ISOLATION, SPECTROSCOPIC CHARACTERIZATION AND COMPUTATIONAL MODELING OF CHEMICAL CONSTITUENTS OF PIPER LONGUM NATURAL PRODUCT

P. Mishra,
Department of chemistry, University of Delhi, Delhi-110007.
***E-mail:** prmmishra@rediffmail.com

ABSTRACT

A simple, rapid and efficient method has been developed for the isolation of piperine from the fruits of Piper nigrum. The method involves extraction of the fruit powder with glacial acetic acid, from which piperine is partitioned into chloroform and subsequently crystallized. The identity of the compound was confirmed by its melting point, comparison of IR, ^{1}HNMR, XRPD, mass spectral and molecular modeling. The purity of the compound was ascertained by TLC.

Keywords: Piperine, Piper longum Linn, Piperaldehyde, Rasayana

INTRODUCTION

Piper longum Linn. popularly known as Pippali belonging to the family Pipperacea, an important medicinal plant is used in traditional medicine in Asia and Pacific islands especially in Indian medicine [1]. P. longum is a component of medicines which is reported as good remedy for treating gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, chronic gut-related pain and arthritic conditions [14]. Other reported beneficial effects of P. longum include analgesic and diuretic effects, relaxation of muscle tension and alleviation of anxiety [2]. Since a long time P. longum has been used to possess immunomodulatory and antitumor activity [3].

It is one of the herbs mentioned in all ancient scriptures of Ayurveda. In Sanskrit, it possesses various synonyms, describing its properties and specialities, like sana – pungent, capala – quickly acting, krsna – black, magadhi – from Magadha region, upakulya – growing near water resources, kola improving the test sensation etc. The great sage Caraka has categorized it as dipaniya – an appetizer, kanthya – beneficial for the throat, uptighna – anti-saturative, asthapanao-paga – an adjunct to decoction enema, sirovirecaniya – a cleansing nasal therapy, purisa sangrahaniya – give form to the faeces, purisa virajaniya – give color to the stool, sita prasamana – relieve cold sensation on the skin, sulaghna – anti colic, rasayana – a rejuvenator, kasahara-anti-tussive, vamaka- emetic, hikka nigrahana – mitigates hiccup. The root of pippali, pippal mula is cited as dipaniya – an appetizer and sulaghna – anti colic. Pippali is on of the ingredients of trikatu – three pungent viz. sunthi, marica and pippali, which is the most commonly, used combination for the remedy of kapha dosha. Trikatu is anti-cold, anti tussive a well as anti-asthmatic in its properties. Pippali is a specially recommended rasayana for respiratory system (Pranavaha srotasa) and is the best rasayana rejuvenate to kappa dosha.

The plant grows all over Indian subcontinent. A small shrub with a large woody root and numerous creeping, jointed stems, thickened at the nodes. The leaves are alternate, spreading, without stipules and blade varying greatly in size. The lowest leaves are 5-7 cm long, whereas, the uppermost 2-3 cm long. The flowers are in solitary spikes. The fruits, berries, in fleshy spikes 2.5-3.5 cm long and 5 mm thick, oblong, blunt and blackish green in color. The mature spikes collected and dried, form the commercial form of pippali and the root radix is known as pippalimula.

The botanical name of pippali is piper longum and it belongs to family piperaceae. Piper longum L. has been used as a crude drug for the treatment of the disorder of peripherally poor blood circulation in domestic medicine. Piper longum is a component of Indian traditional medicine reported to be used as a remedy for treat in gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infection, chronic gut-related pain and arthritic conditions [3].

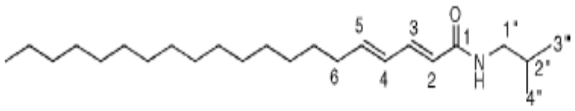
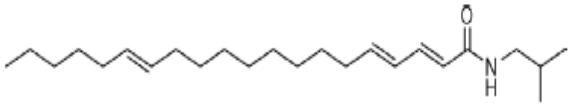
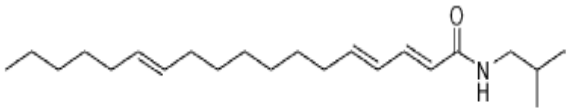
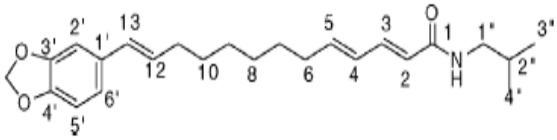
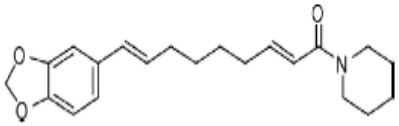
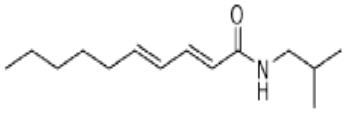
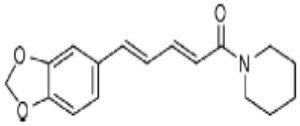
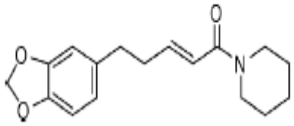
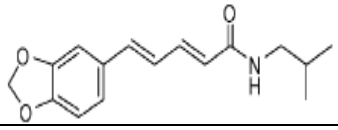
Other reported beneficial effects of piper longum include analgesic and diuretic effects, relaxation of muscles tension and alleviation of anxiety [4] Piper extracts and piperine possess inhibitory activities on prostaglandin and leukotrienes COX-1 inhibitory effect and thus exhibit anti-inflammatory activity [5] Recently, biochemical activities of some important medicinal plants including Piper species and their metabolites have been described [6-10].However, very little is done to elucidate the possible targets of its action. The fruits of Piper longum have been widely used since time immemorial in household spices and also in various traditional systems of medicine. According to Ayurvedic system of medicine, P. longum fruits are anathematic, antiasthmatic, alterative, and used to treat pain, piles, insomnia, and epilepsy [11].

Studies have revealed anticonvulsant [12] and bioavailability-enhancing properties [13-15] of the drug. The fruits contain 1.0–2.5% volatile oil, 5–9% alkaloids, of which the major ones are piperine, chavicine, piperidine, and piperetine, and a resin [6]. Most of the pharmacological properties of P. nigrum fruits are attributed to a piperidine alkaloid, piperine, which is present in the fruits in amounts of 1.7–7.4% [16]. The structure of piperine is shown in Tab. 1. Pharmacological

and clinical studies have revealed that piperine has CNS depressant, antipyretic, analgesic, anti-inflammatory [17], antioxidant [18], and hepatoprotective [19] activities. Piperine has also been shown to enhance the bioavailability of several drugs, for example sulfadiazine, tetracycline, streptomycin, rifampicin, pyrazinamide, ionized, ethambutol , and phenytoin [20].

