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PHARMACOLOGICAL, PHYTOCHEMICAL AND COMPUTATIONAL STUDIES ON PRENYLATED FLAVONOIDS AND PTEROCARPANS FROM *ERYTHRINA FUSCA* LOUR.

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Abstract

In the present study, the dry powdered bark of *Erythrina fusca* was extracted with methanol. It was subjected to peripheral analgesic study using the acetic acid-induced writhing test in mice model and it demonstrated significant analgesic activity. The methanol extract was partitioned into n-hexane, chloroform and water-soluble fractions. Primarily, the chloroform fraction of the methanol extract was subjected to various chromatographic techniques and four major metabolites, Lupinifolin (1), Lupinifolin-4'-methyl ether (2), Sandwicensin (3) and Licoagrocarpin (4) were purified. Molecular docking studies of 1-4 against cyclooxygenase-2 (COX-2) enzyme suggest that they may interact with COX-2 at different levels. The pharmacokinetic and biological parameters of 1-4 were also checked through in silico tool to understand their drug-likeliness.

Introduction

Erythrina fusca Lour. (Bengali name- Pannya mandar; Family: Fabaceae) is a common tree in Bangladesh. It is a deciduous tree that normally grows up to 10 - 15 metres in height and 60 cm in diameter (Anjum *et al.* 2021). Traditionally, different parts of it are used as analgesic, antipyretic, antiinflammatory, antiinfective, antirheumatic, antitussive, etc. (Anjum *et al.* 2022). Pain and inflammation are the body's defensive response to harmful stimuli and are major targets of scientific research due to their association with human and animal diseases (Soma *et al.* 2019, Ramalingam *et al.* 2020). Prostaglandins are key mediators of both pain and inflammation and produced from arachidonic acid via the cyclooxygenase (COX) enzyme (Rahman 2019, Ramalingam *et al.* 2020). Non-steroidal anti-inflammatory drugs (NSAIDs) target COX-2 and are commonly used to alleviate pain and inflammation (Griswold and Adams 1996) but long-term use can lead to side effects such as gastric ulcers, bleeding and nephrotoxicity. To overcome these side effects, natural products and functional foods are vastly recommended (Devaraj and Karpagam 2011).

In the current investigation, the methanol extract of *E. fusca* was subjected to peripheral analgesic assay. Four major compounds were isolated known as Lupinifolin (1), Lupinifolin-ether (2), Sandwicensin (3) and Licoagrocarpin (4). A molecular docking study of the four isolated compounds was performed against the COX-2 enzyme. Besides, drug- likeliness properties of them were also predicted through in silico study for the first time.

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Materials and Methods

The bark of *Erythrina fusca* was collected from Fulbaria, Mymensingh district, Bangladesh and a voucher specimen (DACB accession no. 45025) has been deposited at Bangladesh National Herbarium, Mirpur, Dhaka for future reference. 0.5 kg of *E. fusca* was soaked in 2.5 L methanol for a period of 15 days and extracted. The methanol extract was concentrated and partitioned into n-hexane, dichloromethane and aqueous soluble fractions (VanWagenen *et al.* 1993).

To assess the peripheral analgesic activity of the methanol extract of *E. fusca*, the acetic acid-induced writhing method was used in mice (Ahmed *et al.* 2019). The animal care facility of the Department of Pharmacy, State University of Bangladesh, Dhaka, Bangladesh was used and the protocol was approved by their Animal Ethics Committee. Results were calculated as mean ± standard deviation. Statistical assessments were done by using Student's t-test. The $p<0.05$ was considered as statistically significant.

A portion of the concentrated methanol extract (5 g) was fractionated into n-hexane, chloroform and aqueous soluble fractions. Based on the TLC pattern, the chloroform soluble fraction (300 mg) was subjected to gel permeation chromatography (GPC) over Lipophilic Sephadex (LH-20) soaked in a mixture of n-hexane-chloroform-methanol (2:5:1) and it yielded compounds 1, 2 and 3. A portion of the concentrated chloroform extract (250 mg) was also subjected to column chromatography over silica gel (Kieselgel 60, mesh 70-230) and the column was eluted with n- hexane and ethylacetate mixtures of increasing polarities and it yielded compound 4. ^{1}H-NMR spectrum was recorded using a Bruker AMX-400 (400 MHz) instrument.

