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Original article

Antipyretic activity of *Caesalpinia digyna* (Rottl.) leaves extract along with phytoconstituent's binding affinity to COX-1, COX-2, and mPGES-1 receptors: *In vivo* and *in silico* approaches

Antipyretic activity of *Caesalpinia digyna* (Rottl.)

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ABSTRACT

Caesalpinia digyna (Rottl.) (Family: Fabaceae) is well known for its numerous medicinal values against several human disorders including fever, senile pruritis, diarrhea, tuberculosis, tonic disorder, diabetes, etc. The current study is intended to investigate the *in vivo* antipyretic activity of the methanol extract of *C. digyna* leaves (MECD) and its carbon-tetrachloride (CTCD) and butanol fraction (BTCD). Besides, *in silico* molecular docking and ADME/T profiling of the selective identified bioactive compounds of *C. digyna* has been also studied to validate the experimental outcomes and establish a better insight into the possible receptor-ligand interaction affinity. *In vivo* antipyretic activity of MECD, CTCD and BTCD were evaluated by employing yeast induced pyrexia technique in mice model and *in silico* analysis of the identified compounds of *C. digyna* has been implemented using PyRx autodock vina, Discovery Studio 2020, UCSF Chimera software and ADME/T online tools. MECD and BTCD unveiled significant antipyretic activity in dose dependent manner whereas, CTCD failed to exhibit significant antipyretic activity. Comparing to other test sample, MECD (400 mg/kg; b.w) ($p < 0.001$) displayed maximum inhibition of pyrexia. In molecular docking approach, docking score between −6.60 to −10.20 kcal/mol have been revealed. Besides, in ADME/T analysis, no compound violated the lipiniski's 5 rules and displayed any toxicity. Biological and computational approaches ascertain the ethno-botanical use of *C. digyna* as a good agent against pyrexia and the compounds of *C. digyna* are primarily proved as safe. Hereafter, further analysis is suggested to validate this research.

1. Introduction

Pyrexia (fever) is an expression of complex immune-physiologic condition triggered by a cascade of biochemical reactions responding to the infectious or inflammatory stimuli in the body (A. Muhammad et al. 2019). Fever is a physiological term referring to the increase of the body's core temperature in response to the introduction of pathogenic agents into the body (Blatteis 2006). Consequently, Inflammatory and infectious pathogens i.e., CRF, IL-6, ET-1, IL-1β, chemokines and particularly PGE2 lead to generate several endogenous pyrogens (Roth et al. 2009). The key eicosanoid component in the central nervous system during fever in

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mammals is prostaglandin E2 (PGE2) (Gaetano et al. 2010). Nevertheless, previous attempts to minimize PGE2 development were focused on inhibition of the isoforms of the cyclooxygenase (COX-1 and COX-2) enzyme (Malvar et al. 2014; Tavares and Miñano 2002). Prostaglandin synthesis is an essential element for fever responding to endogenous and exogenous pyrogens followed by COX-2 activation in brain endothelium (Islam 2019). However, latest antipyretic medicaments of COX-2 selective inhibitors may cause cardiovascular damage to the patients, whereas the non-selective inhibitors mediate detrimental gastrointestinal/renal system complications to the patients (Bacchi et al. 2012). Microsomal prostaglandin E synthase-1 (mPGES-1) is also a possible object owing to its terminal position in prostaglandin's biosynthesis pathway, which also responsible to catalyzes transformation of prostaglandin H2 (PGH2) to PGE2 (Koeberle and Werz 2015). Several studies recently examined the valuable targets for new antipyretic, anti-inflammatory, or anti-cancer agents for prostaglandin E2 synthases (PGES; i.e., mPGES-1, mPGES-2 and cPGES) (Lauro et al. 2017).

In current era, docking methodology became popular as a standard computational tool to find out novel active molecules and their binding affinities to the specific receptors (Parenti and Rastelli 2012). To have a better understanding of how small ligands bind with macromolecules, molecular docking is considered as an invaluable approach in pharmaceutical research and development (Blaney 2012). Docking facilitates simulated screening of broad libraries of compounds, ranking the results, and proposing structural hypotheses about how the ligands inhibit the target, which is incredibly valuable for lead optimization (Morris and Lim-Wilby 2008). Free energy landscape and best ligand poses were investigated using search algorithms are used during molecular docking (Huang and Caflisch 2011). Therefore, interaction affinities and binding modes of the biological molecules towards a particular target receptor were predicted employing structure-based strategy of ligand − receptor molecular docking (Guedes, de Magalhães, and Dardenne 2014). Medicinal plants are well known for offering substantial role in health systems around the globe for people as well as for animals to maintain good health from very ancient era. Herbal medications are the outputs of therapeutic herbs either preventing and treating infections and illnesses or to support health and healing (Pathak and Das 2013). Numerous recent investigations indicated significant understandings between the ethnopharmacological utilization of the herbs by natives and novel drug development to cure different disease conditions (Fabricant and Farnsworth 2001). The scope and uses of plants with such potentiality have been generally utilized by native healers in the treatment of different maladies (Konaté et al. 2015).

Caesalpinia digyna (Rottl.) is one of the valuable species in the Fabaceae family possessing notable ethnopharmacological importance. The species is usually found in Bangladesh, India, Nepal, Myanmar and southern part of China. It is a large, annual, stubborn climbing shrub and known as 'Kochoi' in Bengali and 'Teri Pods' in English. The plant mostly occurs in the forest areas among shrubberies on mountain inclines and seashores. This herb is a part of the native drug recipe 'Geriforte' (Emon, Jahan, and Sayeed 2020), which has passable efficacy in the treatment of senile pruritus. Ancient Indian practitioners had also found antipyretic, tonic, astringent and anti-respiratory properties within this species (Srinivasan et al. 2007; Emon, Alam, Sawon, et al. 2021). In terms of bioactive phytochemicals, this plant comprises of caesalpinine A, cesalpinine C, intricatinol, isointricatinol, E-8-methoxybonducelline, Z-8 methoxybonducelline, bonducellin, isobonducellin, e-eucomine and z-eucomine (Somendu et al. 2012; Mahato et al. 1985). Moreover, the stem of *C. digyna* is well known for having antidiabetic properties and also used to cure fever (Kumar et al. 2012). The powder of this plant is also used to heal diarrhea and other chronic fluxes by the indigenous communities in India and Bangladesh (S. K. Roy et al. 2012a; herbpathy). To our best knowledge, no experimental work has been performed till date to evaluate the antipyretic property of *C. digyna* leave extract which is it's most common ethnopharmacological use. The present study attempts to rationalize its indigenous use by investigating the *in vivo* anti-pyretic efficacy of methanol extract (MECD) of *C. digyna* leaves along with its carbon-tetrachloride fraction (CTCD) and butanol fraction (BTCD) in yeast-induced pyrexia method. Besides, computational analysis (molecular docking and ADME/T) has been implemented to characterize previously defined compounds of the plant.

