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Research article

An alkaloid derivative from *Coscinium fenestratum* exhibit dual COX/LOX inhibition

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Key words: *Coscinium fenestratum*, Cyclooxygenase, Lipoxygenase, Aspirin, Nimesulide.

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Abstract

Agents that inhibit both Cyclooxygenase (COX) and Lipoxygenase (LOX) are highly recommended in the development of anti-inflammatory drugs. An alkaloid derivative from *Coscinium fenestratum* was isolated and characterized by UV Visible, IR, ^{1}H NMR, ^{13}C NMR and LC-MS spectroscopy. Dual COX/LOX inhibitory study was performed by high throughput screening assay. In the control (maximum enzyme activity) experiment COX activity of 73 µmol/min was noted, which is reduced to 50 µmol/min by the compound isolated. The standard inhibitors show 38 and 29 µmol/min respectively for Aspirin and Nimesulide. Compound isolated from *Coscinium fenestratum* also possess significant LOX inhibition when compared to standard inhibitor vanillin.

Introduction

Acute inflammation is a short-term response that will heal the damaged tissue and removing the stimuli. The prolonged inflammation leads to chronic conditions and should be treated, otherwise it is associated with many chronic human conditions and diseases, including allergy, atherosclerosis, cancer, arthritis and autoimmune diseases [1]. Among the most widely prescribed drug worldwide, Nonsteroidal anti-inflammatory drugs (NSAIDs) are the prime one and drugs of the first choice for the treatment of rheumatic disorders and other degenerative inflammatory joint diseases [2]. NSAIDs have a number of potentially toxic effects on the gastrointestinal (GI) tract, damage the entire GI tract in humans mainly in stomach and duodenum. Also shows small bowl ulceration, bleeding with consequent anemia and perforation. NSAIDs cause erosive damage and ulceration in the colon [3]. These pathological conditions were noticed in those who use NSAIDs for long term. NSAID damage is silent in the majority of people, therefore the problems often occurs without warning symptoms [4]. The mechanism by which NSAIDs reduce the inflammation and pain is by inhibiting Prostaglandin synthesis. Cyclooxygenase (COX) is the key enzyme in the prostaglandin pathway. This enzyme exists in two isoforms, constitutive form COX-1 and inducible one COX-2. The COX-2 is the main target for anti-inflammatory drug, in addition it is also a promising target for the prevention and treatment of many human cancers [5]. The main side effects of NSAIDs were raised by inhibiting constitutive isoform COX-1.Hence any agents that selectively inhibit COX-2 are highly desirable. Various selective COX-2 inhibitors such as celecoxib and rofecoxib have been developed to reduce side effects. They are potent anti-inflammatory drugs having less gastrointestinal side effects when compared to non selective COX inhibitors [6, 7] even though, long-term use of COX-2 selective inhibitors causes cardiovascular side effects [8]. Hence the development of new drugs with safety profile is still necessary.

Lipoxygenase (LOX) is another marker enzyme in the inflammatory pathway. It's coming under dioxygenase group of enzyme, which inserts one molecule of oxygen at the different site of arachidonic acid. The site of incorporation is tissue and enzyme specific. In animal tissues, four main types of LOXs with positional specificities are found, 5-LOX, 8-LOX, 12-LOX, and 15-LOX [9]. Agents that inhibit LOX enzyme have a potent role in the development of anti-inflammatory drugs.

Currently, the natural products continue to be widely used in traditional systems of medicine mainly due to their no side effects. *Coscinium fenestratum* commonly known as tree turmeric belongs to *Minispermaceae* family and widely seen in Western Ghats of India and Sri Lanka [10]. It is a strong woody, dioecious, flowering and fruiting in August to October. This plant is widely used in the traditional systems of medicine especially in Ayurveda and Sidha. A number of research reports were available for the various therapeutic effects of this plant. In ethnomedicine, the stem is used for treating poultice cuts sores and snakebite, jaundice, fever, ulcers etc [11, 12]. This plant is recommended to have thermogenic, anti-inflammatory, antiseptic, tonic effects and is used in ophthalmology, inflammation and general debility [13]. The decoction of the stem is used for snake bites and the

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stem bark used to treat intermittent fevers [14]. An yellow crystalline alkaloid 'Berberine' is the major constituent of *Coscinium fenestratum* [15]. In addition to berberine, stem constituents are ceryl alcohol, hentriacontane, sitosterol, palmittic acid, oleic acid, and saponin. In the roots of the plant tertiary alkaloids, berlambine, dihydroberlambine and noroxyhydrastinine were reported [16]. It exhibits multiple pharmacological activities such as being active against hypertension, tumors, bacteria, inflammation, and Human Immuno deficiency Virus (HIV) mainly due to the presence of berberine [13]. From the earlier research reports it is evident that this plant possesses numerous pharmacological activities.

