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Investigations toward new lead compounds from medicinally important plants*

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Abstract: Extensive phytochemical investigations on 30 *Piper* species growing in India and other medicinal plants have revealed the presence of a large number of novel compounds belonging to different classes. The antiviral activity of several lignans and neolignans belonging to different structural types has been evaluated against six different viral strains. Further, the effects of ethanol, chloroform, and hexane extracts of *Piper longum* and *Piper galiatum* on TNF-α induced expression of intercellular adhesion molecule-1 (ICAM-1) on human umbilical vein endothelial cells have been studied, a novel aromatic ester was isolated from the most active extract of *P. longum*. A potential antifungal compound having implications in treating aspergillosis was isolated from an important Indian medicinal plant, *Datura metel*.

INTRODUCTION

Plants have been the source of medicines for thousands of years, species of the genus *Piper* are among the important medicinal plants used in various systems of medicine [1–3]. *Piper* species are widely distributed in the tropical and subtropical regions of the world and are of high commercial and economical importance, e.g., black pepper from *Piper nigrum* has world-wide spice market. Some of the plants belonging to the genus *Piper* are reputed in the Indian Ayurvedic system of medicine for their medicinal properties and in folklore medicines of Latin America and the West Indies. Chloroform extract of the stems of *P. aborescens* was found to display significant activity against the KB cell culture system and the P-388 lymphocytic leukemia system in cell culture [4]. *Piper amalago*, distributed from Mexico to Brazil, is used to alleviate chest pain and inflammation [5]. *Piper sylvaticum* roots are used as an effective antidote to snake poison in the indigenous system of Indian medicine. *Piper chaba* roots and fruits find numerous applications in medicines, particularly for asthma, bronchitis, fever, and abdominal pain, as a stimulant, and in hemorrhoidal afflictions [6]. *Piper futokadsura* is a medicinal plant that grows in Fuchein and Taiwan provinces. The West African black pepper (*P. guineense*) is a woody

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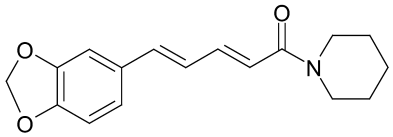
climber distributed throughout West Africa; its fruits have been used as a flavorant, while preparations of leaves, roots, and seeds have been used internally as medicinal agents for the treatment of bronchitis, gastrointestinal diseases, venereal diseases, and rheumatism. An extract of black pepper shows carcinogenesis in mice; the evidence of malignant tumors and of multiple tumors was greater in the pepper-treated mice than in vehicle-treated mice [7]. The extract of *P. betle* has also been reported to show antihypertensive activity and that of *P. argyrophyllum* has shown activity as inhibitor of aflatoxin B_1-DNA binding [8]. An equal part of powdered seeds of embelia ribes, fruit of *P. longum* and borax powder has been used as an Ayurvedic contraceptive [9]. The stem of *P. futokadsura*, known as haifengteng, is widely used in Chinese herbal medicinal prescriptions for the treatment of asthma and arthritic conditions; the benzene extract of its leaves showed anti-feedent activity against the larvae of *Spodoptera litura* F. [10]. *Piper longum* has been used in traditional remedies as well as in the Ayurvedic system of medicine against various disorders [11,12]. A methanolic extract of its fruit powder is found to be effective against mosquito larvae [13], whereas an ethanolic extract has an anti-amoebic activity [14].

Many potential insecticidal amides have been isolated from the genus *Piper*, e.g., pipercide, isolated from *Piper nigrum*, which has been found to be just as active against adzuki bean weevils as the pyrethroids [15]. *Piper brachystachyum* shows insecticidal properties [16]. The petroleum ether and dichloromethane extracts of the leaves and stems of *P. falconeri* have shown insecticidal activity against *Musca domestica* (flies) and *Aedes aegyptii* (mosquitoes) [17]. *Piper rotundistipulum* has been used traditionally as an insecticide and as a fish poison [18]. *Piper longum*, *P. cubeba*, and *P. peepuloides* are known to have insecticidal activity against mosquitoes and flies [19] and were shown to repel grain pests [20]. Neurotoxic amides and lignans appear to be mainly responsible for the anti-insect activities of *Piper* species [21,22]. Several bioactive constituents have been isolated from *Datura metel*, one of the important medicinal plants grown in India. It contains tropane alkaloids, which have been used as sedatives and antispasmodic agents [23].

Herein, we present an overview of our work carried over two decades under a major project being investigated in collaboration between Indian and Danish Universities. We have carried out the phytochemical investigations on a majority of *Piper* species [3] growing in India, *Taxus* species [24–27], *Gardenia* species [28], *Uvaria* species [29,30], *Cephalotaxus* species [31], *Aristolochia* species [32], *Prunus* species [33–38], *Fraxinus* species [39–41], *Tamarix* species [42–46], *Tephrosia* species [47–52], and *Agave americana* [53–55]. In addition, recent unpublished work on the antiviral activity evaluation of lignans and neolignans, anti-inflammatory activity guided separation of an active constituent from the chloroform extract of *P. longum* and isolation of a potent antifungal pyrrole derivative from the chloroform extract of *D. metel* are also reported.

PHYTOCHEMICAL INVESTIGATION OF INDIAN *PIPER* SPECIES AND OTHER MEDICINAL PLANTS

Since the isolation of piperine from *P. nigrum* [56], scientists have been searching for new physiologically active compounds in plants from the family Piperaceae and hundreds of compounds belonging to different classes have been isolated [3,57–63].



Piperine

As part of our research program on the isolation and structure elucidation of naturally occurring bioactive compounds from Indian *Piper* species, we collected 30 *Piper* species (Table 1), mainly from Western Ghats, Andaman, and Nicobar islands and northeastern parts of India, and extracted them suc-

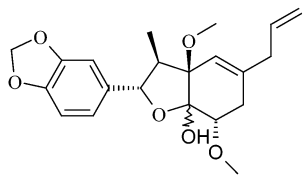
cessively with petroleum ether, dichloromethane, and methanol to isolate pure compounds from different extracts. It was discovered that *Piper* species are a rich source of different classes of compounds, viz. lignans and neolignans, long-chain esters and amides, alkaloids, terpenoids, steroids, kawapyrones, piperolides, chalcones, dihydrochalcones, flavonoids, and pyrrole derivatives [3,57,58].