2. Materials and methods

2.1. Drugs and Chemicals

Methanol, carbon-tetrachloride and butanol have been obtained from Sigma Chemicals Co. (St. Louis, MO, USA). Tween-80 was collected from BDH Chemicals Ltd. (Poole, UK). Indomethacin was obtained from Aristopharma Ltd (Dhaka, Bangladesh). Yeast was found from Angel Yeast Co. (China). Therefore, analytical grade chemicals were used throughout time frame of the study.

2.2. Collection and identification of the plant

For this present research, plant sample (leaves) of *C. digyna* was collected in February 2019 from Sonaichori area of Sitakund Upazila (sub-district), Chattogram (previously known as Chittagong), Bangladesh. The plant specimens were appropriately identified by Mr. Sajib Rudra, Taxonomist, Department of Botany, University of Chittagong, Chattogram-4331, Bangladesh (Accession number: CTGUH SR 2793). The plant leaves were initially cleaned by systematic wash with tap water. Then the plant materials were dried in natural shade for fourteen days to protect the thermo labile phytoconstituents and the temperature in natural shade was kept constant at 23 ± 2 °C (room temperature). Thereafter, the plant materials were further dried in the oven for 1 h at considerably low temperature (40 ^{0}C) for better grinding. Then dry materials were milled and grinded to powder substances utilizing a high-capacity grinding machine. At that point, powdered plant materials were stored in cool, dark, ventilated, and well-closed plastic container.

2.3. Extraction of the plant material

Around 500 gm of the dried powder of plant materials were soaked in 2.5 L of methanol in a separate clean glass compartment. The materials in container were kept for 14 days at 23 ± 2 °C by fixing the container with aluminum foil and a container lid and were going for shaking and mixing occasionally. The materials were filtered through cotton using no. 1 Whatman double rings filter paper (Bibby RE200, Sterilin Ltd., UK) and the filtrates were bathed and concentrated at 40 °C to evaporate the solvent. Then 18 g crude methanol was extracted from leaves. Finally, rest of the fractions (CTCD, BTCD) had been extracted from crude methanol by following Kupchan partitioning method (Qureshi et al. 2019). The crude extract and fractions were preserved at the refrigerator (4^{0}C).

2.4. Animals and ethical consideration

Swiss albino mice of around 25–30 g weight and 7–8 weeks aged were collected from BCSIR (Bangladesh Council of Scientific and Industrial Research), Chattogram, Bangladesh. The collected

mice were facilitated with laboratory grade foods, mineral water, proper ventilated house and natural day-night cycle was also maintained. All the animals were kept at plastic cases under the 20 ± 2 °C temperature and facilitated with a 12 h light–dark cycle. All the examinations were carried out in isolated and silent condition. The mice were acclimatized for 10 days prior of experiment in laboratory conditions. The handling and taking care of animals was carried out by universal rules for the utilization and maintenance of experimental animals (Care, Animals, and Resources 1985).

2.5. Acute oral toxicity test

All the experiments during the study were carried under standard laboratory conditions following guidelines of Organization for Environmental Control Development (Viran et al. 2003). The mice were grouped as 10 in each and mice in each group received oral doses of 1000, 2000 and 3000 mg/kg of MECD, CTCD and BTCD, while vehicle was supplied to the control group. The experiment was continued for 48 h and the typical behavior and any sign of harmness of mice was monitored in regular interval and changes of body weight were also noted every day.

2.6. Yeast-induced pyrexia

Fever induction by Brewer's yeast method was applied in experimental animals by injecting 20% yeast suspension subcutaneously with a dose of 10 mL/kg which were kept under fasting condition overnight (Loux, DePalma, and Yankell 1972; Emon, Alam, Rudra, et al. 2021). The primary temperature was recorded via rectal route with a digital thermometer. After a time period of 18 h, the animal models which showed a rectal temperature uplifting of 0.3 – 0.5 °C (32.54 to 32.9 °F) were chosen for antipyretic activity testing. The plant extract of different concentrations i.e. 50, 100, 200 and 400 mg/kg body weight were given to conduct the test along with indomethacin (4 mg/kg) as reference drug and TWN–80 was provided to animal models of control group. After this treatment, rectal temperature was again recorded at an interval of one hour for three hours straight (Abena et al. 2003) and every reading was documented as a mean value of three readings.

2.7. In silico study

2.7.1. Molecular docking: Protein preparation

Three dimensional crystal structure includes cyclooxygenase-1 (PDB ID: 3N8V) (Sidhu et al. 2010), human cyclooxygenase-2 (PDB ID: 5KIR, 3PGH) (Orlando and Malkowski 2016; Kurumbail et al. 1996), mPGES-1 (PDB ID: 5YK5) (Luz et al. 2015) have been retrieved in PDB format from RCBS Protein Data Bank (https://www.rcsb.org/structure). The proteins were being free of water and heteroatom employing Discovery studio 2020 (S. Alam et al. 2020). Gasteiger charge was assigned, and non-polar hydrogens were combined with proteins for further arrangement. Moreover, all the proteins were converted to the least energy state in Gasteiger mode of UCSF Chimera keeping standard residues in AMBER ff14sB before further analyses (Shapovalov and Dunbrack Jr 2011).

2.7.2. Molecular docking: Ligand preparation

The structure of five selected molecules isolated (Fig. 1) from *C. dignya* leaves namely Intricatinol (PubChem CID: 6439175), Bonducellin (PubChem CID: 14079439), Isobonducellin (PubChem CID: 10423880), Caesalpinine A (PubChem CID: 5458904), Caesalpinine C (PubChem CID: 100949741) have been retrieved from the PubChem database. The ligands (2DSDF format) were downloaded and then minimized and converted (pdbqt format) to quest best optimal hit against mentioned targets using PyRx tools. The virtual screening was carried out using default settings of PyRx (Herowati and Widodo 2014; S. Alam, Rashid, et al. 2021) retrieved from MGLTools (https://ccsb.scripps.edu/mgltools/).

