



Phytochemical investigation Afforded a novel Cycloartanetriterpenoid from *Piper thomsoni*

Goswami Rajeev* and Jain S.C.

Department of Chemistry, University of Delhi, Delhi-110 007, INDIA

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Abstract

A Novel alkenyl phenol, 4-(7E-dodecenyl)phenol (**2**) and triterpenoid cycloart-23-en-3-one (**4**) and also first time from *Piper* genus 3 β -22-dihydroxylanosta-8,24-dien-26-oicacid- δ -lactone (**8**) were isolated from the leaves and stems DCM:MeOH (1:1) extracts of *Piper thomsoni*. All isolated compounds structures were defined using modern spectral techniques.

Keywords: *Piper thomsoni*; Piperaceae; cycloart-23-en-3-one; 4-(7E-dodecenyl)phenol; anticancer.

Introduction

Since ancient time, plants have been the source of medicines and the plants belong to the genus *Piper* are known for their medicinal importance^{1,2}. *Piper* species are geographically disseminated in the tropical and subtropical climatic zones and are explored as folk medicines in several ways. *P. nigrum* ripened fruit is used as white pepper, whereas black pepper is sourced from its unripe fruit. Isobutyl amides isolated from *P. nigrum* fruits showed larvicidal effect on *Aedes aegypti*, *A. togoi* and *Culex pipiens pallens* larvae³. *P. amalagois* used as anti-inflammatory agent for alleviating chest pain⁴. *P. aborescens* stems chloroform extract exhibited good activity against P-388 lymphocytic leukemia cells and KB cell⁵. *P. cubeba* has long been a source of folk and herbal medicine⁶. The *P. longum* fruit has also been a source of indigenous medicines, which includes Indian Ayurvedic medicine, to treat bronchitis, diarrhea, malaria, viral hepatitis and tumors⁷. An amide, separated from the fruit of *P. longum* L., displayed inhibitory activity against the fourth-instar larvae of *Aedes aegypti*⁸.

Alkaloids isolated from CH₃OH extract of *P. lolot* exhibited good inhibition of thrombocyte clustering caused by polyunsaturated ω -6 fatty acid (arachidonic acid) and PAF-acether⁹. Rats treated with pipermethystine, abundant in *Piper methysticum*, showed increased cytosolic superoxide dismutase, cachectin mRNA expression, hepatic glutathione, CYP 1A2 and 2E1 which suggested adaptive feature to induce oxidative stress and likely drug-drug interactions¹⁰.

Piper thomsoni is one of the forty-five species of the family Piperaceae and is being used as traditional and folk medicines². It is well documented in the Indian Ayurvedic system of medicine. *P. thomsoni* leaves are the source of Pan, which is also applied to wounds and swellings; whereas its aqueous root extract is used as diuretic. Previously *P. thomsoni* phytochemical explorations have resulted in the isolation and characterization of several alkaloids and terpenoids^{11,12}. Here,

we describe the isolation and characterization of bioactive secondary metabolites from the leaves and stems combined extract of this species, which afforded two new compounds an alkenyl phenol and one cycloartanetriterpenoid, additionally seven earlier reported compounds (figure-1). However, out of seven known compounds, lanostanetriterpenoid (**8**) is first time reported from the *Piper* genus. Herein, extraction, compounds isolation and their detailed characterization are discussed.

Material and Methods

Solvents used for column chromatography (CC) were procured from Merck and used after distilling them. 60-120 mesh silica-gel was used for CC from Merck. Melting points were checked using Fischer Johns equipment and are uncorrected. The ¹H, ¹³C NMR and 2D NMR spectra were recorded with tetramethylsilane as an internal standard on a Bruker-300 Spectrometer in deuterated solvents as required. Perkin-Elmer Infra-red Spectrometer was used to record IR spectra either as KBr pellets or film. The ESI mass spectra were determined using Jeol Spectrophotometer and elemental analysis was done on GmbH Vario EL V3.00 elemental analyzer.

Plant Material: *Piper thomsoni* leaves and stems (650g) were collected from the Botanical Survey of India, Shillong forests and identified by Dr. B.M. Wadhwa.