Table 1 *Piper* species collected from different parts of India.

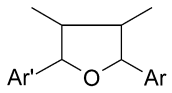
1.	*Piper falconeri* Dc.	16.	*Piper griffithi* DC.
2.	*Piper clarkii* Dc.	17.	*Piper pedicellosum* Wall.
3.	*Piper acutisleginum* C. Dc.	18.	*Piper beetileoides* C. Dc.
4.	*Piper betle* Linn.	19.	*Piper hymnophyllum* Miq.
5.	*Piper khasiana* C. Dc.	20.	*Piper thomsoni* hook f.
6.	*Piper peepuloides* Wall.	21.	*Piper brachystachyum* Wall.
7.	*Piper nigrum* Linn.	22.	*Piper argyrophyllum* Miq.
8.	*Piper schmidtii* hook f.	23.	*Piper sylvaticum* Roxb.
9.	*Piper wightii* Miq.	24.	*Piper mullesua* D. Don.
10.	*Piper hookeri* Miq.	25.	*Piper boehmeriaefolium* Miq.
11.	*Piper attanuatum* Ham.	26.	*Piper gamblei* C. Dc.
12.	*Piper colubrinum*	27.	*Piper nepalense* Miq.
13.	*Piper longum* Linn.	28.	*Piper galiatum* C. Dc.
14.	*Piper manii* C. Dc.	29.	*Piper rebiseoides*
15.	*Piper diffusum* vahl.	30.	*Piper aduncum*

LIGNANS, NEOLIGNANS, LONG-CHAIN ESTERS, AND AMIDES FROM *PIPER* SPECIES

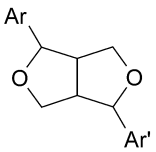
The terms "lignans" and "neolignans" have been defined by Gottlieb and Yoshida [64], and Ayres and Lioke [65]. The lignans and neolignans isolated from Piperaceae until 1992 have been reviewed by Jensen et al. [57]. During 1994–1999, we have reported the isolation of 38 lignans and neolignans from different *Piper* species out of which 18 are new compounds, viz. **1**, **4**, **9**–**11**, **16**, **18**, **20**, **21**, **23**–**27**, **29**, **34**, **37**, and **38**, this perhaps is the largest number of compounds of this class reported from any genus. Lignans and neolignans can be divided into five different structural types (Scheme 1), e.g., 2,5-bisaryl-



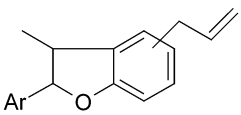
Structure of schmiditin [Joshi et al. *J. Nat. Prod.* 53, 479 (1990)]



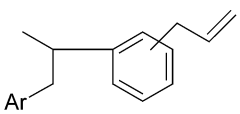
2,5-bisaryl-3,4-dimethyltetrahydrofuran



2,6-bisaryl-3,7-dioxa[3,3,0]bicyclooctane



benzofuran



1,2-diarylpropane

Scheme 1

3,4-dimethyltetrahydrofurans **1**–**5**, 2,6-bisaryl-3,7-dioxa[3.3.0]bicyclooctanes **6** and **7**, benzofurans **8**–**23**, 1,2-diarylpropanoids **24**–**36**, and miscellaneous **37** and **38** as given in Figs. 1–5. We have isolated 22 open-chain and cyclic amides **39**–**55** (Fig. 6) in addition to several long-chain esters and other miscellaneous compounds (Fig. 7) from different *Piper* species, out of which nine are novel compounds, i.e., **39**, **42**, **48**, **56**, and **61**–**65**.

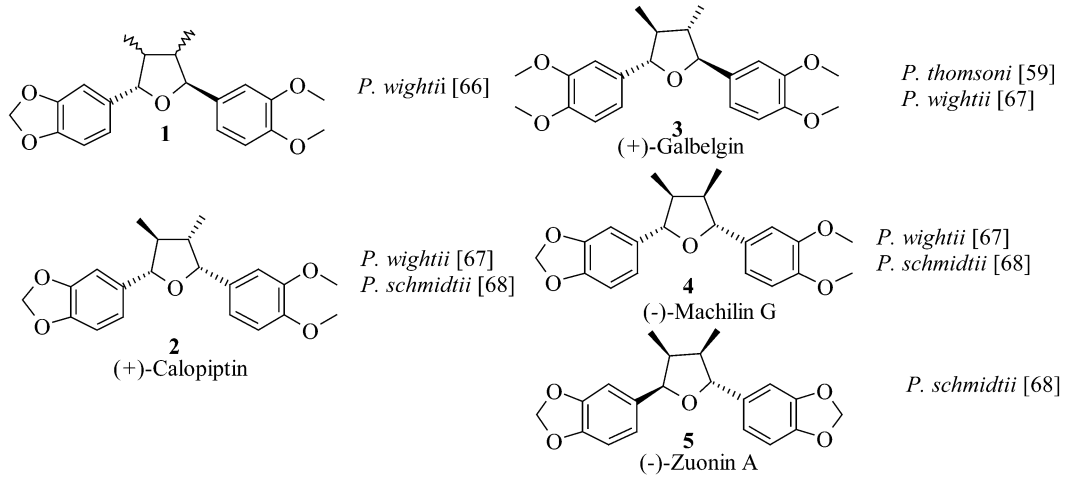


Fig. 1 2,5-Bisaryl-3,4-dimethyltetrahydrofurans.