2.7.3. Molecular docking: Docking analysis

The protein–ligand linking process of the selected protein–ligand complexes were achieved by using PyRx Autodock Vina with a semi-flexible docking system (Herowati and Widodo 2014; M.M. Alam, Emon, et al. 2021). Transformation of proteins and other molecules from PDB to PDBQT format were done using PyRx AutoDock software. The rigidity of proteins and the flexibility of ligands were maintained throughout the study. Ligand molecules were possessed of 10 degrees of liberty. Ttransformation of the molecules into pdbqt format, sort of box, grid box creation, etc were defined by AutoDock. During transformation, grid box was created with an active site in the center of the box. In the end, the docking positions for the best linking approaches were assessed using BIOVIA Discovery Studio Visualizer 2020 (Biovia 2017).

2.7.4. Ligand based pharmacokinetics and toxicity measurement

The pharmacokinetic properties of three major compounds were determined using an online tool, SwissADME (http://www.swissadme.ch/). Favorable drug-like properties of all compounds were evaluated following Lipinski's rule of five (M.W not more than 500; H-bond donors $\leq$ 5; H-bond acceptors $\leq$ 10; Lipophilicity < 5 and molar refractivity ranging from 40 to 130) (Lipinski et al. 1997). Moreover, an online web tool admet SAR (http://lmmd.ecust.edu.cn/admetsar2) were employed to determine toxicological properties of the compounds.

3. Statistical analysis

Statistical analysis of data was controlled using Graph pad prism version 5.0. and exhibited as mean ± SEM (Standard Error Mean). One-way Analysis of variance (ANOVA) was used to dictate statistical significance and accomplished by Dunnett's multiple comparison tests. Significance level were considered when P-value<0.05, 0.01 and 0.001. Therefore, dose dependent antidiarrheal activity was observed applying linear regression analysis based on suitability.

4. Results

4.1. Acute toxicity of MECD, CTCD and BTCD

Morphological characteristics (fur, face, eyes and nose) of the experimented animals have not been changed in acute oral toxicity study. Physiological characteristics such as tremors, hallucinations, salivations, diarrhea lethargy have not been observed and behavioral characteristics such as self-mutation, regressive actions, gait and attitude, sensory stimuli etc. were also natural. The weight was remaining unchanged in both control and the treatment groups. Food and water supplies were same to all experimented animals. In such controlled conditions, effects of MECD, CTCD and BTCD were steady till 3000 mg/kg of body weight dose. Henceforth, MECD, CTCD and BTCD (50, 100, 200 and 400 mg/kg) doses were chosen for antipyretic activity.

4.2. Effect of MECD, CTCD and BTCD on yeast induced pyrexia

Upon the administration of brewer yeast suspension through subcutaneous route, rectal temperature was highly raised after 18 h. Significant (p < 0.05, 0.01, 0.001) dose dependent pyrexia inhibition has been observed in the treated mice upon the application of indomethacin (4 mg/kg, i.p), MECD and BTCD (100, 200 and

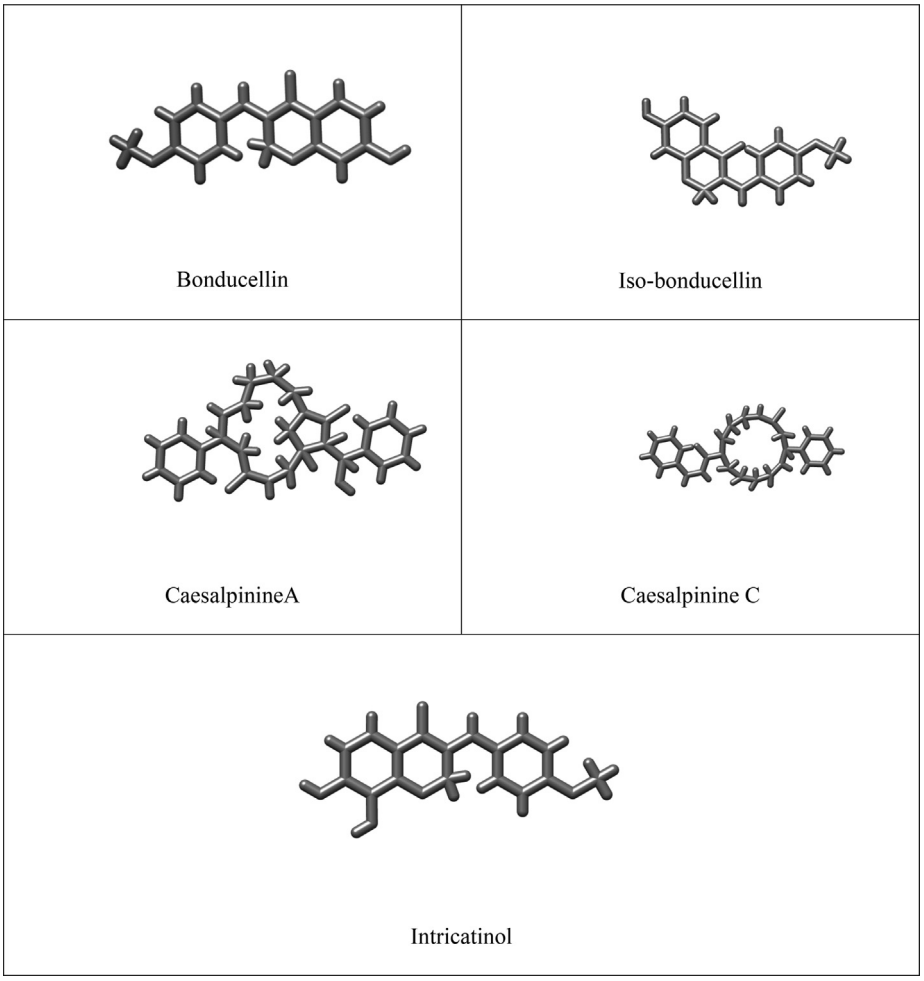


Fig. 1. Structure of all selected phytoconstituents (Bonducellin, Isobonducellin, Caesalpinine A, Caesalpinine C, Intricatinol).

Table 1
Report on inhibition of Brewer's yeast induced pyrexia by MECD, CTCD, BTCD and control samples in mice model.