Lupinifolin (1): Yellow mass; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 12.24 (1H, s, OH-5), 7.32 (2H, d, J = 8.4 Hz, H-2', H-6'), 6.87 (2H, d, J = 8.4 Hz, H-3', H-5'), 6.62 (1H, d, J = 10.1 Hz, H-4"), 5.50 (1H, d, J = 10.1 Hz, H -3"), 5.33 (1H, dd, J = 12.8, 2.8 Hz, H-2), 5.13 (1H, t, J = 7.2 Hz, H -2'"), 3.19 (2H, d, J = 7.2 Hz, H -1'"), 3.04 (1H, dd, J = 17.1, 12.5 Hz, H-3α), 2.80 (1H, dd, J = 17.1, 3.0 Hz, H - 3β), 1.66 (3H, s, CH_3-4'"), 1.66 (3H, s, CH_3 -5'"), 1.46 (3H, s, CH_3 -6"), 1.45 (3H, s, CH_3 -5") .

Lupinifolin-4'-methyl ether(2): Yellow mass; ^{1}H-NMR (400 MHz, $CDCl_3$): 12.26 (1H, s, 5-OH), 7.34 (2H, d, 8.4 Hz, H-2', H-6'), 6.88 (2H, d, 8.4 Hz, H-3', 6.63 (1H, d, 10.0 Hz, H-4''), 5.49 (1H, d, 10.0 Hz, H-3''), 5.35 (1H, dd, 3.0, 13.0 Hz, H-2), 5.14 (1H, t, 7.2 Hz, H-2'''), 3.90 (3H, s, OCH_3-4'), 3.21 (2H, d, 7.2 Hz, H-1'''), 3.05 (1H, dd, 13.0, 17.0 Hz, H-3ax), H-5'), 2.80 (1H, dd, 3.0, 17.0 Hz, H-3eq), 1.65 (3H, s, H-4'''), 1.65 (3H, s, H-5'''), 1.46 (3H, s, H-6''), 1.44 (3H, s, H-5'').

Sandwicensin (3): Yellow mass; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.41 (1H, d, J=8.4 Hz, H-1), 7.02 (1H, d, J = 8.1 Hz, H-7), 6.58 (1H, dd, J = 8.4, 2.4 Hz, H-2), 6.44 (1H, d, J = 2.4 Hz, H-4), 6.42 (1H, d, J = 8.1 Hz, H -8), 5.46 (1H, d, J = 6.8 Hz, H-11a), 5.26 (1H, t, J = 7.2 Hz, H-2'), 4.23 (1H, t, J = 10.9, H -6β), 3.81 (3H, s, OCH_3-9), 3.66 (1H, dd, J = 10.9, 5.1 Hz, H -6α), 3.52 (1H, m, H-6a), 3.31 (1H, d, J = 7.9 Hz, H-1'), 1.78 (3H, s, CH_3-4'), 1.68 (3H, s, CH_3 -5').

Licoagrocarpin (4): Yellow mass; ^{1}H-NMR (400 MHz, $CDCl_3$): 7.40 (1H, d, J=8.4 Hz, H-1), 6.97 (1H, d, J=8.0 Hz, H-7), 6.56 (1H, d, J=8.0 Hz, H-8), 6.40 (1H, d, J=8.4 Hz, H-2), 6.38 (1H, m, H-10), 5.47 (1H, d, J=6.8 Hz, H-11a), 5.30 (1H, br.s, OH-3), 5.23 (1H, t, J=6.8 Hz, H-2'), 4.22 (1H, m, H-6α), 3.80 (3H, s, OCH_3-9), 3.62 (1H, m, H-6β), 3.55 (1H, m, H-6a), 3.24 (2H,d, J= 6.8 Hz, H-1'), 1.81 (3H, s, CH_3-4'), 1.75 (3H, s, CH_3-5').

Docking of 1-4 with COX-2 was performed as per previously published method (Khan *et al.* 2022). The drug-likeness properties were assessed using Molinspiration Cheminformatics online property calculator tool kit (http://www.molinspiration.com).