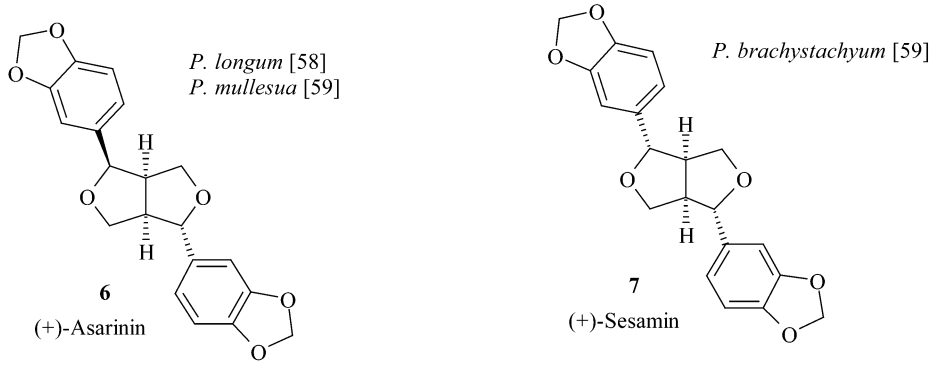


Fig. 2 2,6-Bisaryl-3,7-dioxa[3.3.0]bicyclooctanes.

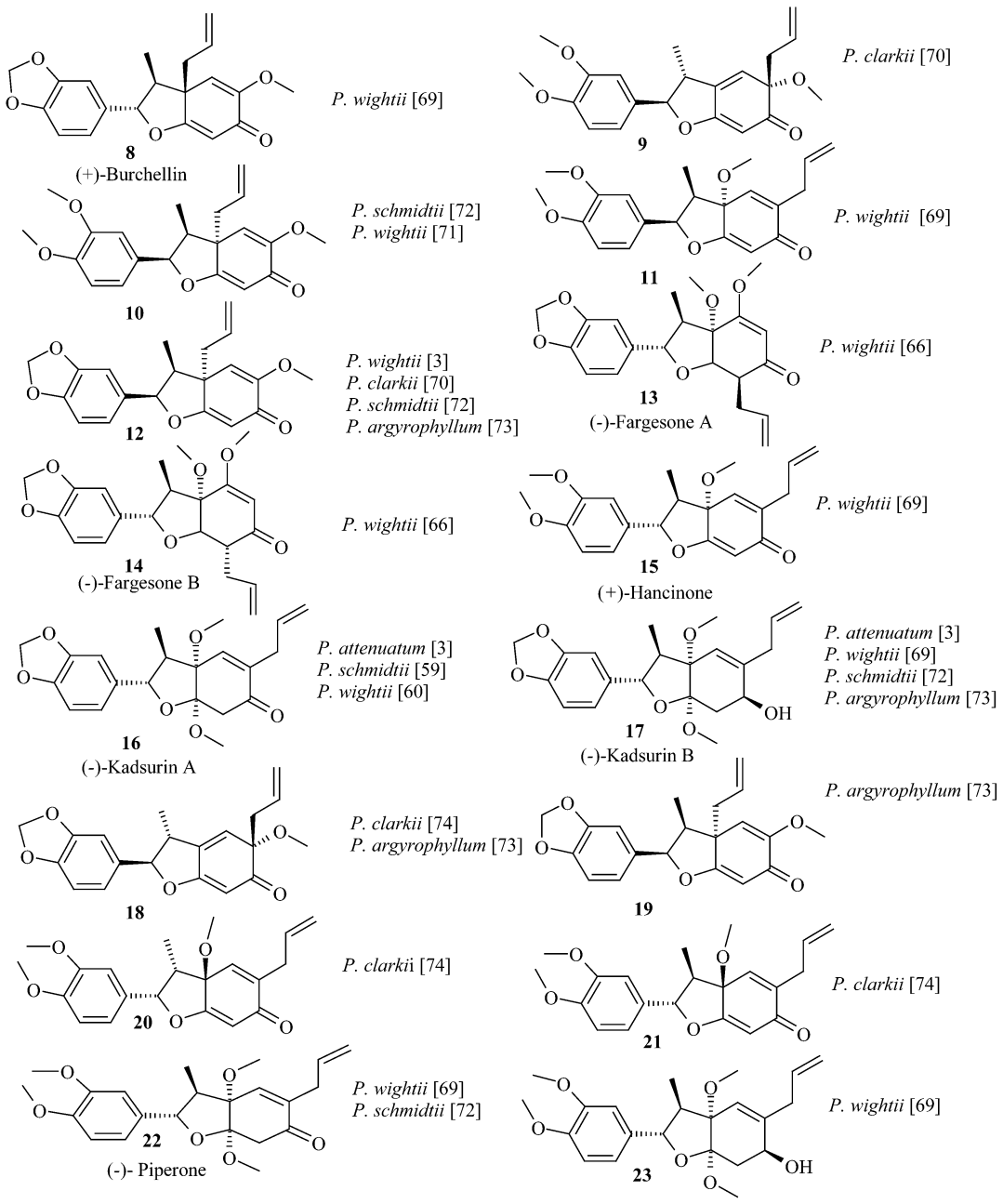


Fig. 3 Benzofuran lignans.