Treatment	Dose (mg/kg-b.w; p.o)	Normal rectal temperature (^{O}F)	Rectal temperature (^{O}F) after yeast administration	Rectal temperature (^{O}F) after drug administration		
				60 min	120 min	180 min
TWN − 80	10 mL/kg	97.24 ± 0.23	103.13 ± 0.86	104.90 ± 0.34$^{\#}$	104.21 ± 0.23$^{\#}$	104.34 ± 0.36$^{\#}$
IDM	4 (i.p)	98.25 ± 0.32	104.20 ± 0.73	97.17 ± 1.54***	97.18 ± 0.47***	97.53 ± 0.83***
MECD	50	97.90 ± 0.45	104.10 ± 1.20	104.24 ± 0.77	105.48 ± 0.54	103.41 ± 0.45
CTCD	50	97.24 ± 0.54	105.44 ± 0.72	105.38 ± 1.58	105.78 ± 0.65	105.34 ± 0.84
BTCD	50	98.12 ± 0.82	104.50 ± 0.28	105.23 ± 0.16	105.44 ± 0.48	104.10 ± 1.25
MECD	100	96.88 ± 1.25	104.66 ± 1.24	103.36 ± 0.80*	103.12 ± 0.48*	103.56 ± 0.59*
CTCD	100	96.50 ± 0.56	104.22 ± 0.33	104.60 ± 1.88	104.34 ± 0.67	104.00 ± 0.74
BTCD	100	97.25 ± 0.44	104.87 ± 0.44	103.36 ± 0.64	103.20 ± 1.45	103.44 ± 0.50
MECD	200	98.40 ± 0.65	104.02 ± 1.76	102.24 ± 0.65**	101.12 ± 0.46**	101.78 ± 0.54**
CTCD	200	98.15 ± 0.32	105.62 ± 1.70	105.44 ± 0.33	104.20 ± 0.27	104.25 ± 1.20
BTCD	200	97.85 ± 0.76	105.42 ± 0.68	103.22 ± 0.90*	101.20 ± 1.64*	102.82 ± 0.56**
MECD	400	95.45 ± 0.44	105.10 ± 1.2	99.74 ± 0.44**	97.18 ± 0.54***	98.00 ± 0.60***
CTCD	400	96.23 ± 0.36	104.41 ± 1.07	103.88 ± 0.80	103.80 ± 0.44	103.22 ± 1.68*
BTCD	400	98.60 ± 0.66	104.66 ± 0.90	100.88 ± 0.63***	98.68 ± 1.30***	98.85 ± 0.74***

All the values are presented as mean ± SEM; One-way analysis of variance (ANOVA) followed by Dunnett's test. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ is considered as significant compared with the control, where # is designated as control. MECD = methanol extract of *Caesalpinia digyna,* CTCD = carbon tetrachloride fraction of *Caesalpinia digyna,* BTCD = butanol fraction of *Caesalpinia digyna,* TWN = 1% Tween 80, and IDM = Indomethacin.

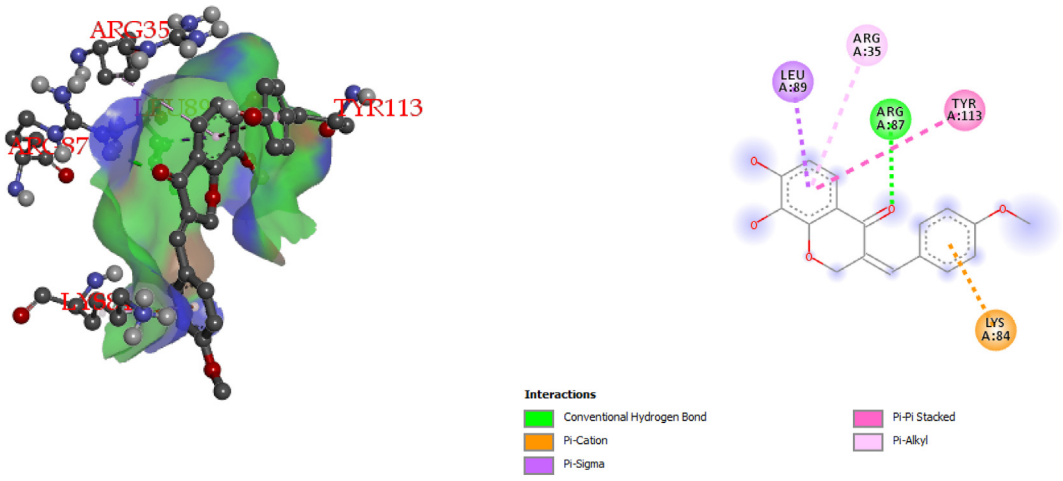
400 mg/kg). In contrast, CTCD failed to reduce yeast-induced pyrexia on mice model. The maximum inhibition of pyrexia has been by MECD 400 mg/kg which is close to the prescribed dose of Indomethacin-4 mg/kg (table 1).

4.3. Molecular docking analysis

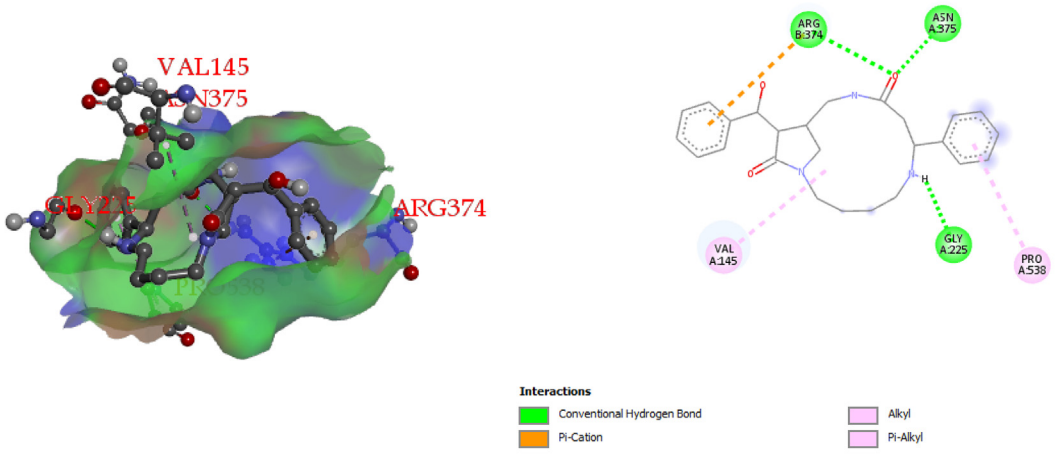
The Antipyretic activity has been investigated by docking analysis and shown in table 2 and Fig. 2, where three receptors namely

Table 2
Docking scores of the experimental compounds for the antipyretic activity.