Results and Discussion

The acetic acid-induced writhing test (Ahmed *et al.* 2019) was conducted on mice to evaluate the peripheral analgesic activity of the methanol extract of bark of the *E. Fusca* (Table 1). The two experimental groups receiving the test materials at doses of 200- and 400- mg/kg body weight exhibited a significant reduction in the number of writhing in a dose-dependent manner, indicating the presence of analgesic principles that might be intervening with the prostaglandin pathways (Uddin *et al.* 2018, Henneh *et al.* 2021).

Table 1. Effect of methanol extract of *E. fusca* on acetic acid-induced writhing in mice.

Group	Doses (mg/kg body weight)	Number of writhing	% Inhibition
1% Tween 80 in normal saline (Negative control)	10 #	18.25 ± 0.96	-
Diclofenac (Positive control)	50	5.3 ± 1.5*	71.2 ± 1.5
Methanol extract of bark of *E. fusca*	200	7.0 ± 1.2*	61.6 ± 1.2
	400	6.3 ± 1.7*	65.47 ± 1.7

Values are presented as mean ± standard deviation; n = 4; Standard and test groups were compared with the vehicle treated group; *$p < 0.05$; #used in ml/kg body weight.

The ^{1}H-NMR spectrum of compound 1 displayed a characteristic a sharp singlet at δ 12.24 (1H, s, OH-5) of a chelated hydroxyl group at C-5 in a flavonoid skeleton. The proton resonances at δ 3.04 (1H, dd, *J* = 17.1, 12.5 Hz), 2.80 (1H, dd, *J* = 17.1, 3.0 Hz) and 5.33 (1H, dd, *J* = 12.8, 2.8 Hz) could be attributed to H- 3α, H-3β and H-2, respectively, of the flavanone nucleus. Two doublets at δ 5.50 (1H, d, *J* = 10.1 Hz, H-3") and 6.62 (1H, d, *J* = 10.1 Hz, H-4") along with two singlets at δ 1.45 (3H) and 1.46 (3H) were typical for the *cis*-double bond protons and *gem*-dimethyl groups of a 2,2-dimethyl-chromene moiety, respectively (Amabeoku and Kabatende 2011). Two *ortho*-coupled doublets (*J* = 8.4 Hz) centered at δ 7.32 (2H) and 6.87 (2H) could be assigned to the protons at C-2' and C-6', and C-3' and C-5' of the *para*-disubstituted benzene ring (C ring). Two singlets at δ 1.66 (3H, s, CH_3-4''') and 1.57 (3H, s, CH_3-5'''), a doublet at 3.19 (1H, d, *J* = 7.2 Hz, H-1''') and a triplet at 5.13 (1H, t, *J* = 7.2 Hz, H-2''') indicated the presence of an isoprenyl group. These proton NMR spectral features of compound 1 were comparable with the NMR data of Lupinifolin (Yusook *et al.* 2017). The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound 2 displayed similar pattern like compound 1 except having a methoxy signal at δ 3.90 (s) of C-4' of B ring and it was deduced as Lupinifolin-4'-methyl ether (Fig. 1) (Yenesew *et al.* 2009).

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound 3 displayed four proton signals at δ 3.66 (1H, m, H-6α), 4.23 (1H, t, J = 10.9, H-6β), 3.52 (1H, m, H-6a) and 5.46 (d, J=6.8 Hz, H-11a) confirmed the spectral features of the characteristic –O-CH_2-CH-CH-O- moiety connecting the rings B and C of a pterocarpan central skeleton. The ^{1}H-NMR also displayed a pair of *ortho*-coupled doublets (J=8.4 Hz) of one proton intensity at δ 7.41 and 6.58 assignable to H-1 and H-2, respectively in ring A indicating the substitution at C-3 and C-4. Coupling of two other aromatic protons at δ 7.02 (d, J=8.1 Hz, H-7) and 6.42 (d, J=8.1 Hz, H-8) indicated their presence at ring D. In addition, a triplet of one proton signal at δ 5.26 (t, J = 7.2 Hz, H-2'), two proton signals at 3.31 (d, J= 7.9 Hz, H-1') and two methyl signals at δ 1.68 (s) and 1.78 (s) at C-3' indicated the presence of an isoprenyl moiety at C-4. Based on the above spectral features and comparing to a previously published data (Tjahjandarie *et al.* 2015), the structure of compound 3 was deduced as

Sandwicensin (Fig. 1). Compound 4 also showed the similar NMR spectral pattern except the position of isoprenyl moiety at C-4 and it was deduced as Licoagrocarpin (Fig. 1) (Anjum *et al.* 2021).