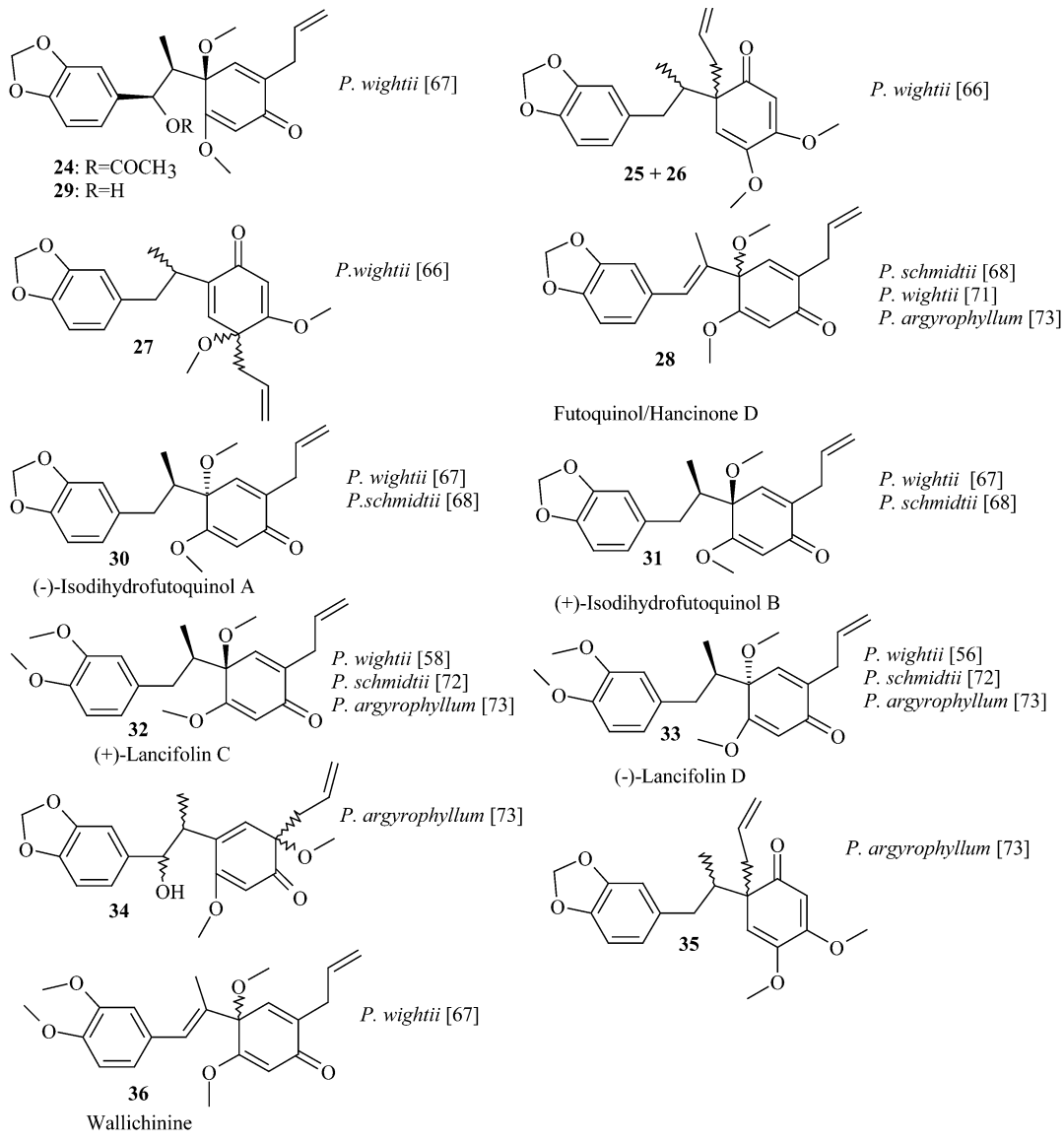


Fig. 4 1,2-Diarylpropanoids.

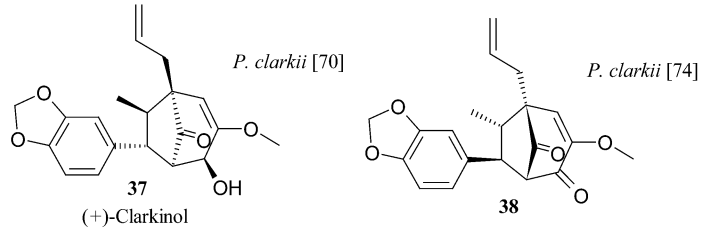


Fig. 5 Miscellaneous.