Docking Score				
Compounds	**Anti-pyretic activity**			
	COX-1	COX-2		mPGES-1
	3N8V	5KIR	3PGH	4YK5
Intricatinol	−8.5	−8.6	−7.0	**−8.0**
Bonducellin	−8.3	−8.2	−6.7	−7.8
Isobonducellin	−8.5	−8.0	−6.6	−7.4
Caesalpinine A	**−9.9**	**−10.2**	**−8.9**	−7.3
Caesalpinine C	−8.6	−9.1	−8.2	−7.9



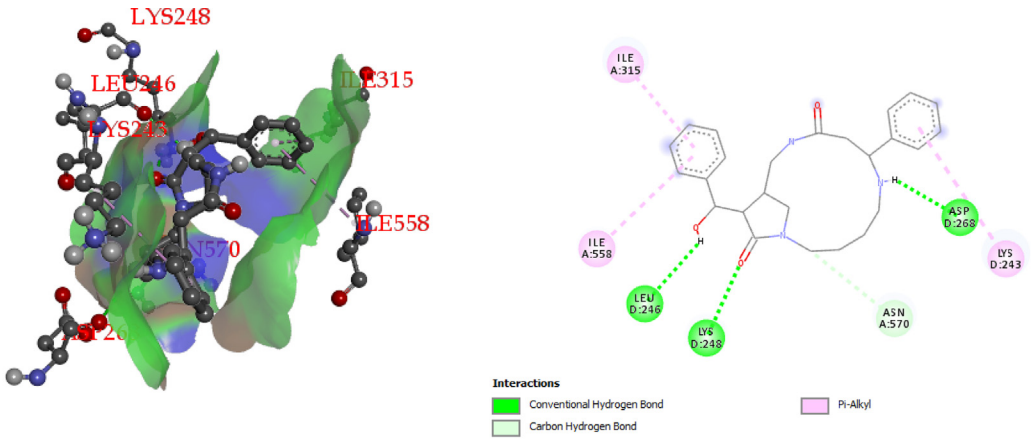
(a) 4KY5 - Intricatinol



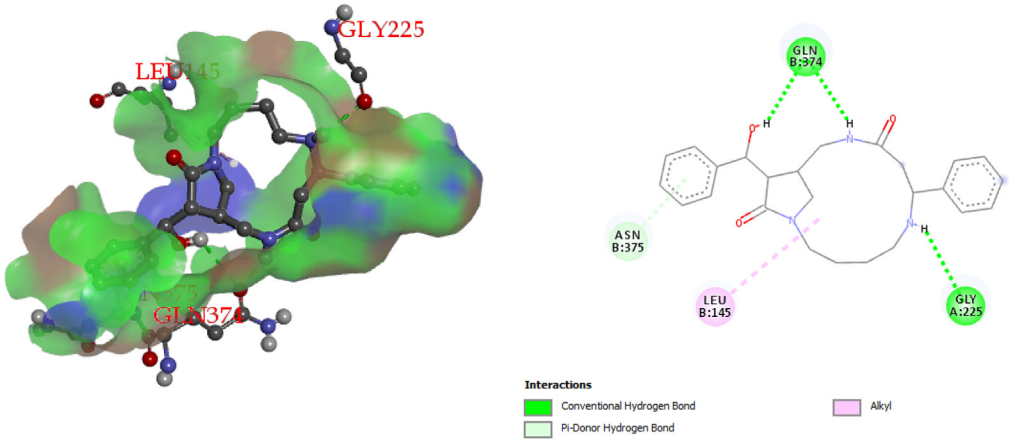
(b) 3N8V - Caesalpinine A

Fig. 2. The best ligand- receptor interactions (a, b, c, and d represent 4KY5 – Intricatinol, 3N8V - Caesalpinine A, 3PGH - Caesalpinine A and 5KIR - Caesalpinine interactions respectively) presented in 3D and 2D view.

cyclooxygenase-1 (PDB ID: 3N8V), cyclooxygenase-2 (PDB ID: 5KIR and 3PGH) and microsomal prostaglandin E synthase-1 (4YK5) were employed to find out binding affinities with some particular compounds of *C. digyna*. Caesalpinine A shows best binding affinity with cyclooxygenase-1 (3N8V) and cyclooxygenase-2 (5KIR, 3PGH) proteins. The docking score on 3N8V ranked as Caesalpinine A > Caesalpinine C > Intricatinol > Isobonducellin > Bonducellin. The docking ranking for 5KIR and 3PGH were same. The ranking



(c) 3PGH - Caesalpinine A



(d) 5KIR - Caesalpinine A

Fig. 2 (*continued*)

of binding affinity for the 5KIR and 3PGH was as follows: Caesalpinine A > Caesalpinine C > Intricatinol > Bonducellin > Isobonducellin. The best interaction of 3N8V with the Caesalpinine A was possessed through the conventional hydrogen bond (arg374, asn375 and gly225), alkyl (val145) and pi-alkyl (pro538) bonds. Caesalpinine A also shown best binding affinity to the 5KIR and 3PGH through a series of bonds: Caesalpinine A – 5KIR (leu145, asn375, gln374 and gly225) and Caesalpinine A – 3PGH (ile558, ile315, lys243, asp268, asn570, lys248 and leu246) respectively. Besides, Intricatinol yielded best binding interaction with the mPGES-1 (4YK5) by the formation of series of bonds namely: conventional hydrogen bond (arg87), pi-cation (lys84), pi-sigma (arg35), pi-pi stacked (tyr113 and pi-alkyl (leu89). The ranking of the docking score on mPGES-1 were follows: Intricatinol > Caesalpinine C > Bonducelline > Isobonducellin > Caesalpinine A.

4.4. ADME/T analysis

An online tool SwissADME was used employing Lipinski's rules of five to calculate the pharmacokinetic characteristics of the selected compounds. According to declaration of the Lipinski, A drug has to follow some criteria (molecular weight < 500 amu, Hydrogen bond donor < 5, Hydrogen bond acceptor sites ≤ 10 and Lipophilicity value LogP ≤ 5) to be orally bioavailable. As per declaration of the Lipinski, all the experimented compounds show strong oral bioavailability. Prediction about toxicological properties admet SAR, an online tool also showed that the selected compounds (Intricatinol, Bonducellin, Isobonducellin, Caesalpinine A, Caesalpinine C) of *C. digyna* were neither toxic nor carcinogenic (table 2). Hereafter, these compounds were proven biologically stable as a drug source.