Due to its diverse pharmacological properties, piperine is important as a biomarker for standardization of fruit of P. nigrum and Piper longum and of polyhedral formulations containing these raw materials. The bioavailability-enhancing property of piperine indicates its potential to be used as an adjuvant with therapeutic drugs in chronic ailments, to reduce the effective dose of the drug and, hence, subsequent adverse effects [21-35]. Inspired by the various pharmacological attributes of piperaldehyde. In the present study, isolation, spectroscopic characterization and molecular modeling of isolated new compound. And it may be useful as a lead compound for the prevention or treatment of thrombosis. The inhibitory mechanism and other pharmacological actions of piperaldehyde are also currently under investigation.

Table 1
Chemical structure of amides purified from *Piper longum* L.

Compound	Name	Chemical structure
1	(2*E*,4*E*)-*N*-Isobutyleicosa-2,4-dienamide	
2	(2*E*,4*E*,14*Z*)-*N*-Isobutyleicosa-2,4,14-trienamide	
3	(2*E*,4*E*,12*Z*)-*N*-Isobutylocatadeca-2,4,12-trienamide	
4	Guineensine	
5	Pipernonaline	
6	Pellitorine	
7	Piperine	
8	Piperanine	
9	Piperlonguminine	

MATERIALS AND METHODS

Method for isolation of piper-longum-L

All the chemicals used in this study were of analytical grade and used as procured. Solvents used were of analytical grade and were purified by standard procedures. Piper–longum-L was purchased from local market and washed with AR grade methanol then dried to laboratory temperature. P. longum fruit powder (100 g) was dissolved in 150 ml of 50% methanol and incubated at room temperature (28–30ºC) for 16 h. The supernatant (140 ml) collected by centrifugation at 14,000 rpm was dried in vacuum (3.5 g), designated as methnolic extract (F001). This was further fractionated using hexane (35 ml, b.p: 68–70ºC), soluble fraction dried under vacuum and designated as hexane extract (F002). The insoluble

fraction was further dissolved in chloroform (40 ml, b.p. 65ºC), the supernatant was separated by using a separator funnel. The lower fraction was dried under vacuum, and designated as PlCE (F003). Finally, all the extracts were dissolved in DMSO individually, and used for testingICAM-1 inhibitory activities. The stoichiometric analyses (C, H and N) of the isolated compound were performed using Elementar vario EL III (Germany) model. Their IR spectra were recorded on Perkins–Elmer FTIR spectrophotometer in KBr and polyethylene pellets. ^{1}HNMR spectra were recorded in DMSO-d6 solvent on a Bruker Advance 400 instrument The XRD powder pattern were recorded on a vertical type Philips 1130/00 x- ray diffractometer, operated at 40kVand 50Ma generator using the Cu kα line at 1.54056 A° as the radiation sources. Sample was scanned between 5° to 70° (2θ) at 25°C. The crystallographic data was analyzed by using the CRYSFIRE –2000 powder indexing software package and the space group was found by the Check -Cell program. Debye – Scherer relation with the help of 100% peak width determined the particle size. The density was determined by Archimedes method. Mass spectrum carried out by TOF-Mass spectrometer. 3D modeling of the proposed structure of isolated compound was performed using CsChem3D Ultra-11 programme package. The correct stereochemistry was assured through the manipulation and modification of the molecular coordinates to obtain reasonable low energy molecular geometry. The potential energy of the molecule was the sum of the following terms=Estr +Etor +Evdw +Eoop +Eele, where all Es represent the energy values corresponding to the given types of interaction (kcal/mol). The subscripts str, ang, tor, vdw.oop and ele denote bond stretching, angle bonding, torsion deformation, Vander waals interactions, out of plain bending and electronic interaction respectively.

RESULTS AND DISCUSSION

Elemental analysis C, 68.38%; H, 4.83%; F, 2.46%; N, 3.62%; O, 16.56%; S, 4.15%, hence the molecular formula is $C_{44}H_{37}FN_2O_8S$ and m.pt is 65^0C.

Vibrational spectroscopy

The isolated molecule exhibits absorptions 3180(m) aromatic primary amine, 3019(m), 2931(w)CO, 2855(w) Ar-O-Ar, 2733(w) Ar-OH, 1668(s) Ar-NO_2, 1591(s), 1511(s), 1511(s), 1430(s) Ar-NO2, C-F, 1298(s), 1266(s), 1153(s), 1028(m), 732(m), 631(m), 588(w), 551(w)cm-1. These bands are indicated C-H and C=C stretching for trans –CH=CH-, C-S.

^{1}H N M R

^{1}HNMR data were carried out DMSO-d_6 with 400Mz resolution δ7.767 ppm Ar-H, 7.761-7.394ppm[s], Ar-H, 6.577-5.539[m] ppm, 3H, CH, 5.304-653, 24H, CH2, 2.184-4.447ppm CH, 2.1.36-0.73ppm, 18H

XRPD Crystal structure analysis

From the XRPD data the isolated compound is good crystalline form having orthorhombic crystal system with a (Å) = 19.1830, b (Å) =10.7633, c(Å) = 4.4107, $\alpha = 90^0$, $\beta = 90^0$, $\gamma = 90^0$, V = 911.59 (Å^3), index $0 \leq h \leq 11, 0 \leq k \leq 8$, $0 \leq l \leq 4$, space group C222, reflections is 324, particles size 44.24 nm.