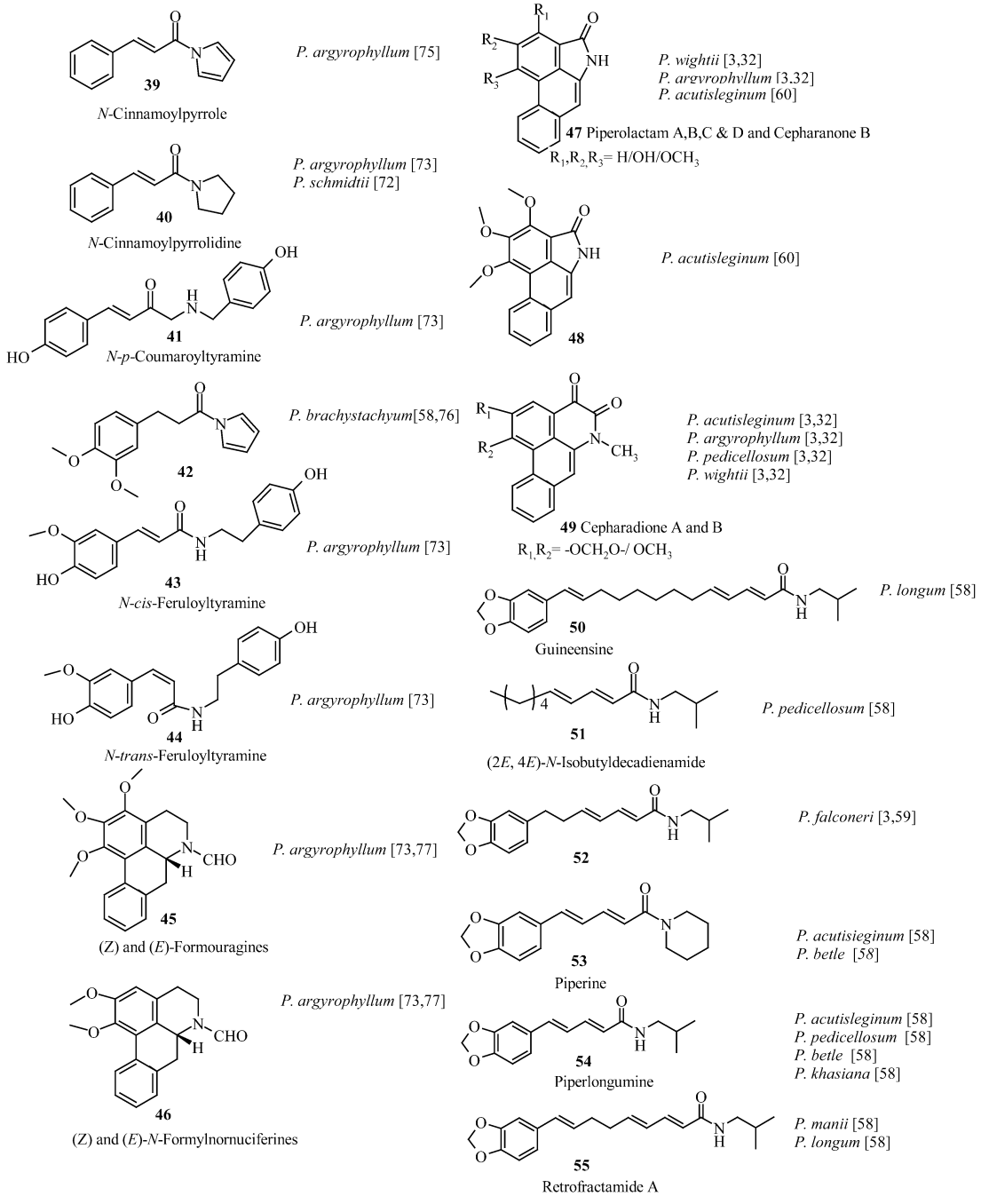

39
N-Cinnamoylpyrrole
P. argyrophyllum [75]
40
N-Cinnamoylpyrrolidine
P. argyrophyllum [73]
P. schmidtii [72]
41
N-p-Coumaroyltyramine
P. argyrophyllum [73]
42
P. brachystachyum[58,76]
43
N-cis-Feruloyltyramine
P. argyrophyllum [73]
44
N-trans-Feruloyltyramine
P. argyrophyllum [73]
45
(Z) and (E)-Formouragines
P. argyrophyllum [73,77]
46
(Z) and (E)-N-Formylnornuciferines
P. argyrophyllum [73,77]
47 Piperolactam A,B,C & D and Cepharanone B
R1,R2,R3= H/OH/OCH3
P. wightii [3,32]
P. argyrophyllum [3,32]
P. acutisleginum [60]
48
P. acutisleginum [60]
49 Cepharadione A and B
R1,R2= -OCH2O-/ OCH3
P. acutisleginum [3,32]
P. argyrophyllum [3,32]
P. pedicellosum [3,32]
P. wightii [3,32]
50
Guineensine
P. longum [58]
51
(2E, 4E)-N-Isobutyldecadienamide
P. pedicellosum [58]
52
P. falconeri [3,59]
53
Piperine
P. acutisieginum [58]
P. betle [58]
54
Piperlongumine
P. acutisleginum [58]
P. pedicellosum [58]
P. betle [58]
P. khasiana [58]
55
Retrofractamide A
P. manii [58]
P. longum [58]

Fig. 6 Amides.

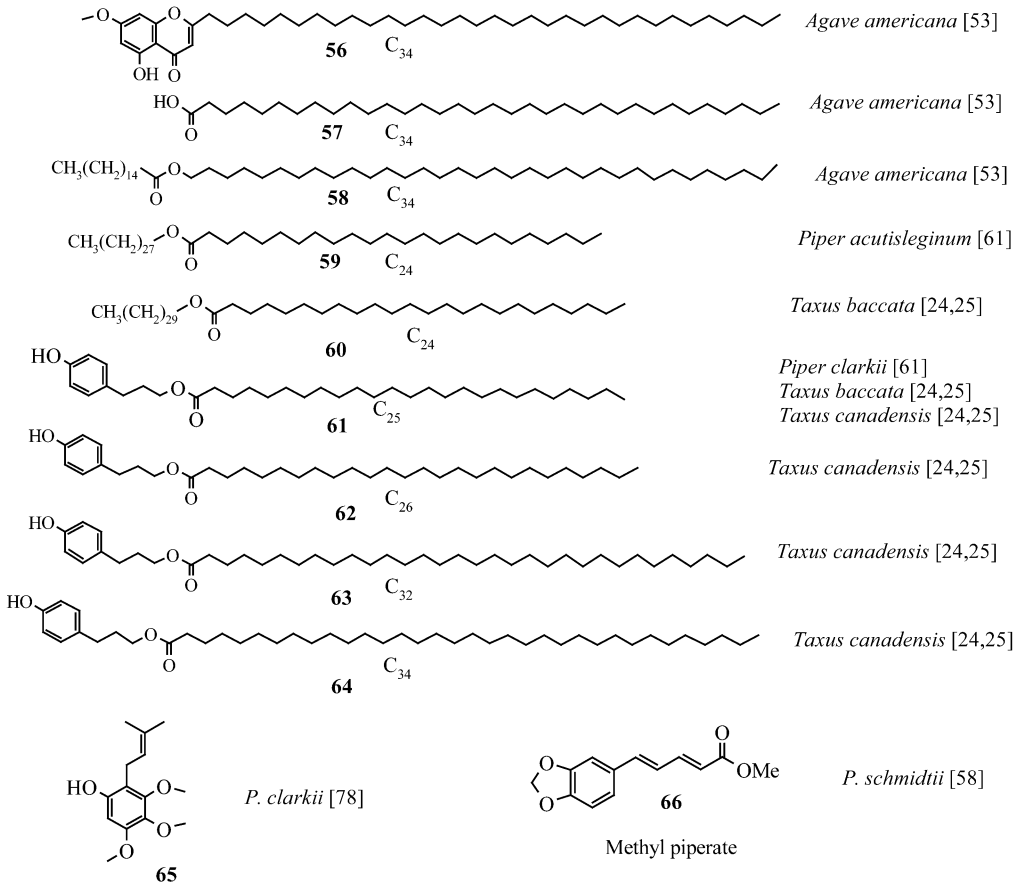


Fig. 7 Esters and other compounds.

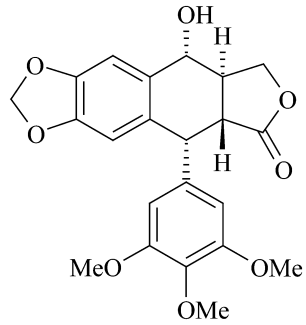
We have revised the structures of several compounds reported earlier in the literature from natural sources, one example of relevance in this paper is about the occurrence of a new lignan, viz. schmiditin from *P. schmidtii* reported by Joshi et al. [*J. Nat. Prod.* **53**, 479 (1990)]. According to our detailed studies, the structure assigned to the compound isolated by Joshi et al. is (–)-kadsurin B (**17**), and we suggested the removal of the name schmiditin from the literature [Tyagi et al. *Acta Chem. Scand.* **49**, 142 (1995)].