In this present study, we concentrate on isolation of a compound from *Coscinium fenestratum* having Anti-inflammtory potential which has been explained by dual LOX/COX inhibition and elucidate the structure through sophisticated analysis such as UV-Visible spectroscopy, IR Spectroscopy, LC-MS, ^{1}H NMR and ^{13}C NMR.

Experimental

Materials and methods

Plant

The experimental plant *Coscinium fenestratum* were collected from ayurvedic raw material shop, Thrissur, Kerala. Collected plant was botanically authenticated by Dr. N. Sasidharan, Scientist Kerala Forest Research Institute (KFRI) and kept a voucher specimen in the herbarium (No 28493) of Kerala Forest Research Institute, Peechi, Thrissur, Kerala. The stem was used for the study.

Chemicals

All biochemicals were purchased from Sigma-Aldrich and chemicals used were procured from Merck India.

Extraction

Collected plant was washed with water, shade dried and chopped into small pieces. Hot soxhlet extraction method was employed for this study. 50 g powdered sample was used for the thimble preparation. Gradient elution was performed initially with petroleum ether and gradually enriched with solvents based on increasing polarity. Chloroform fraction was used for the study.

Structural characterization

Isolated compound was structurally elucidated by sophisticated instruments such as UV-Visible spectroscopy, IR Spectroscopy, LC-MS, ^{1}H NMR and ^{13}C NMR. Purity of isolated compound was done by one dimensional thin layer chromatography (TLC) using solvent system diethyl ether: acetone: ethanol: water in the ratio 1:1:1:1, v/v/v/v as the mobile phase. Shimadzu Pharma spec UV-1700 was used for recording the UV-VIS spectra. Scanned the compound from 200-800 nm using ethanol as solvent.IR spectrum recorded in between 500cm^{-1} and 3500cm^{-1} using Shimadzu FT-IR 8400S. Proton (^{1}H) Nuclear Resonance (NMR) Spectrum was recorded on a NMR-JEOL GSX-400 spectrometer. Xevo G2 (Waters) Quadrapole- Time-of-flight (Q-TOF) spectrometer equipped with an electrospray ion source (ESI) was used to measure the mass spectrum of the compound. The MS in ESI positive mode was performed. ESI conditions: mass spectral range, m/z 100–1000; capillary voltage, 3.50KV; sample cone voltage, 30V and extraction cone voltage, 1 V. 2.4.

In vitro COX activity assay

In vitro COX activity assay was performed with slight modification of high-throughput screening assay [17].

Cell culture

HeLa cells were cultured using DMEM media (Gibco) supplemented with 10% Tet system approved FBS (Clonothec) and 1X pen/strep (100 units of penicillin, 100 µg of streptomycin; Gibco). Cells were maintained in 10cm dishes at 37°C and 4% CO_2 until 60-80% confluence. When confluent, cells were split in 1:10–1:20 dilution.

Assay

Collected the cell pellet after treating with 2 deoxy glucose. Lysed the cells using lysis buffer which contained 50mM tris HCl pH 8.0, 150mM NaCl, 1% IGPAL and 1µl protease inhibitor cocktail. Collected the supernatant by centrifugation at 10000 rpm for 20 minutes. Assay buffer constitutes 100mM tris HCl pH 8, Hematin 15µM and EDTA 3µM. To this assay buffer added 25 µl cell supernatant. Then this was incubated at 27^{o}C for 15 minutes. Reaction was initiated by the addition of arachidonic acid 100µM and TMPD 100µM. Immediately after adding TMPD, started measuring the absorbance at 590nm in kinetic mode for first 30 minutes. Total COX activity was calculated by the following equation.