5. Discussion

Literature searches revealed that, some isolated phytocompounds of *C. digyna* such as bergenin, glutathione and flavonoids were attributed to their anti-inflammatory and antioxidant properties as active substances (Chaudhury 1957; S. Roy et al. 2012b; Somendu et al. 2012). In addition, caesalpinine A, cellallocinie, gallic acid, ellagic acid, pipecolic acid and tannin, isolated from *C. digyna* have been reported to demonstrate antimicrobial, antitubercular, antidiabetic, radioprotective, hypoglycemic and hypolipidemic properties (S. Roy et al. 2012b; Narkhede et al. 2011; Srinivasan et al. 2007). This study aimed to evaluate the antipyretic potential of *C. digyna* leaves extract by administering subcutaneous injection of brewer's yeast which can result in prostaglandin synthesis. This method is gaining considerable

Table 3
Absorption, digestion, metabolism, excretion, and toxicological (ADME/T) properties of the compounds for good oral bioavailability.

Molecules	PD	MW (g/mol)	HBD	HBA	LogP (o/w)	HIA	AM	CAR (binary)	PPB	AOT (kg/moL)
Intricatinol	6,439,175	298.29	2	5	2.77	0.9911	0.5600	0.9078	1.243	2.041
Bonducellin	14,079,439	282.29	1	4	3.06	0.9941	0.5600	0.8936	1.13	1.75
Isobonducellin	10,423,880	282.29	1	4	3.06	0.9941	0.5600	0.8936	1.13	1.75
Caesalpinine A	5,458,904	421.5	3	4	2.43	0.9687	0.6000	0.8429	0.932	2.501
Caesalpinine C	100,949,741	405.5	2	3	3.55	0.5714	0.6900	0.8286	0.923	1.779

PD = PubChem ID, MW = Molecular Weight (acceptance range : ⟨5 0 0), HBD = Hydrogen Bond Donor (acceptance range: $\leq$ 5), HBA = Hydrogen Bond Acceptor: (acceptance range: $\leq$ 10), LogP = High Lipophilicity (acceptance range : < 5), HIA = Human Intestinal Absorption, AM = AMES Mutagenesis, CAR = Carcinogens, PPB = Plasma Protein Binding, AOT = Acute Oral Toxicity.

attention for the identification of plant materials and synthetic pharmaceutical goods for their antipyretic effect (N. Muhammad, Saeed, and Khan 2012). Yeast-induced pyrexia is responsible for pathogenic fever whereas, secretion of prostaglandins has been considered as its etiology (Moltz 1993). The possible mechanism of antipyretic action might be inhibition of prostaglandin synthesis like paracetamol blocked the syntheses of prostaglandin by diminishing cyclooxygenase enzyme's activity. Antipyretic activity might be denoted by inhibition of pyrexia inducing mediators (N. Muhammad, Saeed, and Khan 2012). Besides, NSAIDs, non-steroidal anti-inflammatory antipyretic drugs due to its inhibition capability of prostaglandin synthesis can also exert antipyretic efficacy (Vane and Botting 1998). Hence, MECD and BTCD might be considered as antipyretic agent as it possessed of bioactive phytochemicals, capable to inhibit the prostaglandin synthesis and to reduce the rectal temperature induced by yeast in mice model. Nowadays, investigation through molecular docking has become a popular method to investigate ligand-receptor binding affinities and to understand biological activity of the natural products. Molecular docking is a common and highly applied approach in the field of drug development. There are several articles detailing a wide range of approaches in which the discoveries of novel bioactive molecules are defined (Ferreira et al. 2015). In structure-based virtual scanning, the consolidated database is docked to a pre-selected target site (Lionta et al. 2014) and the ligand-based virtual screening approach is based on the examination of molecular descriptors derived from recognized active compounds (Geppert, Vogt, and Bajorath 2010). It is an effective method to achieve better knowledge on binding mechanisms and binding potentiality of different proteins (Khan et al. 2019). Considering the facts, obtained experimental results in biological study were further evaluated and validated by analyzing through molecular docking. Hence, analysis through molecular docking provides deeper insights in terms of ascertain the antipyretic activity of the five representative molecules of the *C. digyna*. In antipyretic molecular docking study, several selected compounds showed docking values between −6.60 to −10.20 kcal/mol indicate that, they are possessed of anti-pyretic properties (table 2**)**. Among these compounds, Caesalpinine A, Caesalpinine C, and Intricatinol have been proven highly active to the COX – 1, COX – 2 and mPGES −1. They showed best binding interactions with the reactants (enzymes/receptors) through the group of residues. Moreover, oral bioavailability of the selected compounds was proven following the declaration of Lipinski's five rules **(**table 3**)**. Toxicological properties of the selected phytochemical were evaluated using admetSAR and ensuring safety of the compounds, which is vital criterion to be a good drug candidate (Cheng et al. 2012; Emon, Alam, Sawon, et al. 2021). Again, the pharmacokinetic/drug likeliness, least toxicity and oral bioavailability of the experimented compounds were significant in terms of biological review, new drug development and clinical trial (Lipinski 2004).

6. Conclusion

The study was aimed to validate the conventional application of *C. digyna* as an antipyretic candidate. Comparing to the molecular docking and the biological experiments, the finding confirms the antipyretic activity of *C. digyna*. In our investigation, it can be inferred that, MECD and BTCD can be the source of antipyretic therapies and the research also agrees with the folk application of this species. Further extensive biological and clinical research on the experimented compounds is required to ensure efficacy and safety profile of selected phytocompounds. In addition, advanced studies are warranted to establish *C. digyna* as a potential antipyretic agents for human welfare and also to reveal the exact mechanism of displayed antipyretic action.

Ethical consideration

The assessment of pharmacological activities has been conducted in accordance with the ethical guidelines outlined in the Declaration of Helsinki 2013 (Millum, Wendler, and Emanuel 2013). Animal models were preserved, administered, and euthanized in accordance with the American Veterinary Medical Association for Animal Euthanasia: 2013 (Leary et al. 2013).

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CRediT authorship contribution statement

Nazim Uddin Emon: Conceptualization, Investigation, Data curation, Formal analysis, Writing- original draft, Writing - review & editing. **Safaet Alam:** Methodology, Formal analysis, Software, Writing - original draft, Writing- review & editing. **Sajib Rudra:** Conceptualization, Methodology, Resources, Validation, Visualization. **Ibrahim Khalil Al Haidar:** Visualization, Writing- Review & editing. **Mohammed Farhad:** Investigation, Data Curation, Validation. **Md. Ezazul Hoque Rana:** Investigation, Data curation, Formal analysis. **Amlan Ganguly:** Conceptualization, Supervision, Project administration, Validation.

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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References

Abena, A.A., Diatewa, M., Gakosso, G., Gbeassor, M., Hondi-Assah, T.H., Ouamba, J.M., 2003. Analgesic, antipyretic and anti-inflammatory effects of essential oil of Lippia multiflora. Fitoterapia 74 (3), 231–236.