Most of the *Piper* species are climbers. Phytochemical investigation of *Piper acutisleginum* and *Piper clarkii* has led to the isolation of two long-chain esters **59** and **61**, which may be the constituents of the waxes present in these climber plants. Phytochemical investigation of *Agave americana* has led to the isolation of the biogenetically related compounds: chromone **56**, long-chain acid **57**, and ester **58**. Plants of the *Taxus* species are famous for the isolation of anticancer compounds, i.e., taxol and other taxanes. Phytochemical investigation of *Taxus baccata* and *Taxus canadensis* has led to the isolation of five long-chain esters **60–64**.

BIOLOGICAL ACTIVITIES OF LIGNANS AND NEOLIGNANS ISOLATED FROM DIFFERENT *PIPER* SPECIES, AND CHROMONE, LONG-CHAIN ALCOHOL, AND ESTER ISOLATED FROM *AGAVE AMERICANA*

Lignans and neolignans possess a variety of biological activities. Some of the lignans isolated from *Piper* species possess antifeedant activity against stored pests [79]. The lignans, sesamin and sesamolin

combined with certain synthetic compounds, synergize their insecticidal activities [80]. In one report, the inflorescence material of *P. mullesua* (syn. *P. brachystachyum*), commonly known as *pahari peepal*, was examined for sesamin content where the insecticidal and growth inhibitory effects of purified sesamin were quantified against the larvae of *Spilarctia oblique* Walker [81]. The recent identification of lignans in human urine and blood indicated their possible roles in human physiology [82]. Some of the important biological activities shown by lignans and neolignans are antitumor, antimitotic, aflatoxin-DNA binding inhibition, antiviral, insecticidal, etc. The antitumor activity of podophyllotoxin, a lignan isolated from *Podohyllum* species and other related compounds, has aroused considerable interest in compounds of this class [83–85]. A close inspection of structures of active compounds belonging to these classes has revealed that the presence of some structural features, e.g., a five-membered lactone ring, a 3,4,5-trimethoxyphenyl group, and a methylenedioxyphenyl group, are responsible for their activity [82].



Podophyllotoxin

Encouraged by the interesting biological profile of lignans and neolignans, we evaluated antiviral activities of representative examples of each structural types of lignans and neolignans, i.e., (–)-machilin G (**4**), belonging to 2,5-bisaryl-3,4-dimethyltetrahydrofuran structural type; (+)-asarinin (**6**) and (+)-sesamin (**7**), belonging to 2,6-bisaryl-3,7-dioxa[3,3,0]bicyclooctane structural type; (–)-kadsurin A (**16**) and (–)-kadsurin B (**17**), belonging to benzofuran structural type; and (–)-isodihydrofutoquinol A (**30**) and (+)-isodihydrofutoquinol B (**31**), belonging to 1,2-diarylpropane structural type. Antiviral activity of these compounds was tested against herpes simplex virus type 1 (HSV-1), coxsakie B2 (Cox B2), measles edmondston A (MEA), poliomyelitis virus type 1 (Polio 1), semliki forest L10 (SF L10), and vesicular stomatitis virus (VSV) at five different concentrations, 100, 50, 25, 10, and 1 μg/ml. (+)-Asarinin (**6**) was found to be highly active against Cox B2, MEA, and Polio 1 viruses, and it was weakly active against SF L10 and VSV viruses. (+)-Sesamin (**7**) showed high activity against SF L10 virus, it was inactive against other viruses. (–)-Kadsurin A (**16**) and (+)-isodihydrofutoquinol B (**31**) have shown moderate activity against HSV-1 virus, they were however inactive against other viruses. (–)-Machilin G (**4**), (–)-kadsurin B (**17**), and (–)-isodihydrofutoquinol A (**30**) either did not exhibit appreciable activity against any of the viruses under study or precipitated in the growth medium [unpublished results].

We undertook the chemical investigation of *A. americana* L. because of its traditional use as drug in the Indian system of medicine (used as diuretic, antisyphilitic, laxative, emmenagogue, and antiscorbutic). Antibacterial activity of compounds isolated from *A. americana* has been tested against four bacteria viz. *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus faecalis*, and it was found that 2-tritriacontyl-5-hydroxy-7-methoxychromone (**56**) is highly active against *P. aeruginosa* [53].

STUDY OF EFFECT OF EXTRACTS OF *PIPER LONGUM* AND *P. GALIATUM* ON TNF-α INDUCED EXPRESSION OF ICAM-1 ON HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

Inflammation is caused by soluble antigen, live organisms and chemical or mechanical stress upon tissue, which serves to destroy and/or dilute the injurious materials, and remove the injured tissues. It is characterized pathologically by an increased supply of blood to the affected area, increased capillary permeability caused by retraction of the endothelial cells and infiltration of phagocytic, monocytic and polymorphonuclear cells into the site of tissue insult. The accumulation and subsequent activation of leukocytes is one of the central events in the pathogenesis of all forms of inflammation.

The migration of the leukocytes to the site of inflammation is regulated in part by the expression of cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and E-selectin [86]. These cell-adhesion molecules are induced on endothelial cells by various pro-inflammatory cytokines like IL-1β and TNF-α and also by bacterial LPS [87]. It is well established that in various inflammatory diseases, the expression of these proteins is upregulated on endothelial cells [88,89]. Various synthetic drugs have been demonstrated to inhibit the expression of these molecules but they have been reported to have many side effects [90]. Therefore, there is a need to develop a remedy that is safer and has fewer or negligible side effects. We have examined the effect of ethanol, chloroform and hexane extracts of *P. longum* and *P. galiatum* on TNF-α induced expression of ICAM-1 on human umbilical vein endothelial cells [unpublished results].