Total COX activity = OD at 590/5min/0.00826x5.25+2

In vitro LOX activity assay

LOX inhibitory activity was measured by minor modification of the spectrometric method developed by Anthon and Barrett [18]. Enzyme solution of 0.93 µM was prepared in 0.2 mM borate buffer of pH 9.0. The substrate, linoleic acid, was dissolved by adding equal amount of Tween 20, at 0.32-mM concentration. The substrate solution was prepared in the same borate buffer. The assay mixture was made of 50 µl of the 13-LOX and 360 µl of the substrate. The final volume was made up to 2 ml with 1.59 ml borate buffer. The activity of LOX was measured as the formation of hydroperoxy

octadecadienoic acid which was monitored at 234nm on a spectrophotometer, HITACHI U-2900. The same procedure was repeated with different fractions for the identification of LOX inhibitory activity.

Statistical analysis

Values are expressed as mean ±SD.

Results and Discussion

Yield and purity

4.5 g of extractives was obtained from 50 g of sample. Its purity was checked through one dimensional thin layer chromatography and was found to be a single spot.

Structural studies

10-(allyloxy)-15-((1Z,3Z)-hexa-1,3,5-trien-1-yl)-5-((E)-pent-2-en-1-yl)-4,5,9,9a,13a,15-hexahydro-[1,3]dioxino[5,4-g]isoquinolino[3,2-a]isoquinolin-11-ol (Figure 1).

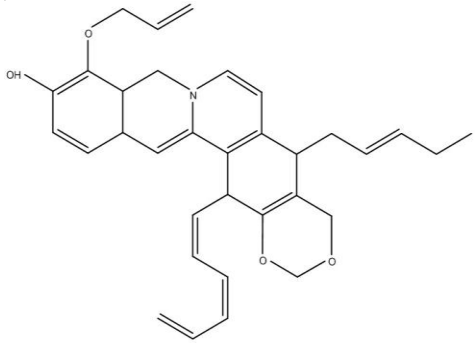


Figure 1. Structure of isolated compound.

Appearance : Yellow solid; Melting point 170; UV-Vis: (MeOH) λ_{max} 260 (ε 0.010), 340 (ε 0.008) nm (Figure 2); IR (KBr) ν max 3407, 3390, 3365, 2933, 1708, 1720, 1649, 1602, 1582, 1508, 1232, 827 cm^{-1} (Figure 3); ^{1}H NMR (MeOD, 500 MHZ: δ 8.80–8.30, 3.5-4, 6.3, 7.5 and 8.3 ppm (Figure 4); ^{13}C NMR (MeOD, 125 MHz) δ 30, 60, 105-110, 120-150 ppm (Figure 5). (ESI- MS) m/z = 511 (calculated for $C_3H_{37}NO_4$) (M + 1); positive ion, TOF MS ES+ 5.90e 7, 401,480,518 (Table 1, Figure 6).

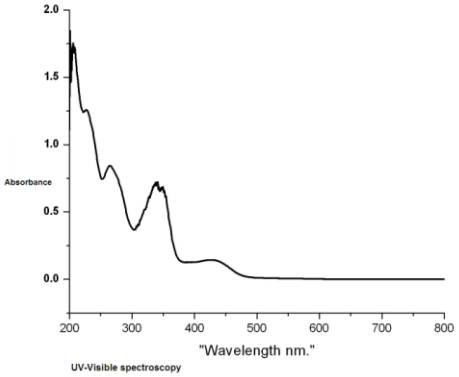


Figure 2. UV-Visible spectroscopy.