Molecular Modeling

3D molecular modeling of the proposed structure of the complexes was performed using CsChem3D program package. The correct stereochemistry was assured through the manipulation and modification of the molecular coordinates to obtain reasonable low energy molecular geometries. The potential energy of the molecule was the sum of the following terms: $E = E_{str} + E_{ang} + E_{tor} + E_{vdw} + E_{oop} + E_{ele}$. Where all E's represent the energy values corresponding to the given types of interaction. The subscripts str, ang, tor, vdw,oop and ele denote bond stretching, angle bonding, torsion deformation, vander waals interactions, out of plain bending and electronic interaction, respectively. The optimized energy is -22.35 Jmol^{-1}

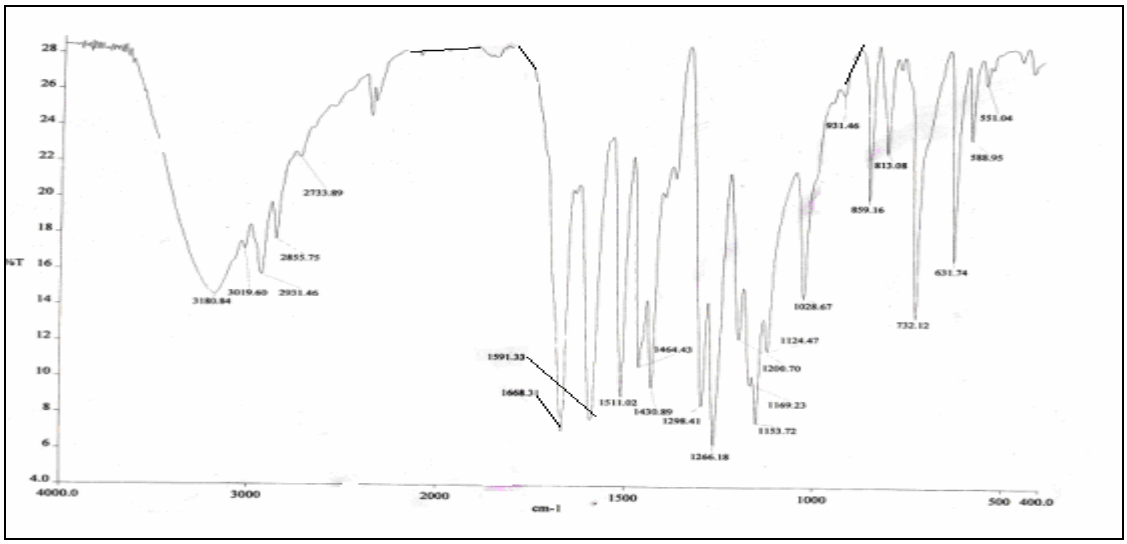


Figure 1: FT-IR spectra of isolated P. longum

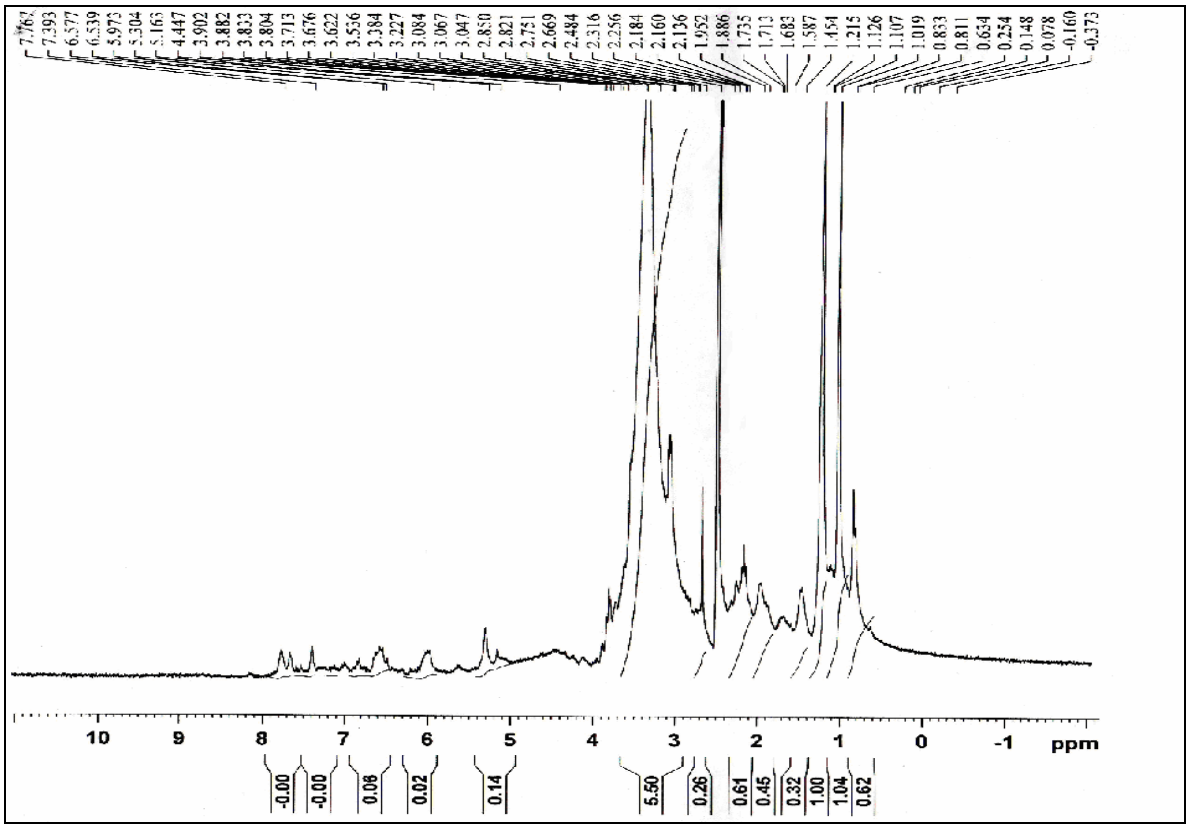


Figure 2: [1]HNMR-spectra of isolated compound

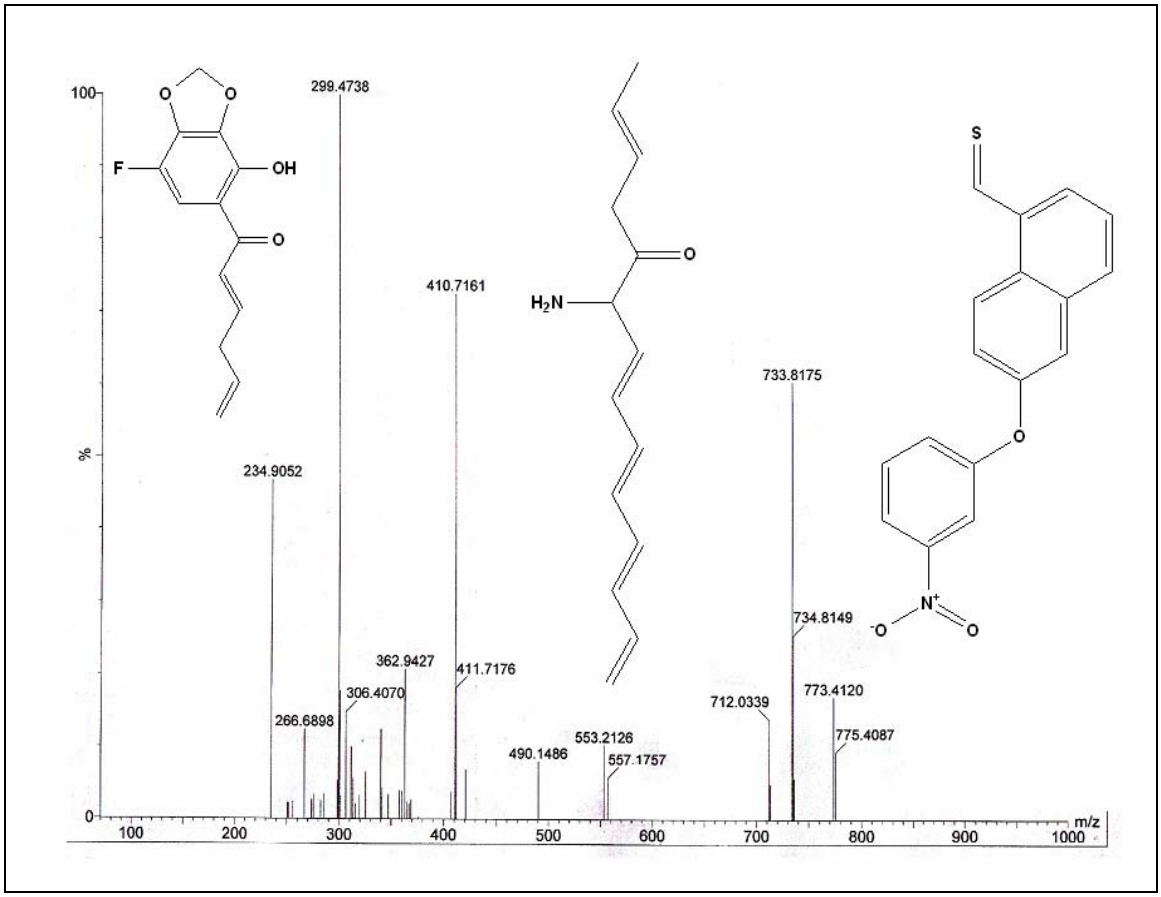


Figure 3: TOF-Mass spectra of isolated compound [7-((1E,3E,5E,7E,12E,15E,18E)-9-amino-20-(7-fluoro-4-hydroxybenzo[d] [1,3] dioxol-5-yl)- 10, 20-dioxoicosa-heptaenyl)-6- (3-nitrophenoxy) naphthalene-1- carbothialdehyde [piperalehyde]

