacity of an antioxidant protein, β-lactoglobulin (βLG), in covalent conjugation (Li *et al*., 2019). Recent study found the present of catechin, gentisic acid, protocatechuic acid, quinic acid, rutin, and other phenolic compounds in the *F. religiosa* leaves extract. The compounds were proved to be excellent antioxidants (Suriyakalaa *et al*., 2022).

The four chemicals have hydroxyl groups as the primary structural motifs that contribute to their antioxidant action. The most prevalent pharmacophore characteristics in antioxidants are highly conjugated OH groups. These antioxidants function through the HAT (hydrogen atom transfer) and SET (single electron transfer) pathways and sometimes in combination. Certain conditions may preferentially enhance one mechanism over the other (Charlton *et al*., 2023).

This study investigated the antioxidant activity through suppressed NO production and increased CAT activity. The antioxidant activity level is categorized according to Phongpaichit *et al*. (2007) as very strong, strong, mild, weak, and inactive free radical if the IC_{50} values are <10 g/mL, 10-50g/mL, 50-100g/mL, 100-250g/mL and >250 g/mL, respectively (Phongpaichit *et al*., 2007). The study revealed that the extract of *F. religiosa* exhibited mild antioxidant activity to suppress NO generation.

NO is a dual molecule that plays important roles in cell signaling and oxidative/nitrosative stress, depending on the timing and dose (Vane *et al*., 1994). This molecule may be produced physiologically by activating NO synthases, then inducing cytoprotective and anti-inflammatory pathways. However, NO may also enhance the production/release of proinflammatory mediators, such as prostanoids (Sautebin *et al*., 1995), reactive oxygen species (Marcinkiewicz *et al*., 1995), and cytokines, thereby promoting the inflammatory response.

The strong NO^{+} nature of SNP-generated NO can change the structure and functionality of several cellular components. The peroxynitrite anion (-ONOO-), which is a potentially powerful oxidant that can break down to yield ^{-}OH and NO_2 (Pacher *et al*., 2007), is formed when superoxide and NO^{-} combine, increasing the NO^{-} toxicity.

NO is a crucial chemical mediator produced by neurons, macrophages, and endothelial cells. It is important in controlling many physiological processes, including inflammation. It also contributes to oxidative damage although NO may have positive effects. Numerous disorders are linked to excessive NO production and release (Knowles *et al*., 1989). The development of compounds that stop NO overproduction has become a new area of study for treating chronic inflammatory illnesses.

Besides successfully demonstrating its capability to suppress NO production, the extract can also increase CAT activity. CAT is one of the natural antioxidant enzymes in the body that work to fight free radicals. However, oxidative stress occurs if the number of free radicals formed is so large that it exceeds the ability of antioxidant enzymes to overcome them. CAT is a part of inner/natural antioxidant enzymes. This CAT enzyme is most abundant in the liver and is produced by peroxisomes (Murray, 2003). CAT enzyme is an endogenous antioxidant that can capture and decompose free radicals in cells into less reactive substances. This enzyme also has a vital role in catalyzing hydrogen peroxide into H_2O and O_2 and preventing CO_2 bubble formation in the blood (Afiati *et al*., 2016). A decreased CAT enzyme activity can cause adverse effects due to free radical accumulation.

This study performed in silico-based analysis to support the data from *in vitro* test. PASS Online, which is a software for *in silico* screening was used. The results were in line with the in vitro tests. A new mechanism of action was also predicted.

Software for in silico screening is made to analyze compounds virtually and it frequently includes tools that predict theoretical selectivity and recognition for particular sites of action or bioreactions. Additionally, it frequently has access to extensive databases of small compounds for structural comparison (Muegge and Oloff, 2006). Predictive tools, also known as computational prediction models, are a key component of the methodology toolkit that directs pharmaceutical technological research. These tools research potential and real chemicals, forecasting pharmacological, toxicological and pharmacokinetic outcomes (Vedani *et al*., 2006). Regulatory agencies have already proposed such predictive tools for technological advancement studies to verify the fictitious toxicity of substances in the mammalian metabolic environment (Marchant, 2012), as well as to enable database creation of relationships between chemical substances and human health outcomes and relationships between chemical structure and biological activity (Auld *et al*., 2002).

PASS Online was used to anticipate the compound's biological action based on compounds similar to drugs. The Pa value (to be active) represented the likelihood of being active, whereas the Pi value represented the likelihood of being passive. The value ranges from 0 to 1,

and the higher the Pa value, the more accurate the forecast (Lagunin *et al*., 2018).

This biological activity prediction of a chemical molecule followed the premise that the structure is directly related to the activity. The parameter Pa (probability "to be active") expresses the likelihood that activity is present and calculates the likelihood that the investigated molecule falls into the category of active compounds. Hence, the estimated likelihood that the molecule would exhibit a certain activity increases as the computed Pa value approaches one. The way these forecasts were used and understood was flexible.

This study proved the antioxidant mechanism of the action of *F. religiosa* through *in vitro* and *in silico* experiments. However, *in vitro* experiments fail to replicate cell conditions in an organism because CAT activity and NO production were affected by many factors. This study could further research about in vitro and *in vivo* analyses. However, the findings of this study could be preliminary data of *F. religiosa* for other studies concerned with various diseases related to oxidative stress.

CONCLUSION

The current investigation indicates that the leaves of *F. religiosa* have antioxidant characteristics and could operate as free radical scavengers or inhibitors by reducing NO generation and increasing CAT activity. The activity was supported by some antioxidant agents present in the extract such as corchorifatty acid F, D-(-)-Quinic acid, 2,5-dihydroxybenzaldehyde and gentisic acid. Treating and preventing human damage from occurring due to free radicals would be easier with this kind of investigation. Therefore, studies are needed to better characterize the critical active constituents responsible for antioxidant activity.

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