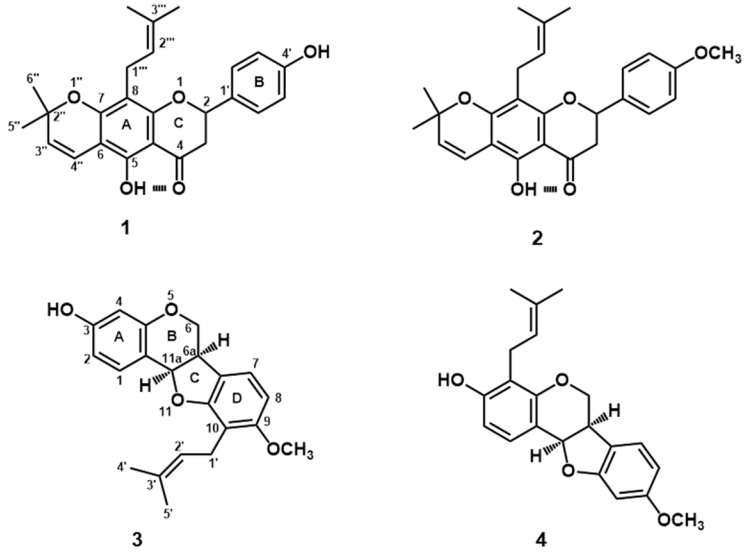

Fig. 1. Structure of the isolated compounds 1-4 from *E. fusca.*

The results of the molecular docking study (Khan *et al.* 2022) of compounds 1-4 are summarized in Table 2, which showed the binding free energy (kJ/mol) of each compound with COX-2. Compound 3 had the lowest binding free energy (-28.45 kJ/mol), suggesting the strongest interaction with COX-2. 1 (-24.69 kJ/mol) and 4 (-22.59 kJ/mol) also showed relatively strong interactions with COX-2. However, 3 had a less negative binding affinity (-17.15 kJ/mol), indicating a weaker interaction with COX-2 compared to the other three compounds. The molecular docking results also provided insight into the binding modes of the compounds with COX-2 (Fig. 2). Compound 1 forms hydrogen bonding with Trp373 and Leu338 and displayed van der Waals interactions with several residues of COX-2. Similarly, compounds 2-4 also showed several van der Waals interactions.

Table 2. Binding free energy (kJ/mol) of compounds 1-4 with COX-2.

Compounds	Binding free energy (kJ/mol)
1	-24.69
2	-17.15
3	-28.45
4	-22.59

The pharmacokinetics and biological properties of compounds 1- 4 were evaluated using Molinspiration Cheminformatics online property calculator tool kit (http://www.molinspiration.com) (Table 3). In the present calculated values, parameters for 1-4 were in harmony with the proposed limits of Lipinski's rule (Lipinski 2004) presenting maximum one violation. Compounds

1-4 showed the computational Topological polar surface area (TPSA) in the range 47.93 to 76.00 Å, indicating their capacity to demonstrate acceptable intestinal absorption and blood–brain barrier penetration (Deswal *et al.* 2022). In the predicted bioactivities, compounds (1-4) also showed prominent scores for the parameters nuclear receptor ligand and enzyme inhibitor. It indicated their mode of action like non-steroidal anti-inflammatory drugs (NSAIDs) to a bit. NSAIDs were capable to bind with nuclear receptor (e.g. peroxisome proliferator-activated receptor-γ) and inhibited the appearance of a wide range of proinflammatory genes (Sastre *et al.* 2003). Besides, NSAIDs could inhibit cyclooxygenase-2 enzyme very strongly (Chakraborti *et al.* 2010). Taken together, compounds (1-4) might serve as lead compounds and might be considered to test them for analgesic potentials.