Alam, Safaet, Nazim Uddin Emon, Saimon Shahriar, Fahmida Tasnim Richi, Mohammad Rashedul Haque, Mohammad Nazmul Islam, Shahenur Alam Sakib, Amlan Ganguly, Pharmacological and computer-aided studies provide new insights into Millettia peguensis Ali (Fabaceae), Saudi Pharmaceutical J. (2020).

Bacchi, S., Palumbo, P., Sponta, A., Coppolino, M.F., 2012. Clinical pharmacology of non-steroidal anti-inflammatory drugs: a review. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents) 11 (1), 52–64.

Biovia, Dassault Systèmes, 2017. Discovery studio visualizer. San Diego, CA, USA, p. 936.

Blaney, Jeff, 2012. A very short history of structure-based design: how did we get here and where do we need to go?. J. Comput. Aided Mol. Des. 26 (1), 13–14.

Blatteis, Clark M., 2006. Endotoxic fever: new concepts of its regulation suggest new approaches to its management. Pharmacol. Ther. 111 (1), 194–223.

Care, Institute of Laboratory Animal Resources. Committee on, Use of Laboratory Animals, and National Institutes of Health. Division of Research Resources (1985). Guide for the care and use of laboratory animals. National Academies.

Chaudhury, G.R., 1957. Chemical Examination of the Roots of Caesalpinia digyna. Identy of Vakerin with Bergenin. J. Sci. Indust. Res. (India) B 16, 511.

Cheng, Feixiong, Li, Weihua, Zhou, Yadi, Shen, Jie, Zengrui, Wu., Liu, Guixia, Lee, Philip W, Tang, Yun (Eds.), 2012. admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. ACS Publications.

Emon, Nazim Uddin, Jahan, Israt, Sayeed, Mohammed Aktar, 2020. Investigation of antinociceptive, anti-inflammatory and thrombolytic activity of Caesalpinia digyna (Rottl.) leaves by experimental and computational approaches. Adv. Traditional Med., 1–9.

Fabricant, Daniel S, Farnsworth, Norman R, 2001. The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect. 109 (suppl 1), 69–75.

Ferreira, Leonardo G., Dos Santos, Ricardo N., Oliva, Glaucius, Andricopulo, Adriano D., 2015. Molecular Docking and Structure-Based Drug Design Strategies. Molecules 20 (7), 13384–13421. https://www.mdpi.com/1420-3049/20/7/13384.

Gaetano, L., Watanabe, K., Barogi, S., Coceani, F., 2010. Cyclooxygenase-2/microsomal prostaglandin E synthase-1 complex in the preoptic-anterior hypothalamus of the mouse: involvement through fever to intravenous lipopolysaccharide. Acta Physiol. 200 (4), 315–324.

Geppert, Hanna, Vogt, Martin, Bajorath, Jürgen, 2010. Current trends in ligand-based virtual screening: molecular representations, data mining methods, new application areas, and performance evaluation. J. Chem. Inf. Model. 50 (2), 205–216.

Guedes, Isabella A, de Magalhães, Camila S, Dardenne, Laurent E, 2014. Receptor–ligand molecular docking. Biophys. Rev. 6 (1), 75–87.

herbpathy. https://herbpathy.com/Uses-and-Benefits-of-Caesalpinia-Digyna-Cid6634.

Herowati, Rina, Widodo, Gunawan Pamudji, 2014. Molecular Docking studies of chemical constituents of Tinospora cordifolia on glycogen phosphorylase. Procedia Chem 13, 63–68.

Huang, Danzhi, Caflisch, Amedeo, 2011. The free energy landscape of small molecule unbinding. PLoS Comput Biol 7, (2) e1002002.

Islam, Muhammad Torequl, 2019. Antipyretic effect of phytol, possibly via 5KIR-dependent COX-2 inhibition pathway. Inflammopharmacology 27 (4), 857–862.

Khan, Saima, Nazir, Mamona, Raiz, Naheed, Saleem, Muhammad, Zengin, Gokhan, Fazal, Gazala, Saleem, Hammad, Mukhtar, Mahreen, Tousif, Muhammad Imran, Tareen, Rasool Baksh, 2019. Phytochemical profiling, in vitro biological properties and in silico studies on Caragana ambigua stocks (Fabaceae): A comprehensive approach. Ind. Crops Prod. 131, 117–124.

Koeberle, Andreas, Werz, Oliver, 2015. Perspective of microsomal prostaglandin E2 synthase-1 as drug target in inflammation-related disorders. Biochem. Pharmacol. 98 (1), 1–15.

Konaté, Kiessoun, Kassi Yomalan, Oksana Sytar, Marian Brestic, Antidiarrheal and antimicrobial profiles extracts of the leaves from Trichilia emetica Vahl. (Meliaceae), Asian Pacific J. Tropical Biomed. 5 (3) (2015) 242–248. https://doi.org/https://doi.org/10.1016/S2221-1691(15)30012-5. http://www.sciencedirect.com/science/article/pii/S2221169115300125.

Kumar, Rajesh, Patel, Dinesh K, Prasad, Satyendra K, Laloo, Damiki, Krishnamurthy, Sairam, Hemalatha, S., 2012. Type 2 antidiabetic activity of bergenin from the roots of Caesalpinia digyna Rottler. Fitoterapia 83 (2), 395–401.

Kurumbail, Ravi G, Stevens, Anna M, Gierse, James K, McDonald, Joseph J, Stegeman, Roderick A, Pak, Jina Y, Gildehaus, Daniel, Penning, Thomas D, Seibert, Karen, Isakson, Peter C, 1996. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature 384 (6610), 644–648.

Lauro, Gianluigi, Manfra, Michele, Pedatella, Silvana, Fischer, Katrin, Cantone, Vincenza, Terracciano, Stefania, Bertamino, Alessia, Ostacolo, Carmine, Gomez-Monterrey, Isabel, De Nisco, Mauro, 2017. Identification of novel microsomal prostaglandin E2 synthase-1 (mPGES-1) lead inhibitors from Fragment Virtual Screening. Eur. J. Med. Chem. 125, 278–287.

Leary, Steven L, Underwood, Wendy, Raymond Anthony, S., Cartner, D Corey, Grandin, T., Greenacre, C., Gwaltney-Brant, S., McCrackin, M.A., Meyer, R., 2013. "AVMA guidelines for the euthanasia of animals.

Lionta, Evanthia, Spyrou, George, Vassilatis, Demetrios K, Cournia, Zoe, 2014. Structure-based virtual screening for drug discovery: principles, applications and recent advances. Curr. Top. Med. Chem. 14 (16), 1923–1938.