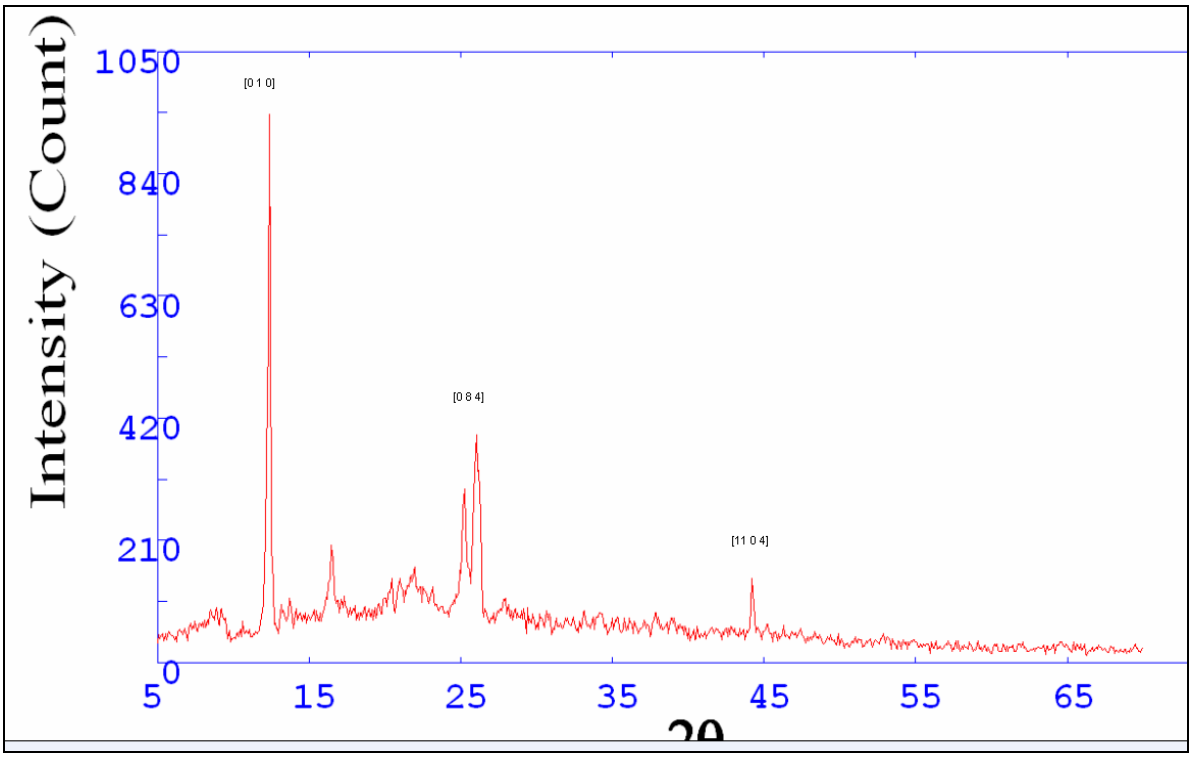


Figure 4: XRPD spectra of isolated compound

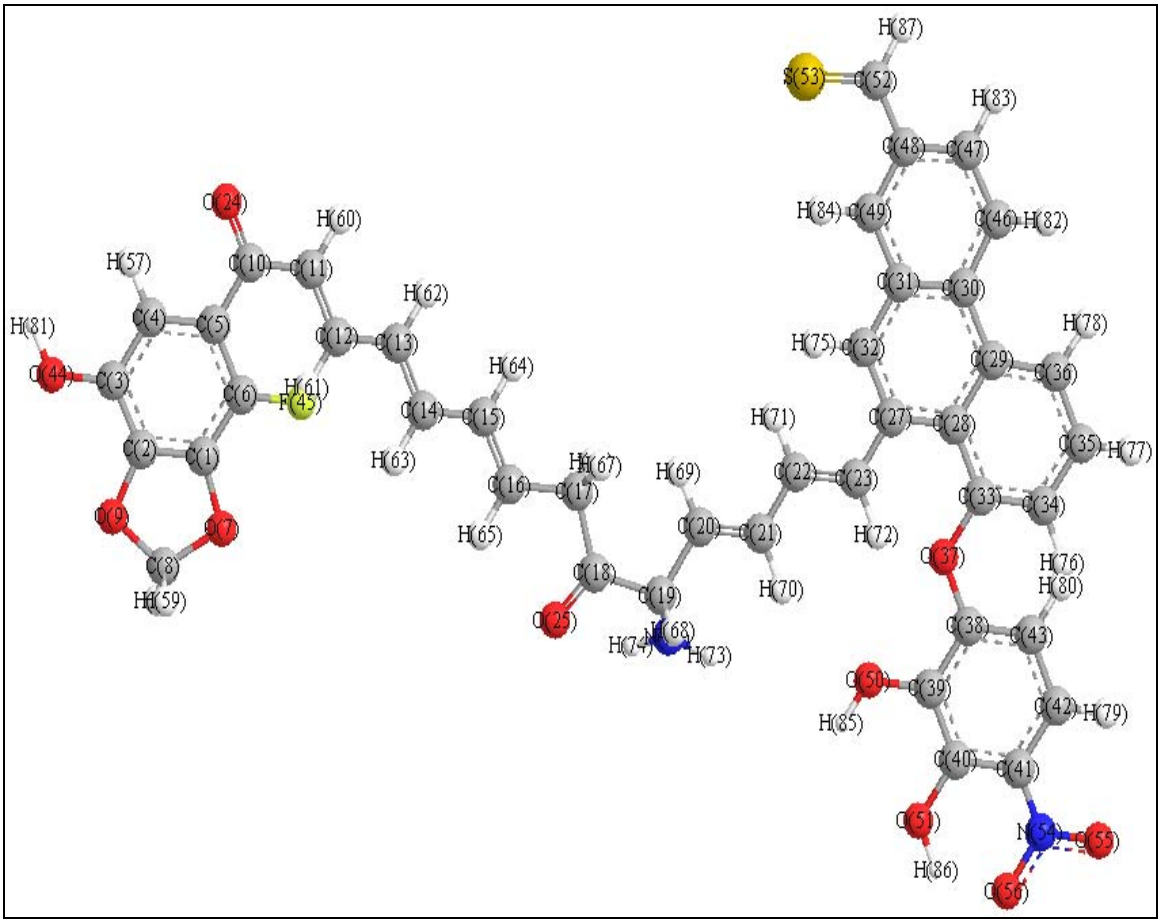


Figure 5: Optimised structure of isolated compound

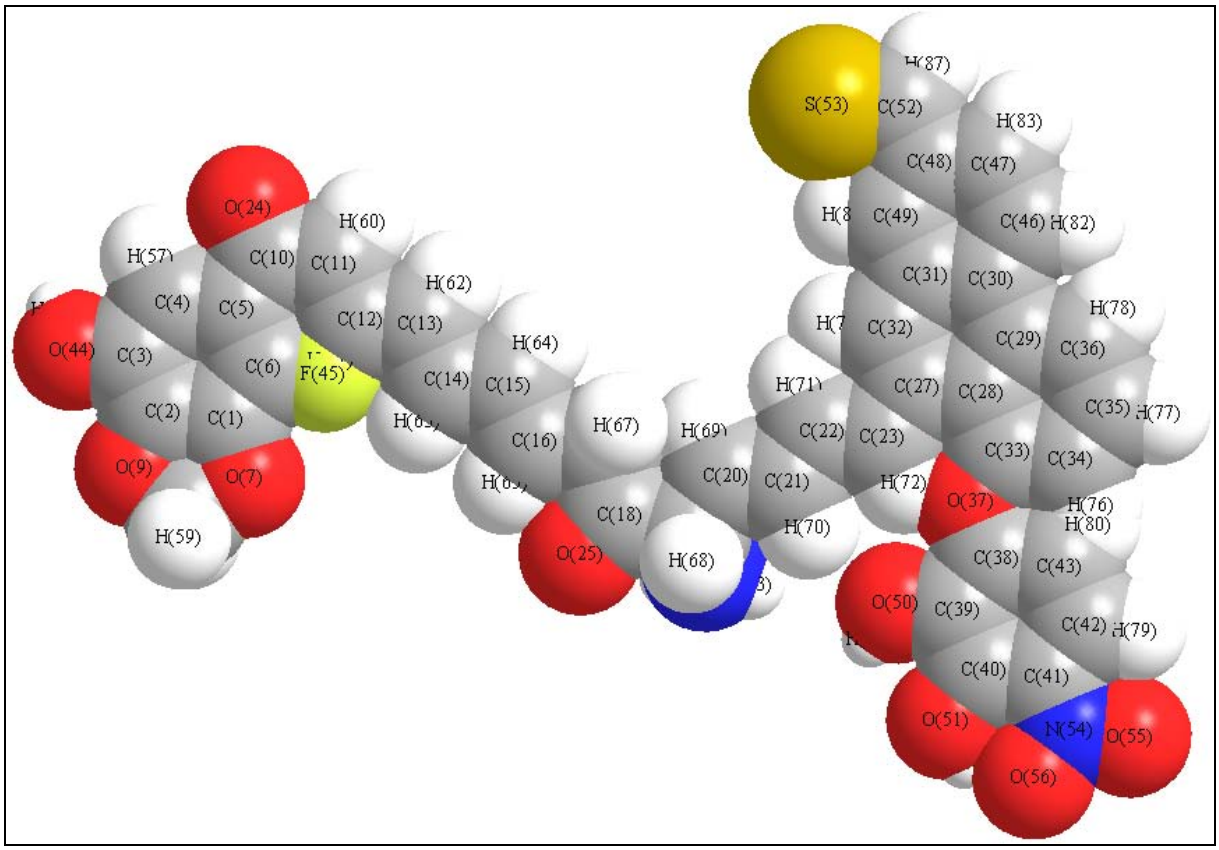


Figure 6: Space filled structure of piperaldehyde from P.longum

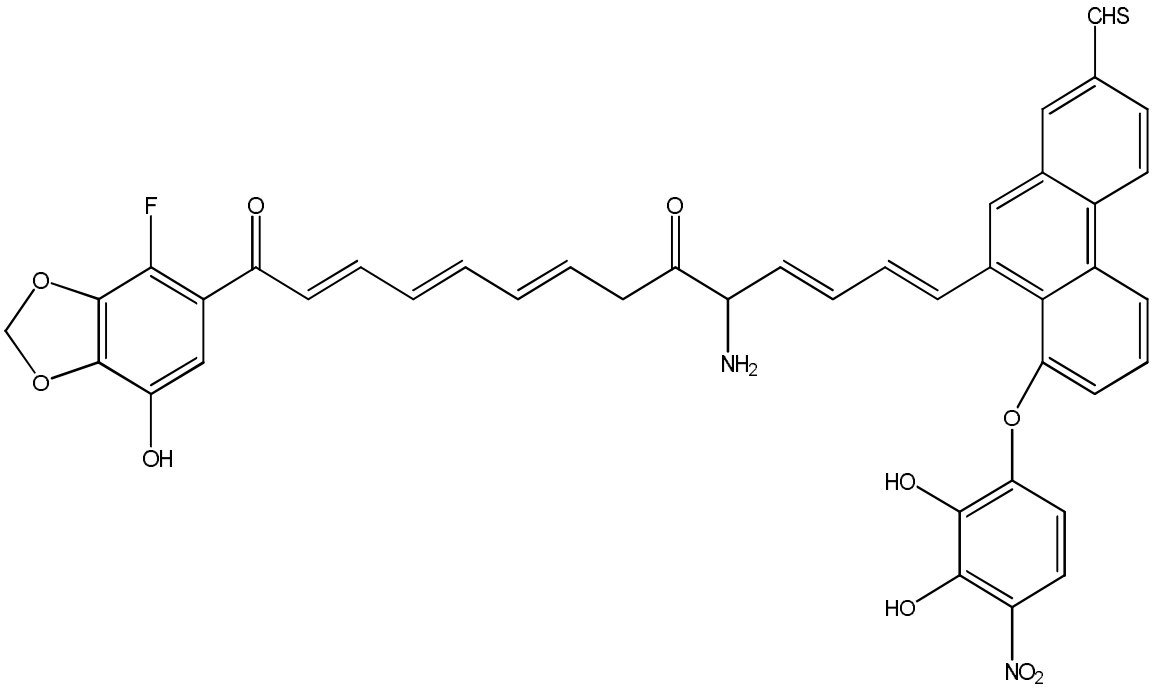


Figure 7: Graphical structure of isolated compound and molecular formula is $C_{12}H_{31}FN_2O_{10}S$ and the IUPAC name is 9-(1E,3E,10,12E)- 5-14-(4-fluoro-7-hydroxybenzo[d][1,3] dioxol-5-yl}-6,14-dioxotetradeca -1,3,8,10,12-pentaenyl)-8-(2,3-dihydroxy-4-nitrophenoxy)phenanthrene-2-carbothialdehyde.[piperaldehyde]

Inhibitory effects of the isolated compound from the fruit of piper

Longum. Rabbit (male) blood was collected from the ear aorta with a one-tenth volume of 1% EDTA and centrifuged for 10 min at 230g. Platelet suspension was prepared from this EDTA-anticoagulant platelet-rich plasma according to the washing procedures described previously. The platelet number was counted using a Coulter Counter [36,37] and adjusted to a concentration of3-108platelets/ml. Platelet aggregation was measured using an aggregometer as described previously[38]. Briefly, washed platelet suspension (WPS) was incubated at 37ºC for 3 min with DMSO (0.5%, control) or various concentrations of tested compounds for 3 min in the