Piper longum and P. galiatum extracts inhibit ICAM-1 expression on endothelial cells: ICAM-1 was expressed at low levels on unstimulated endothelial cells, and its expression was induced over five-fold by TNF-α stimulation. To determine the effect of *P. longum* and *P. galiatum* extracts on the expression of ICAM-1 on endothelial cells, the cells were incubated with or without extracts at different concentrations for 1 h prior to treatment with TNF-α for 16 h. Using cell-ELISA, it was observed that chloroform extract of *P. longum* exhibited 70 % inhibition of TNF-α induced ICAM-1 expression on endothelial cells, followed by hexane and ethanol extracts which showed about 40 % inhibition (Fig. 8). The most active chloroform extract was subjected to column chromatography leading to the purification of two compounds, piperine and a novel aromatic ester. The study of the effect of piperine and novel aromatic ester on the expression of ICAM-1 on endothelial cells and characterization of the ester is in progress.

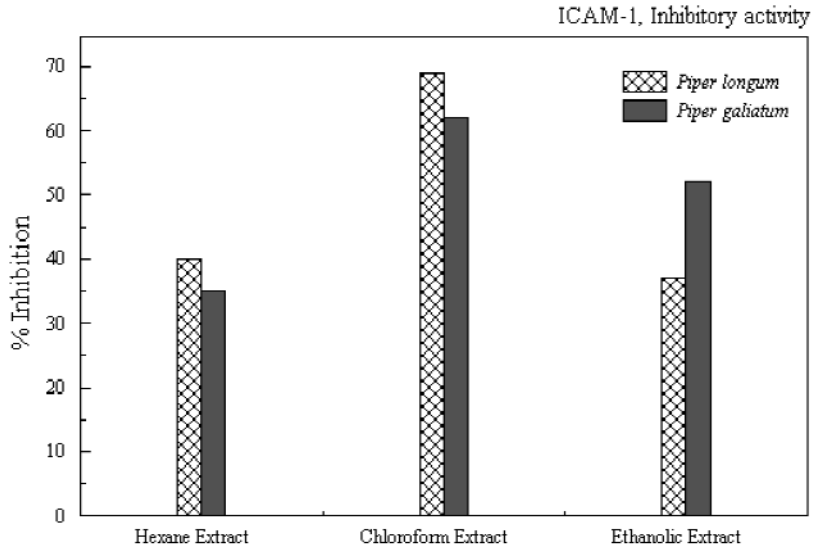


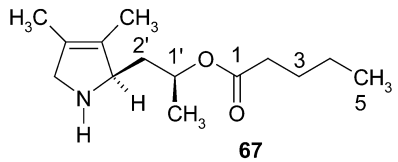
Fig. 8 Inhibition of ICAM-1 expression on endothelial cells by *P. longum* and *P. galiatum* extracts.

The hexane and the chloroform extracts of *P. galiatum* exhibited 35 and 65 % inhibition, respectively, of TNF-α induced ICAM-1 expression on endothelial cells, which is little less than the activity of hexane and chloroform extracts of *P. longum*. However, the inhibitory activity of ethanolic extract of *P. galiatum* is more than the activity of ethanolic extract of *P. longum*. This indicated that ethanolic extract of *P. galiatum* might have another active component present in it. Preliminary toxicity study has revealed that *P. longum* extracts are nontoxic to endothelial cells [unpublished results].

BIOACTIVE PYRROLE DERIVATIVE, 2'-(3,4-DIMETHYL-2,5-DIHYDRO-1*H*-PYRROL-2-YL)-1'-METHYLETHYL PENTANOATE FROM *D. METEL*: ANTIFUNGAL AND IMMUNOPHARMACOLOGICAL PROPERTIES

Antifungal activity

Datura metel is an important medicinal plant, which contains a large number of bioactive constituents. The chloroform fraction of leaves of *D. metel* inhibited the growth of pathogenic *Aspergilli* (*Aspergillus fumigatus*, *A. niger*, and *A. flavus*) up to a concentration 12.5 μg $disc^{-1}$ by disc diffusion (DD), 1.25 mg ml^{-1} each by microbroth dilution assay (MDA) and percent spore germination inhibition (PSGI) assays [91]. Further, phytochemical investigation on the chloroform extract of leaves of *D. metel* Linn. led to the isolation of a new pyrrole derivative, which was characterized as 2'-(3,4-dimethyl-2,5-dihydro-1*H*-pyrrol-2-yl)-1'-methylethyl pentanoate (**67**). Compound **67** was endowed with antifungal activity, and its MIC value was found to be 87.5 μg/ml by MDA and PSGI and 5.0 μg/disc by disc diffusion assay. It was interesting to observe that compound **67** was manifold less toxic to RAW cells than standard drug amphotericin B [92].



67

IMMUNOPHARMACOLOGICAL PROPERTIES: EFFECT OF COMPOUND 67 ON CYTOKINE PROFILE

Initially, the experiments were performed to find out the time of optimum expression of IFN-γ and IL-4. Three mice were drawn from the *Aspergillus fumigatus* infected group and sacrificed every 24 h after infection to find the optimum expression of interleukins. It was observed that the interleukin level started increasing on the third day and reached maximum on the sixth day after infection. Therefore, the animals of test groups (Group I: normal; II: infected; III: infected–amphotericin B treated; IV: infected–compound **67** treated; and V: normal–compound **67** treated) were sacrificed on the sixth day to determine the levels of interleukins in infected–treated groups. The *A. fumigatus* infection evoked increased production of IL-4 (88.8 pg/ml) in animals; in normal animals, the endogenous level of IL-4 was found to be 5.75 pg/ml of serum (Fig. 9). The treatment of infected animals with compound **67** significantly reduced the production of IL-4 (40.1 pg/ml) (Fig. 9). The treatment of infected animals with amphotericin B also reduced the production of IL-4 to 31.9 pg/ml. In animals uninfected but treated with compound **67**, the serum levels of IL-4 were found to be 8.6 pg/ml [unpublished results].