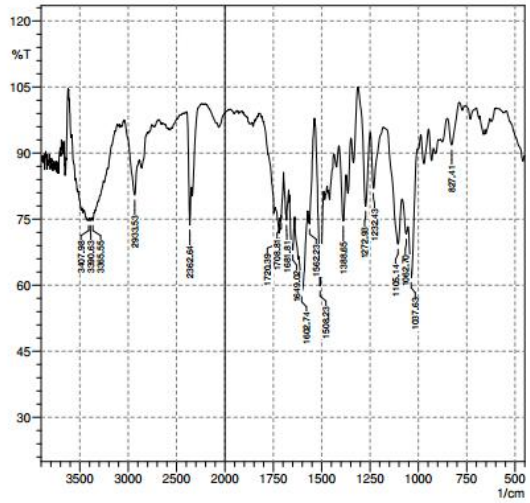


Figure 3. IR spectra,

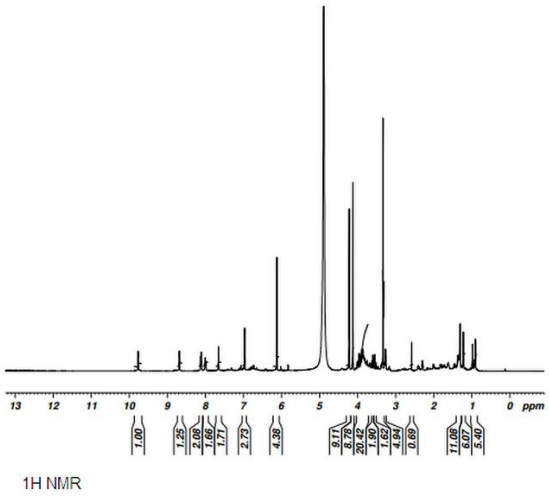


Figure 4. ^{1}H NMR Spectra.

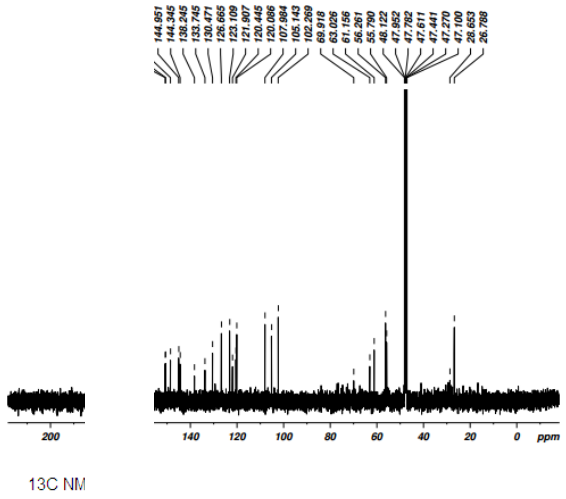


Figure 5. ^{13}C NMR Spectra.

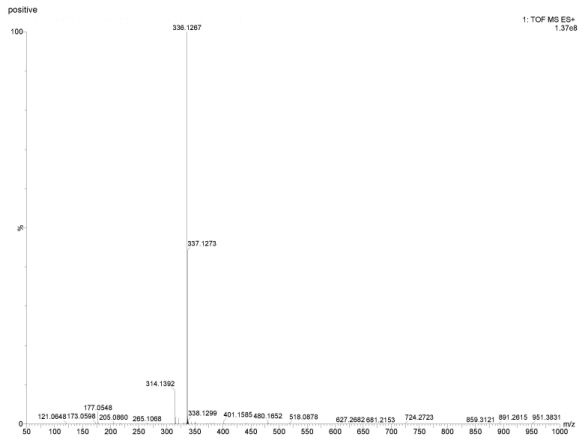
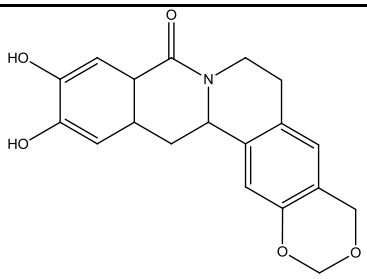
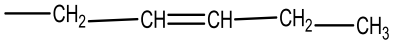
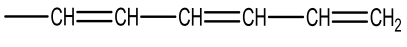
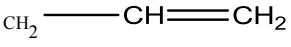


Figure 6. LC-MS.

Table 1. Ionic breakage of the compound.

Peek Value	Structure	Existing Peek
336		Base Peek
401		401-336 =65
480		480-401=79
518		518-480=38

Among the number of compounds reported in the *Coscinium fenestratum,* Berberine is the major constituents of the stem bark. In addition, protoberberine alkaloids, oxypalmatine, berberrubine [19], (-)-8-oxotetrahydrothalifendine, (-)-8- oxoisocorypalmine, palmatine, 1-benzylisoquinoline, jatrorrhizine, tetrahydroberberine, 8-oxocanadine, berberrubine and 8 oxoberberine [16] were also reported. We deduce the structure by eliminating each reported compound based on our spectral datas.

The presence of nitrogen confirms the structure belongs to the class of alkaloids. The absence of sulphur eliminates the tetrahydroberberine. The presence of carbonyl group in IR removes the possibilities of berberine, palmatine, benzylisoquinolone, berberrubine, 8 oxoberberine and oxoisocorypalmine. The presence of phenolic OH in NMR confirms the basic structure contain at least one phenolic hydroxyl group, thus eliminating the presence of 8-oxoberberine and 8 oxo canadine. So the main component of the extract may be (-)-8-oxotetrahydrothalifendine. The main mass spectral peak at 336 gives an information that the major component of the extract has a molecular weight of 336 for the backbone which also in match with the molecular weight of (-)-8- oxotetrahydrothalifendine. So we can confirm that the isolated compound is a derivative of (-)-8-oxotetrahydrothalifendine.