presence of 1mM $CaCl_2$in the aggregometer, and platelet aggregation was then induced by addition of collagen (2 mg/ml), arachidonic acid (AA) (100 mM), platelet-activating factor (PAF) (10 nM), or thrombin (0.1 unit/ml). The resulting aggregation, measured as the change in light transmission, was recorded for 10min.The inhibition rate was obtained from the maximal aggregation induced by the respective agonist at the concentration using the equation inhibition rate ¼ (maximal aggregation rate (MAR) of vehicle-treated WPS-MAR of sample-treated WPS/MAR of vehicle treated)-100. Acetylsalicylicacid (ASA, aspirin) [37-42] was used as a positive control significance of differences between the tested compounds and control. All of the tested Piperaldehyde showed dose dependent inhibitory activities on platelet aggregation induced by collagen, AA, and PAF, except for that induced by thrombin (Table 2). Piperaldehyde had the most potent antiplatelet effect. Piperaldehyde inhibited platelet aggregation induced by collagen with inhibition values of 100, 100, and 49.8, and 19.9% inhibitory effects at 300, 150, 30, and 10 mM, respectively. In a test with AA, Piperaldehyde at 300, 150, and 30m Exhibited 100%, 76.4%, and 12% inhibitory effects, respectively. Furthermore, Piperaldehyde at 300, 150, and 30 mM inhibited platelet aggregation induced by PAF with inhibition values of 100%, 100%, and 29.9%, respectively.

