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

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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, 1HNMR, 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 Piperaceae, 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 gonorrhoea, 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 sangrahami – give form to the faeces, purisa virajaniya – give color to the stool, sita prasamana – relieve cold sensation on the skin, sulagha – anti colic, rasayana – a rejuvenator, kasahara-anti-tussive, vamaka- emetic, hikka nigrahami – mitigates hiccup. The root of pippali, pippal mula is cited as dipaniya – an appetizer and sulagha – 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, pipericine, and piperinene and a resin [6]. Most of the pharmacological properties of P. nigrum fruits are attributed to a pipericine 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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Name</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(2E,4E)-N-Isobutyricosa-2,4-dienamide</td>
<td><img src="image1" alt="Chemical structure" /></td>
</tr>
<tr>
<td>2</td>
<td>(2E,4E,14Z)-N-Isobutyricosa-2,4,14-trienamide</td>
<td><img src="image2" alt="Chemical structure" /></td>
</tr>
<tr>
<td>3</td>
<td>(2E,4E,12Z)-N-Isobutylocarotene-2,4,12-trienamide</td>
<td><img src="image3" alt="Chemical structure" /></td>
</tr>
<tr>
<td>4</td>
<td>Guineensine</td>
<td><img src="image4" alt="Chemical structure" /></td>
</tr>
<tr>
<td>5</td>
<td>Pipernonaline</td>
<td><img src="image5" alt="Chemical structure" /></td>
</tr>
<tr>
<td>6</td>
<td>Pellitorine</td>
<td><img src="image6" alt="Chemical structure" /></td>
</tr>
<tr>
<td>7</td>
<td>Piperine</td>
<td><img src="image7" alt="Chemical structure" /></td>
</tr>
<tr>
<td>8</td>
<td>Piperamine</td>
<td><img src="image8" alt="Chemical structure" /></td>
</tr>
<tr>
<td>9</td>
<td>Pierlonguminine</td>
<td><img src="image9" alt="Chemical structure" /></td>
</tr>
</tbody>
</table>

### 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 (28–30°C) for 16 h. The supernatant (140 ml) collected by centrifugation at 14,000 rpm was dried in vacuum (3.5 g), designated as methanolic extract (F001). This was further fractionated using hexane (35 ml, b.p: 68–70°C), soluble fraction dried under vacuum and designated as hexane extract (F002). The insoluble
fraction was further dissolved in chloroform (40 ml, b.p. 65°C), the supernatant was separated by using a separator funnel. The lower fraction was dried under vacuum, and designated as PICE (F003). Finally, all the extracts were dissolved in DMSO individually, and used for testing ICAM-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.

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 C₄₈H₃₁FN₂O₈S and m.pt is 65°C.

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-NO₂, 1591(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.

¹H N M R

¹HNMR data were carried out DMSO-d₆ with 400Mz resolution 67.767 ppm Ar-H, 7.761-7.394ppm[s], Ar-H, 6.577-5.539[m] ppm, 3H, CH, 5.304-653, 24H, 2.184-4.447ppm CH, 2.136-0.73ppm, 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, α = 90°, β =90°, γ =90°, V = 911.59 (Å³), index 0 ≤  h ≤ 110, 0 ≤  k ≤ 8, 0 ≤  l ≤ 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 geometry. The potential energy of the molecule was the sum of the following terms: Estr +Erot +Evdw +Eoop +Eele, where all E 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.

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 [(1E,3E,5E,7E,12E,15E,18E)-9-amino-20-(7-fluoro-4-hydroxybenzo[d][1,3]dioxol-5-yl)-10,20-dioicosa-heptaenyl)-6-(3-nitrofenoxo) 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 of 3-10^8 platelets/ml. Platelet aggregation was measured using an aggregometer as described previously [38]. Briefly, washed platelet suspension (WPS) was incubated at 37°C 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 10 min. The inhibition rate was obtained from the maximal aggregation induced by the respective agonist at the concentration using the equation inhibition rate = (maximal aggregation rate (MAR) of vehicle-treated WPS-MAR of sample-treated WPS/MAR of vehicle treated)-100. Acetylsalicylic acid (ASA, aspirin) [37-42] was used as a positive control to show significant 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

<table>
<thead>
<tr>
<th>Conc. (µM)</th>
<th>Collagen (2 µg/ml)</th>
<th>AA (100 µM)</th>
<th>PAF (10 nM)</th>
<th>Thrombin (0.1 unit/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>72.7±2.9</td>
<td>69.9±2.9</td>
<td>69.3±1.8</td>
<td>80.2±1.3</td>
</tr>
<tr>
<td>Piperine</td>
<td>62.6±0.3**</td>
<td>2.4±0.4**</td>
<td>1.2±1.1**</td>
<td>75.6±1.5</td>
</tr>
<tr>
<td>30</td>
<td>49.3±1.1**</td>
<td>32.1±3.5*</td>
<td>16.4±2.5**</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>71.1±2.5*</td>
<td>55.3±2.1*</td>
<td>59.2±3.2*</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>3.2±0.3**</td>
<td>2.6±0.3**</td>
<td>3.4±0.2**</td>
<td>76.8±2.1</td>
</tr>
<tr>
<td>Pipernonaline</td>
<td>12.9±0.6*</td>
<td>41.6±3.1*</td>
<td>30.7±0.7*</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>79.2±3.1**</td>
<td>67.4±2.8*</td>
<td>57.2±2.3*</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>14.4±2.1*</td>
<td>29.6±0.8*</td>
<td>21.5±3.8**</td>
<td>76.5±0.2</td>
</tr>
<tr>
<td>300</td>
<td>49.0±1.5*</td>
<td>54.2±1.6*</td>
<td>52.7±1.5*</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>37.2±2.6*</td>
<td>68.8±2.7*</td>
<td>67.6±6.1*</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>0.0±0.0**</td>
<td>0.0±0.0**</td>
<td>0.0±0.0**</td>
<td>61.3±0.9</td>
</tr>
<tr>
<td>250</td>
<td>0.0±0.0**</td>
<td>16.7±2.1*</td>
<td>0.0±0.0**</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>36.5±3.9**</td>
<td>61.5±1.7*</td>
<td>52.0±2.4*</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>58.2±2.4*</td>
<td>70.1±2.4</td>
<td>68.7±2.1</td>
<td></td>
</tr>
<tr>
<td>Acetylsalicylic acid (aspirin)</td>
<td>68.5±3.4</td>
<td>68.2±1.5</td>
<td>81.1±0.7</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>73.1±2.6</td>
<td>17.5±2.4**</td>
<td>69.1±2.1</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>37.8±3.7*</td>
<td>68.2±1.5</td>
<td>81.1±0.7</td>
<td></td>
</tr>
</tbody>
</table>

Washed rabbit platelets were preincubated with DMSO (0.5% control) or each compound at 37°C 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 glutathione 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


18. Yu-Chang Chen, Chang-Hui Liao, Ih-Sheng Chen, Lignans, an amide and anti-platelet activities from...


27. Cheng, Y., Prusoff, W.H. Relationship between the inhibition constant (Ki) and the concentration of inhibitor which causes 50% inhibition (IC50) of an enzymatic reaction. Biochem. Pharmacol. 22, (1973), 3099–3108.


43. M. Abbas Ali, Noor Mahbub Alam, Mast, 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.

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