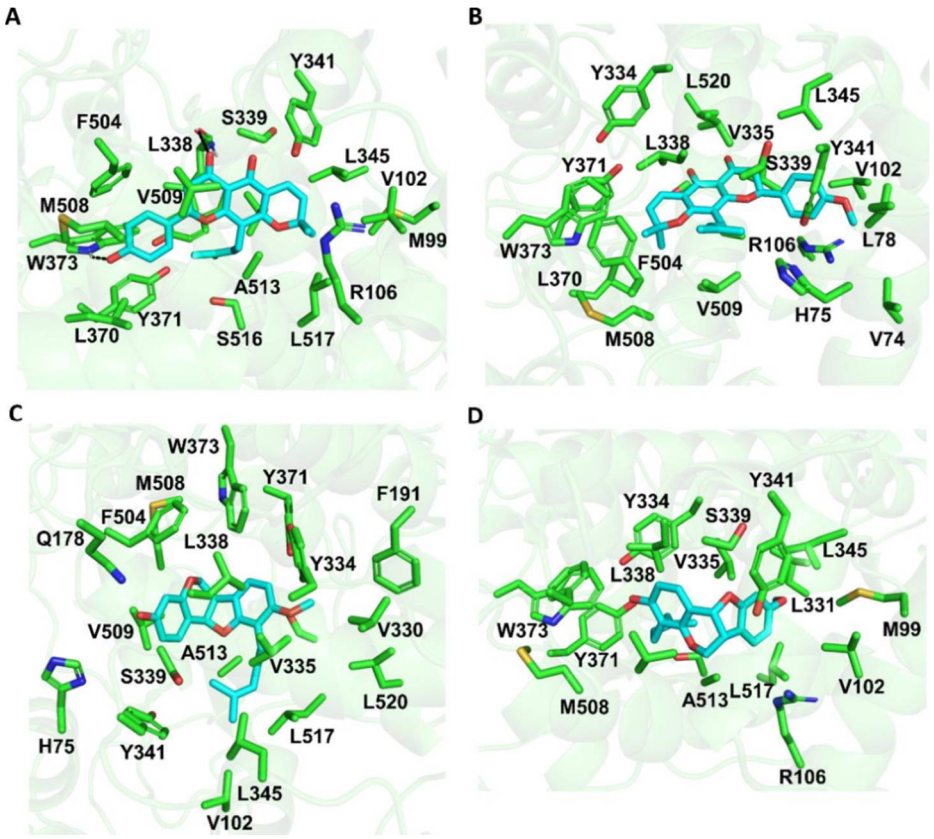

Fig. 2. Interaction of (A) 1 (cyan), (B) 2 (cyan), (C) 3 (cyan)and (D) 4 (cyan) with COX-2 visualized in pymol. Black dash indicates H bonding. Residues from COX-2 are shown in green color.

The present investigation showed that the methanol extract of *E. fusca* exhibited significant analgesic activity in mice model. Four major phytochemicals 1-4 were isolated and purified. Molecular docking study of compounds (1-4) suggested that they might interact with cyclooxygenase-2, which might be connected to the analgesic activity. Additionally, the pharmacokinetic and biological parameters of 1-4 were also checked through in silico tool for the first time to understand their drug-likeliness.

Table 3. Drug likeliness properties of the isolated compounds 1-4 calculated using Molinspiration Cheminformatics (http://www.molinspiration.com).

Parameters	Compounds			
	1	2	3	4
(A) Molecular properties				
No. of atoms	30	31	25	25
MlogP	5.37	5.91	4.54	5.01
MW	406.48	420.50	338.40	338.40
HBA	5	5	4	4
HBD	2	1	1	1
No. rotb	3	4	3	3
Volume	374.77	392.30	313.10	313.10
TPSA	76.00	65.00	47.93	47.93
nV	1	1	0	1
(B) Predicted Bioactivity				
G-protein coupled receptors ligand	0.27	0.22	0.40	0.31
Ion channel modulator	-0.02	-0.08	0.07	-0.06
Kinase inhibitor	-0.10	-0.14	0.03	-0.01
Nuclear receptor ligand	0.82	0.73	0.62	0.54
Protease inhibitor	0.25	0.20	-0.08	-0.08
Enzyme inhibitor	0.61	0.55	0.70	0.65

Here, MlogP, is the octanol-water partitioning coefficient prediction; HBA, sum of N and O (H-bond acceptors); HBD, sum of NH and OH (H-bond donors); No. rotb, number of rotatable bonds; TPSA, topological polar surface area; nV, number of violations.

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