Table 2: Inhibitory effects of the acidamides isolated from Piper longum fruits on washed rabbit platelet aggregation induced by collagen, AA, PAF, and thrombin

	Conc. (μM)	Aggregation (%)			
		Collagen (2 μg/ml)	AA (100 μM)	PAF (10 nM)	Thrombin (0.1 unit/ml)
Control		72.7±2.9	69.9±2.9	69.3±1.8	80.2±1.3
Piperine	300	6.2±0.3**	2.4±0.4**	1.2±1.1**	75.6±1.5
	150	49.3±1.1*	32.1±3.5*	16.4±2.5**	
	30	71.1±2.5*	55.3±2.1*	59.2±3.2*	
Pipernonaline	300	3.2±0.3**	2.6±0.3 **	3.4±0.2**	76.8±2.1
	150	12.9±0.6*	41.6±3.1*	30.7±0.7*	
	30	79.2±3.1**	67.4±2.8*	57.2±2.3*	
Piperoctadecalidine	300	14.4±2.1*	29.6±0.8*	21.5±3.8**	76.5.0±2.5
	150	49.0±1.5*	54.2±1.6*	52.7±1.5*	
	30	71.2±2.6*	68.8±2.7*	67.6±6.1*	
Piperaldehyde	400	0.0±0.0**	0.0±0.0**	0.0±0.0**	61.3±0.9
	250	0.0±0.0**	16.5±2.1**	0.0±0.0**	
	40	36.5±3.9**	61.5±1.7*	52.0±2.4*	
	20	58.2±2.4*	70.1±2.4	68.7±2.1	
Acetylsalicylic acid (aspirin)	300	68.5±3.4	0.0±0.0**	68.2±1.5	81.1±0.7
	150	73.1±2.6	17.5±2.4**	69.1±2.1	
	30		37.8±3.7*		

Washed rabbit platelets were preincubated with DMSO (0.5% control) or each compound at 37ºC for 3 min in the presence of 1Mm $CaCl_2$ and then the inducer was added. Acetylsalicylic acid was used as a positive control. Values are means ± SEM.*$p<0.05$, **$p<0.01$ as compared with the respective control.