In vitro COX activity assay

Compound isolated from *Coscinium fenestratum* showed significant COX inhibitory activity. Standard drugs used for this study were Aspirin and Nimesulide. In the control (maximum enzyme activity) experiment COX activity of 73 µmol/min was noted, which was reduced to 50 µmol/min by the compound isolated. The standard inhibitor showed 38 and 29 µmol/min respectively for Aspirin and Nimesulide (Figure 7).

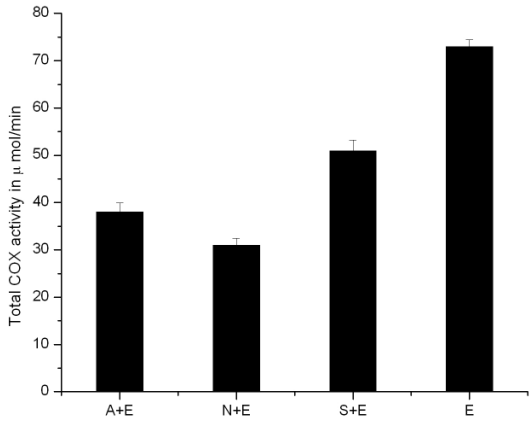


Figure 7. COX inhibitory potential of compound.

The nonsteroidal anti-inflammatory drugs are among the most widely prescribed drug worldwide and it is prime choice in the degenerative inflammatory disorders [20]. The cyclooxygenase enzyme catalyze the conversion of arachidonic acid to prostaglandin. The agents that inhibit the COX will result in the decreased formation of prostaglandins and thereby reduce the inflammation. The anti-inflammatory potential of a drug depends on the ability to inhibit COX. The nonsteroidal anti-inflammatory agents have serious side effects such as damage and ulceration in the small intestine, small bowel ulceration and bleeding with anaemia and perforation. Hence the natural agents (derived from plants, microbes etc) is highly desirable over synthetic one.

In vitro LOX activity assay

Significant LOX inhibition was noted in the compound isolated from *Coscinium fenestratum*. Results were recorded at different time intervals. From the graph

(Figure 8) it is clear that compound exhibit LOX inhibition when compared to standard inhibitor vanillin.
Lipoxygenase proteins have a single polypeptide chain with a molecular mass of; 75–80 kDa in animals and; 94–104 kDa in plants. It oxygenates carbon in the arachidonic acid and thereby converts the arachidonic acid to prostaglandins. LOX is another key enzyme in the arachidonic acid pathway. By inhibiting LOX, we can control the inflammation.

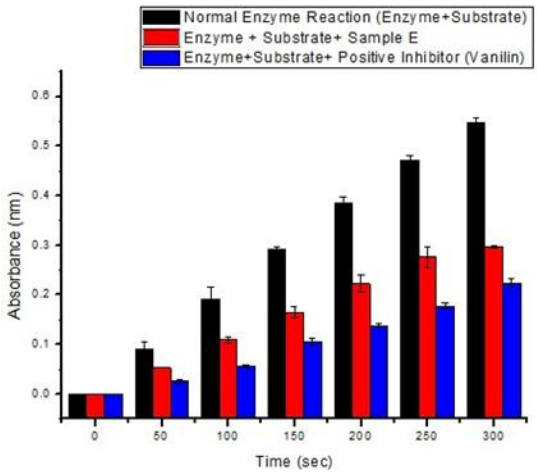


Figure 8. LOX inhibitory potential of compound.

Conclusion

The agents that reduce the inflammation have prime importance in current scenario for the development of anti-inflammatory drugs. COX is the important enzyme in the inflammatory pathway and those agents that block the enzyme can be used for the synthesis of anti-inflammatory drug. In addition to COX, LOX play a pivotal role in the inflammatory pathway. Agents that block both COX and LOX are good enough to manufacture anti-inflammatory drug.
In this paper we concluded that the compound isolated from *Coscinium fenestratum* is an alkaloid derivative of (-)-8- oxotetrahydrothalifendine which possess dual COX/LOX inhibition.