Lipinski, Christopher A., 2004. Lead-and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technol. 1 (4), 337–341.

Lipinski, Christopher A, Lombardo, Franco, Dominy, Beryl W, Feeney, Paul J, 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. 23 (1–3), 3–25.

Loux, J.J., DePalma, P.D., Yankell, S.L., 1972. Antipyretic testing of aspirin in rats. Toxicol. Appl. Pharmacol. 22 (4), 672–675.

Luz, John Gately, Antonysamy, Stephen, Kuklish, Steven L, Condon, Bradley, Lee, Matthew R, Allison, Dagart, Xiao-Peng, Yu., Chandrasekhar, Srinivasan, Backer, Ryan, Zhang, Aiping, 2015. Crystal structures of mPGES-1 inhibitor complexes form a basis for the rational design of potent analgesic and anti-inflammatory therapeutics. J. Med. Chem. 58 (11), 4727–4737.

Mahato, Shashi B, Sahu, Niranjan P, Müller, Eveline, Luger, Peter, 1985. Stereochemistry of a macrocyclic spermidine alkaloid from Caesalpinia digyna Rottl. X-Ray determination of the structure of caesalpinine C (celallocinnine). Journal of the Chemical Society, Perkin Transactions 2 (2), 193–196.

David do C. Malvar, Fernando A. Aguiar, Artur de LL. Vaz, Débora CR. Assis, Miriam CC. de Melo, Valquíria AP. Jabor, Evanguedes. Kalapothakis, Sérgio H. Ferreira, Giuliano C. Clososki, Glória EP. de Souza, Dipyrone metabolite 4-MAA induces hypothermia and inhibits PGE 2-dependent and-independent fever while 4-AA only blocks PGE 2-dependent fever, British J. Pharmacol. 171 (15) (2014) 3666–3679.

Millum, Joseph, Wendler, David, Emanuel, Ezekiel J, 2013. The 50th anniversary of the Declaration of Helsinki: progress but many remaining challenges. JAMA 310 (20), 2143–2144.

Moltz, Howard, 1993. Fever: causes and consequences. Neurosci. Biobehav. Rev. 17 (3), 237–269.

Morris, Garrett M, Lim-Wilby, Marguerita, 2008. Molecular docking. In: Molecular modeling of proteins. Springer, pp. 365–382.

Muhammad, Akhtar, Khan, Behramand, Iqbal, Zafar, Khan, Amir Zada, Khan, Inamullah, Khan, Kashif, Alamzeb, Muhammad, Ahmad, Nasir, Khan, Khalid, Badshah, Syed Lal, 2019. Viscosine as a Potent and safe antipyretic agent evaluated by yeast-induced pyrexia model and molecular docking studies. ACS Omega 4 (10), 14188–14192.

Muhammad, Naveed, Saeed, Muhammad, Khan, Haroon, 2012. Antipyretic, analgesic and anti-inflammatory activity of Viola betonicifolia whole plant. BMC Complementary Alternative Med. 12 (1), 59.

Narkhede, M.B., Ajimire, P.V., Wagh, A.E., Mohan, Manoj, Shivashanmugam, A.T., 2011. In vitro antidiabetic activity of Caesalpina digyna (R.) methanol root extract. Asian J. Plant Sci. Res. 1 (2), 101–106.

Orlando, Benjamin J, Malkowski, Michael G, 2016. Crystal structure of rofecoxib bound to human cyclooxygenase-2. Acta Crystallographica Section F: Struct. Biol. Commun. 72 (10), 772–776.

Parenti, Marco Daniele, Rastelli, Giulio, 2012. Advances and applications of binding affinity prediction methods in drug discovery. Biotechnol. Adv. 30 (1), 244–250.

Pathak, Kalyani, Das, Ratna Jyoti, 2013. Herbal medicine-a rational approach in health care system. Int. J. Herbal Med. 1 (3), 86–89.

Huma. Qureshi, Tauseef. Anwar, Qasim. Ali, Muhammad Zulqurnain. Haider, Noman. Habib, Sammer. Fatima, Muhammad. Waseem, Yamin. Bibi, Muhammad. Arshad, Steve William. Adkins, Isolation of natural herbicidal compound from Lantana camara, Int. J. Environm. Anal. Chem. (2019) 1–8.

Roth, Joachim, Rummel, Christoph, Barth, Stephan W, Gerstberger, Rüdiger, Hübschle, Thomas, 2009. Molecular aspects of fever and hyperthermia. Immunol. Allergy Clinics 29 (2), 229–245.

Roy, S.K., Agrahari, U.C., Gautam, R., Srivastava, A., Jachak, S.M., 2012. Isointricatinol, a new antioxidant homoisoflavonoid from the roots of Caesalpinia digyna Rottler. Nat. Prod. Res. 26 (8), 690–695. https://doi.org/10.1080/14786419.2010.548813. 10.1080/14786419.2010.548813.

Somendu K. Roy, Amit. Srivastava, M Jachak. Sanjay, Analysis of homoisoflavonoids in Caesalpinia digyna by HPLC-ESI-MS, HPLC and method validation. Natural Product Commun. 7 (9) (2012) 1934578X1200700922.

Shapovalov, Maxim V, Dunbrack Jr, Roland L, 2011. A smoothed backbone-dependent rotamer library for proteins derived from adaptive kernel density estimates and regressions. Structure 19 (6), 844–858.

Sidhu, Ranjinder S, Lee, Jullia Y, Yuan, Chong, Smith, William L, 2010. Comparison of cyclooxygenase-1 crystal structures: cross-talk between monomers comprising cyclooxygenase-1 homodimers. Biochemistry 49 (33), 7069–7079.

Srinivasan, R.M.J.N., Chandrasekar, M.J.N., Nanjan, M.J., Suresh, B., 2007. Antioxidant activity of Caesalpinia digyna root. J. Ethnopharmacol. 113 (2), 284–291.

Tavares, Eva, Miñano, Francisco J, 2002. Differential sensitivities of pyrogenic chemokine fevers to cyclooxygenase isozymes antibodies. Brain Res. Bull. 59 (3), 181–187.

Vane, J.R., Botting, R.M., 1998. Anti-inflammatory drugs and their mechanism of action. Inflamm. Res. 47 (2), 78–87.

Viran, Rukiye, Erkoç, Figen Ünlü, Polat, Hilal, Koçak, Oner, 2003. Investigation of acute toxicity of deltamethrin on guppies (Poecilia reticulata). Ecotoxicol. Environ. Saf. 55 (1), 82–85.