CONCLUSION

Piperaldehyde is one of the important constituent of piper longum Linn. It was isolated from the fruits of the piperlongum and extracting with methanol as solvent. Studies shows that the pet alcoholic extract and piperaldehyde shows significant DPPH scavenging activity. The extract and piperaldehyde were also found to exert protective effective in the myocardial narcotic rats. They have protected myocardium from the harmful effects of lipid per oxidation and even maintained the gluthione levels to normal. Hence it can be concluded that the alcoholic extract as well as piperaldehyde are useful in exerting protective activity in case of myocardial ischemia is treated animals.

REFERENCES

1. Srinivas V. Pullela , Ashok K.Tiwari,Uma Maheswara S. Vanka, Anuradha Vummenthula,Hari B. Tatipaka, Krishna R. Dasari, Ikhlas A. Khanc, Madhusudana R. Janaswamy,HPLC assisted chemo biological standardization of α-glucosidase-Ienzyme inhibitory constituents from Piper longum Linn-An Indian medicinal plant, Journal of Ethno pharmacology 108, (2006), 445–449.
2. Kim, J.S., Kwon, C.S., Son, K.H., Inhibition of alpha-glycosidase and amylase by luteolin, a flavonoid. Bioscience, Biotechnology and Biochemistry 64, 58– 2461, (2000)
3. Courageot, M.P., Frenkiel, M.P., Dos Santos, C.D., Deubel,V., Despres, P., Alpha-glucosidas inhibitors reduce dengue virus production by affectingthe initial steps of virion morphogenesis in the endoplasmic reticulum. Journal of Virology 74, (.2000), 564–572.
4. E.S. Sunila, G. Kuttan, Immunomodulatory and antitumor activity of Piper longum Linn. and piperine, Journal of Ethnopharmacology 90, (2004),339–346.
5. Stohr, J.R., Xiaso, P.G., Bauer, R., Constituents of Chinese piper species and their inhibitory activity on prostaglandin and leukotriene biosynthesis in vitro. Journal of Ethanopharmacology 75, (2001), 133–139.
6. Nongyao Sawangjaroen, Kitja Sawangjaroen, Pathana Poonpanang, Effects of Piper longum fruit, Piper sarmentosum root and Quercus infectoria nut gall on caecal amoebiasis in mice, Journal of Ethno pharmacology 91, (2004),357–360.
7. Seung Woong Lee, Young Kook Kim, Koanhoi Kimc, Hyun Sun Leea, Jung Ho Choi , Woo Song Leeb,Chang-Duk Jun , Jee Hun Park, Jeong Min Lee, Mun-Chual Rho, Alkamides from the fruits of Piper longum and Piper nigrum displaying potent cell adhesion inhibition, Bioorganic & Medicinal Chemistry Letters 18, (2008), 4544–4546.
8. Masaya Iwashita[a], Nobuaki Oka, Satoko, Ohkubo, Masaki Saito and Norimichi Nakahata, Piperlongumine, a constituent of Piper longum L., inhibits rabbit platelet aggregation as a thromboxane A_2 receptor antagonist, European Journal of Pharmacology, 570, (2007). 1-3, 38-42.
9. S.C.Jagdale, B.S.Kuchekar, A.R.Chabuskar, P.D.lokhande and C.G.Raut.Anti-Oxidant activity of piper longum L.Int.J.Biological chemistry, 3(3), (2009),119-125.
10. Kartick,M.and M.P.Stanely,2006,Preventive effect of rutin,a bioflavonoid, on lipid peroxides and antioxidants in isoproterenol-induced myocardial infarction in rats,J.Pharmcol, 58, (2006),701-707.
11. A.S.Wakade, A.S .Shah ,M.P .Kulkarni, and Archana R.Kuvekar,Protective effect of Piper Longun-Lon oxidative stress induced injury and cellular abnormality in adriamycin induced cardio toxicity in rats, Indian J.Exp.Biology, 46, (2009), 528-533.
12. Seung Woong Lee , Young Kook Kim, Koanhoi Kim, Hyun Sun Lee, Jung Ho Choi, Woo Song Lee,Chang-Duk Jun, Jee Hun Park, Jeong Min Lee, Mun-Chual Rho ,Alkamides from the fruits of Piper longum and Piper nigrum isplayingpotent cell adhesion inhibition,Bioorganic & Medicinal Chemistry Letters 18, (2009),4544–4546.
13. Madhusudana Rao, J., Srinivas, P.V., Anuradha, A., Tiwari, A.K., Ali,A.Z., Yadav, J.S., Raghavan, K.V., 2004. US Patent Publication No: US2004/0081711A1 and WO 2004/041295 A1.
14. Mehta, A., Zitzmann, N., Rudd, P.M., Block, T.M., Dwek, R.A., Alphaglucosidase inhibitors as potential broad based anti-viral agents. FEBSLetters 430, (1998),17–20.
15. Omote, Y., Tazawa, H., Fujinuma, Y., Sugiyama, N., Synthesis ofpipataline. Bulletin of Chemical Society of Japan 42, (1969), 569–570.
16. Park, I., Lee, S., Shin, S., Park, J.,Young-Joon, A.H.N., Larvicidal activityof isobutyl amides in Piper nigrum fruits against three mosquito species.Journal of Agriculture and Food Chemistry 50, (2002), 1866–1870.
17. Ratner, L., Vander Heyden, N., Dedera, D., Inhibition of HIV and SIVinfectivity by blockade of alpha-glucosidase activity. Journal of Virology181,(1991) 180–192.
18. Sorbera, L.A., Castaner, J., Garcia-Capdevila, L., Celgosivir, Celgosivir._-glucosidase inhibitor. Antihepatitis-C virus drug. Drugs of the Future 30, 545–552. (2005)
19. Van den Brock, L.A., Kat-van Den Nieuwenhaf, M.W., Butters, T.D., VanBoeokel, C.A., Synthesis of alpha-glucosidase I inhibitors showing antiviral (HIV-1) and immunosuppressive activity. Journal of Pharmacy and Pharmacology 48, (1996.), 172–178.
20. Westhuizen, J.H.V., Ferreira, D., Roux, D.G., Phytochemical deoxygenation of an ketol: the dihydroflavonol- flavanone conversion. Journal of Chemical Society Perkin Transactions I 4, (1980.), 1003–1006.
21. Yasuda,I.,Takeya, K., Itokawa, H.,structures of amides from Asia serum heteropoides MAEK.var.vandshuricum MAEK. Chemical and Pharmaceutical Bulletin 29, (1981), 564–566.
22. Zitzmann, N., Mehta, A.S., Carrouee, S., Butters, T.D., Platt, F.M., McCauley,J.M., Blumberg, B.S., Dwek, R.A., Block, T., Imino sugars inhibitthe formation and secretion of bovine viral diarrhea virus, a pestivirusmodel of hepatitis C virus: implications for the development of broad-spectrum anti-hepatitis virus agents. In: Proceedings of the NationalAcademy of Sciences of the United States of America, vol. 96, (1999). pp. 11878–11882
23. Yu-Chang Chen, Chang-Hui Liao, Ih-Sheng Chen, Lignans, an amide and anti-platelet activities from