Declaration of interest

Declarations of interest: none

Acknowledgements

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References

1. Ursula Weiss. Inflammation. Nature insight. 2008; 454(7203): 427–77.
2. Hawkey CJ: COX-1 and COX-2 inhibitors. Best Pract Res Clin Gastroenterol 2001; 15(5):801–20.
3. Jackson LM, Hawkey CJ: COX-2 Selective Nonsteroidal Anti-Inflammatory Drugs Do They Really Offer Any Advantages ?. Drugs 2000; 59(6):1207–16.
4. Tushar KV, George S, Remashree AB, Balachandran I: Coscinium fenestratum (Gaertn.) Colebr.-A review on this rare, critically endangered and highly-traded medicinal species. Journal of Plant Sciences 2008; 3(2):133-45.
5. Subbaramaiah K, Dannenberg AJ: Cyclooxygenase 2: A molecular target for cancer prevention and treatment. Trends Pharmacol Sci. 2003; 24(2):96–102.
6. Lanza FL, Rack MF, Simon TJ, Quan H, Bolognese JA, Hoover ME, *et al.*: Specific inhibition of cyclooxygenase-2 with MK-0966 is associated with less gastroduodenal damage than either aspirin of ibuprofen. Aliment Pharmacol Ther 1999; 13(6):761–7.
7. Ray W a, Stein CM, Daugherty JR, Hall K, Arbogast PG, Griffin MR: COX-2 selective non-steroidal anti-inflammatory drugs and risk of serious coronary heart disease. Lancet 2002; 360:1071–3.
8. Dogné JM, Supuran CT, Pratico D: Adverse cardiovascular effects of the coxibs. J Med Chem [Internet]. 2005; 48(7):2251–2257. Available from: http://pubs.acs.org/doi/abs/10.1021/jm0402059
9. Poeckel D, Funk CD: The 5-lipoxygenase/leukotriene pathway in preclinical models of cardiovascular disease. Cardiovasc Res. 2010; 86(2):243–53.
10. Eswani N, Kudus K, Nazre M, Noor a. GA: Medicinal Plant Diversity and Vegetation Analysis of Logged over Hill Forest of Tekai Tembeling Forest Reserve, Jerantut, Pahang. J Agric Sci. 2010; 2(3):189–210.
11. Sudharshan S., Prashith Kekuda T., Sujatha M: Antiinflammatory activity of Curcuma aromatica Salisb and Coscinium fenestratum Colebr : A comparative study. J Pharm Res. 2010; 3(10):24–5.
12. Chitra K, Sujatha K, Polisetti H, Karri S, Umamaheswara Reddy C: Standardization of Coscinium fenestratum with reference to berberine by high performance thin layer chromatography. Res J Pharm Biol Chem Sci. 2011; 2(2):226–9.
13. Rai R V., Rajesh PS, Kim HM: Medicinal use of Coscinium fenestratum (Gaertn.) Colebr.: An short review. Orient Pharm Exp Med. 2013; 13(1):1–9.
14. Rojsanga P, Gritsanapan W, Suntornsuk L: Determination of berberine content in the stem extracts of Coscinium fenestratum by TLC densitometry. Med Princ Pract. 2006;15(5):373–8.
15. Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahu A, *et al.*: Indian medicinal herbs as sources of antioxidants. Food Res Int. 2008; 41(1):1–15.
16. Pinho PMM, Pinto MMM, Kijjoa A, Pharadai K, Díaz JG, Herz W: Protoberberine alkaloids from Coscinium fenestratum. Phytochemistry 1992; 31(4):1403–7.
17. Anil Kumar K, Chandramohan Reddy T, Reddy G V., Bharat Kumar Reddy D, Mahipal SVK, Sinha S, *et al.*: High-throughput screening assays for cyclooxygenase-2 and 5-lipoxygenase, the targets for inflammatory disorders. Indian J Biochem Biophys. 2011; 48(4):256–61.
18. Anthon GE, Barrett DM: Thermal inactivation of lipoxygenase and hydroperoxytrienoic acid lyase in tomatoes. Food chemistry 2003; 81:275–9.
19. Tran Q Le, Tezuka Y, Ueda JY, Nguyen NT, Maruyama Y, Begum K, *et al.*: In vitro antiplasmodial activity of antimalarial medicinal plants used in Vietnamese traditional medicine. J Ethnopharmacol. 2003; 86(2–3):249–52.
20. Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR: Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. Proc Natl Acad Sci USA [Internet]. 1999; 96(13):7563–8.