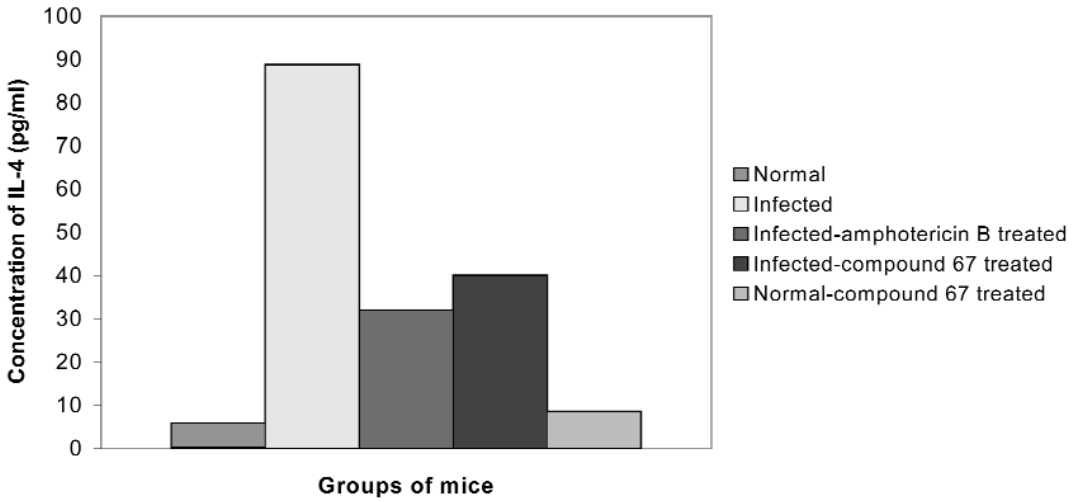


Fig. 9 Level of IL-4 in the serum of different groups of mice.

The level of IFN-γ in infected animals was found to be 1455.0 pg/ml. The treatment of infected animals with compound **67** further boosted the synthesis of IFN-γ, and the level increased up to 1946.0 pg/ml (Fig. 10). The level of IFN-γ in amphotericin B-treated animals was found to be 1731.0 pg/ml. The level of IFN-γ in normal-compound **67** treated and normal, untreated animals was not significantly different [unpublished results].

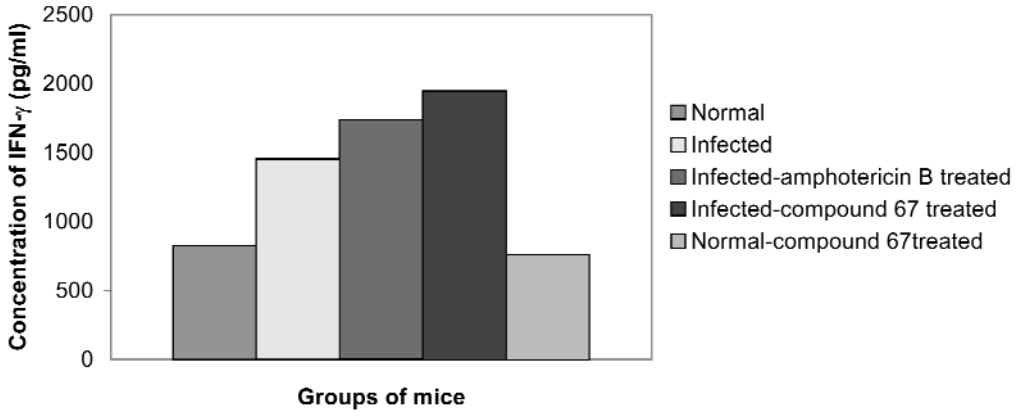


Fig. 10 IFN-γ levels in serum of different groups of mice.

HISTAMINE LEVEL

The whole blood histamine levels in animals of Groups I–V were found to be 148.3, 254.2, 261.3, 190.8, and 154.2 pM, respectively. The infected animals treated with 2'-(3,4-dimethyl-2,5-dihydro-1*H*-pyrrol-2-yl)-1'-methylethyl pentanoate (**67**) showed decreased levels of histamine in the blood (Fig. 11) [unpublished results].

It may be concluded from this study that novel compound **67** isolated from *D. metel* has strong potential against *Aspergillus* species which are pathogenic to humans (cause aspergillosis) and it was manifold less toxic than amphotericin B. The *Aspergillus* infection is known to elicit Th_2 type of im-

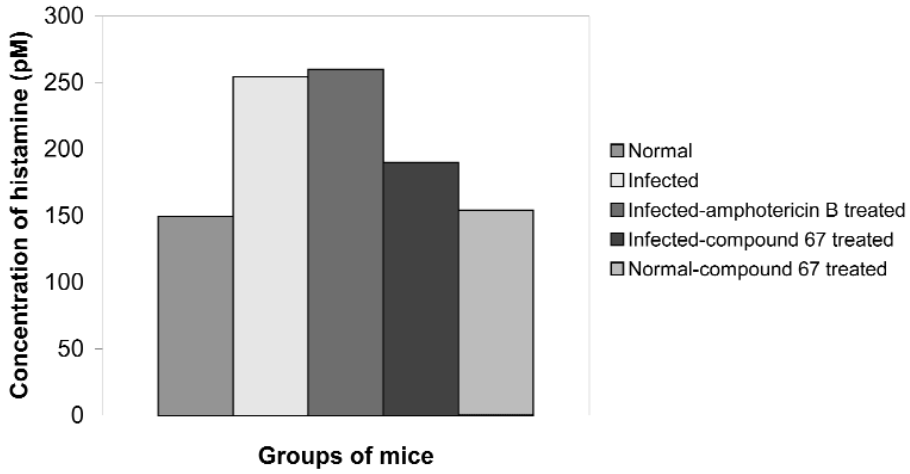


Fig. 11 Whole blood histamine levels in different groups of mice.

mune response. The observation of the current study indicated that the treatment of infected animals with compound **67** might bring a shift from Th_2 to Th_1 type of immune response.

CONCLUSION

Piper species are a rich source of different classes of secondary metabolites, particularly of biologically active amides and lignans and neolignans. As extracts of *Piper longum* inhibit the cytokine-induced ICAM-1 expression on endothelial cells, these extracts and the pure compounds derived from them could be used to develop anti-inflammatory agents in the future. Further, 2'-(3,4-dimethyl-2,5-dihydro-1*H*-pyrrol-2-yl)-1'-methylethyl pentanoate (**67**) isolated from the chloroform extract of *D. metel* had strong inhibitory activity against *Aspergillus* species pathogenic to humans (cause aspergillosis). This compound has manifold less toxicity than the standard drug amphotericin B used clinically to treat aspergillosis.

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