Piper philippinum,Phytochemistry 68, (2007).2101–2111.

24. Masaya Iwashita, Nobuaki Oka, Satoko Ohkubob, Masaki Saito, Norimichi Nakahata, Piperlongumine, a constituent of Piper longum L., inhibits rabbit plateletaggregation as a thromboxane A2 receptor antagonistEuropean Journal of Pharmacology 570, (2007), 38–42.
25. Aronow, W.S, Management of peripheral arterial disease of the lowerextremities in elderly patients. J. Gerontol. 59, (2004). 172–177.
26. Catella-Lawson, F., Reilly, M.P., Kapoor, S.C., Cucchiara, A.J., DeMarco, S.,Tournier, B., Vyas, S.N., FitzGerald, G.A., Cyclooxygenase inhibitorsand the antiplatelet effects of aspirin. .N. Engl. J. Med. 345,(2001) 1809–1817.
27. Cheng, Y., Prusoff, W.H., Relationship between the inhibition constant(Ki) and the concentration of inhibitor which causes 50% inhibition (IC50) ofan enzymatic reaction. Biochem. Pharmacol. 22,(1973), 3099–3108.
28. Djellas, Y.,Manganello, J.M., Antonakis, K., Le Breton,G.C., Identificationof G α13as one of the G-proteins that couple to human platelet thromboxaneA2 receptors. J. Biol. Chem. 274,(1999) 14325–14330.
29. Dorsam, R.T., Kim, S., Murugappan, S., Rachoor, S., Shankar, H., Jin, J.,Kunapuli, S.P., Differential requirements for calcium and Src family kinases in platelet GPIIb/IIIa activation and thromboxane generation downstream of different G-protein path ways. Blood 105, (2005), 2749–2756.
30. Huang, J.S., Ramamurthy, S.K., Lin, X., Le Breton, G.C., Cell signaling through thromboxane A2 receptors. Cell Signal, 16, (2009), 521–533.
31. Kawano, K.I., Hokamura, K., Kondo, K., Ikeda, Y., Suzuki, Y., Umemura, K., Thromboxane A2 synthase inhibitor enhanced antithrombotic efficacyof GPIIb–IIIa receptor antagonist without increasing bleeding. Eur. J.Pharmacol. 417, (2001), 217–222.
32. Klages, B., Brandt, U., Simon, M.I., Schultz, G., Offermanns, S., Activationof G12/G13 results in shape change and Rho/Rho-kinase-mediated myosinlight chain phosphorylation in mouse platelets. J. Cell Biol. 144, (1999) 745–754.
33. McNicol, A., Israels, S.J. Platelets and anti-platelet therapy. J. Pharmacol.Sci. 93, (2009). 381–396.
34. Mitsuhashi, M., Tanaka, A., Fujisawa, C., Kawamoto, K., Itakura, A., Takaku,M.,Hironaka, T., Sawada, S., Matsuda, H., Necessity of thromboxane A2for initiation of platelet-mediated contact sensitivity: dual activation ofplatelets and vascular endothelial cells. J. Immunol. 166, (2001), 617–623.
35. Nagatome, Y., Hirayama, Y., Ito, T., Ebihara, S.,Watanabe, Y,Effects of teacontaining Hihatu (Piper longum) extract on cold constitution. J. Pharmacol.Sci. 97, (2005), 115.
36. Nakahata, N., Matsuoka, I., Ono, T., Nakanishi, H., 1989. Thromboxane A2activates phospholipase C in astrocytoma cells via pertussis toxin-insensitive-protein. Eur. J. Pharmacol. 162, (2009), 407–417.
37. Nakahata, N., Miyamoto, A., Ohkubo, S., Ishimoto, H., Sakai, K., Nakanishi,H., Ohshika, H., Ohizumi, Y., Gq/11 communicates with thromboxane A2 receptors in human astrocytoma cells, rabbit astrocytes and human platelets. Res. Commun. Mol. Pathol. Pharmacol. 87, (1995), 243–251.
38. Offermanns, S., Laugwitz, K.L., Spicher, K., Schultz, G., G proteins of theG12 family are activated via thromboxane A2 and thrombin receptors inhuman platelets. Proc. Natl. Acad. Sci. U. S. A. 91, (1994) ,504–508.
39. Rho, M.C., Park, Y.H., Sasaki, S., Ishibashi, M., Kondo, K.,Kobayashi, J., Ohizumi, Y.,. The mode of rabbitplatelet shape change and aggregation induced by theonezolide-A, a novel polyketide macrolide, isolated from theOkinawan marine sponge Theonella sp. Can. J. Physiol. Pharmacol. 74, (1996) ,193–199.
40. Offermanns, S., Toombs, C.F., Hu, Y.H., Simon, M.I., Defective plateletactivation in G αq-deficient mice. Nature 389, (1997), 183–186.
41. Ohkubo, S., Nakahata, N., Ohizumi, Y., Thromboxane A2-A2-mediated shapechange: independent of Gq-phospholipase C–Ca2+pathway in rabbitplatelets. Br. J. Pharmacol. 117, 1996),1095–1104.
42. Niranjan Kanaki, Mansi Dave Harish Padh, Mandapati Rajani ,A rapid method for isolation of piperine from the fruitsof Piper nigrum Linn. J. Nat Med 62,281-283(2008)
43. M. Abbas Ali, Noor Mahbub Alam, Mst. ,Sarmina Yeasmin, Astaq Mohal Khan, 2M. Abu Sayeed,Antimicrobial Screening of Different Extracts of Piper longum Linn, Research Journal of Agriculture and Biological Sciences, 3(6),(2009), 852-857.

****