Extraction and isolation: Leaves and stems were dried and their powdered mixture (500 g) was extracted with cold CH₂Cl₂:MeOH with a Soxhlet apparatus. Solvent was evaporated from the extract under vacuum and thus obtained residue (8.0 g) was purified by CC using silica-gel. Elutions were made exploiting a linear gradient of hexane-EtOAc-MeOH and totally 101 fractions (400 ml each) were eluted and as per their TLC pattern combined to make 14 fractions (F1-F14). F1 was composed of waxy material. Compound **2** (15 mg; colourless oil) and **4** (25 mg; white amorphous powder) were obtained from fractions F2 and F4, respectively. F8 gave

compound 8 as a white amorphous powder (15 mg). Additionally, six other known compounds viz., dotriacontanol (1), octadec-10Z-enoic acid (3), octacosanoic acid (5), stigmast-6-en-3 β -ol (6), (2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)penta-2,4-dien-1-one (7) and 7-methyl-5H-[1,3]dioxolo [4',5':4,5] benzo [1,2,3-de] benzo [g] quinoline-5,6(7H)-dione (9) were also isolated from other fractions.

(E)-4-(dodec-7-en-1-yl)phenol (2): Colourless oil; $[\alpha]_D -24.1$ (c 0.40, CHCl₃); UV(MeOH) λ_{max} : 274 nm; IR ν_{max} (film) cm⁻¹: 3550, 2919, 2850, 2359, 1638, 1462, 1377, 1265, 1028, 930, 708; ¹H NMR (CDCl₃): δ ppm 0.88 (t, 3H, J = 6.2 Hz, H-12'), 1.28 (brs, 6H, 3x-CH₂-), 1.63 (m, 6H, H-2', H-5' & H-10'), 1.98 (m, 4H, H-6' and H-9'), 2.53 (t, 2H, J = 7.6 Hz, H-1'), 5.1 (brs, 1H, -OH), 5.42 (m, 2H, H-7' and H-8'), 6.74 (d, 2H, J = 8.5 Hz, H-2 and H-6), 7.03 (d, 2H, J = 8.5 Hz, H-3 and H-5); ¹³C NMR (CDCl₃): δ ppm 14.6 (C-12'), 22.5-31.5 (C-2' to C-5', C-10' and C-11'), 34.6 (C-6' and C-9'), 34.9 (C-1'), 130.1 (C-2 and C-6), 131.3 and 131.4 (C-7' and C-8'), 132.9 (C-3 and C-5), 138.1 (C-4), 153.5 (C-1); EIMS (m/z): (M⁺, 260), 242 (M⁺-H₂O), 232, 203 (M⁺-C₄H₉), 177 (M⁺-C₆H₁₁), 167, 149, 135, 120, 106, 95, 85, 83, 74, 57, 43.

Cycloart-23-en-3-one (4): White amorphous powder; m.p. 118-120°C; $[\alpha]_D + 38.1$ (c 0.41, CHCl₃); Elemental analysis: C 84.83%, H 11.52%, O 3.65% (required C 84.87%, H 11.49%, O 3.65%); IR ν_{max} (KBr) cm⁻¹: 2927, 1712, 1451, 1377, 1113, 757; ¹H and ¹³C NMR (CDCl₃): detected via HMBC correlations, see

table 1; EIMS (m/z): 438(M⁺), 423 (M⁺-CH₃), 410 (M⁺-CO), 395 (M⁺-CH₃-CO), 381, 329, 313 (M⁺-C₉H₁₇), 298 (M⁺-C₉H₁₇-CH₃), 286, 270 (M⁺-C₉H₁₇-CH₃-CO), 243, 255, 231, 213, 138, 121, 107, 95, 81, 43.

3 β -22-Dihydroxylanosta-8,24-diene-26-oicacid- δ -lactone (8): White amorphous powder; m.p. 286-288°C; $[\alpha]_D + 27.3$ (c 0.32, CHCl₃); UV (MeOH) λ_{max} : 203, 252 nm; IR ν_{max} (KBr) cm⁻¹: 3531, 2930, 2862, 1709, 1457, 1373, 1261, 1141, 1091, 1036, 855, 803; Elemental analysis: C 79.22%, H 10.23%, O 10.55% (required C 79.25%, H 10.20%, O 10.56%); ¹H and ¹³C NMR (CDCl₃): detected via HMBC correlations, see table 1; EIMS (m/z): 454 [M]⁺, 439 (M⁺-CH₃), 421 (M⁺-CH₃-H₂O), 315, 311, 299, 281, 253, 241, 227, 215, 201, 139 (cleavage of C₁₇-C₂₀ bond), 127, 111 (139-CO), 95, 74.

Results and Discussion

The leaves and stems DCM:MeOH (1:1) extract of *P. thomsoni* was subjected to column chromatography on silica-gel and eluted with different polarity solvent combinations, yielded two new compounds, which include an alkenyl phenol (2) and acycloartaneterpenoid (4), and seven known compounds (1,3,5-9).

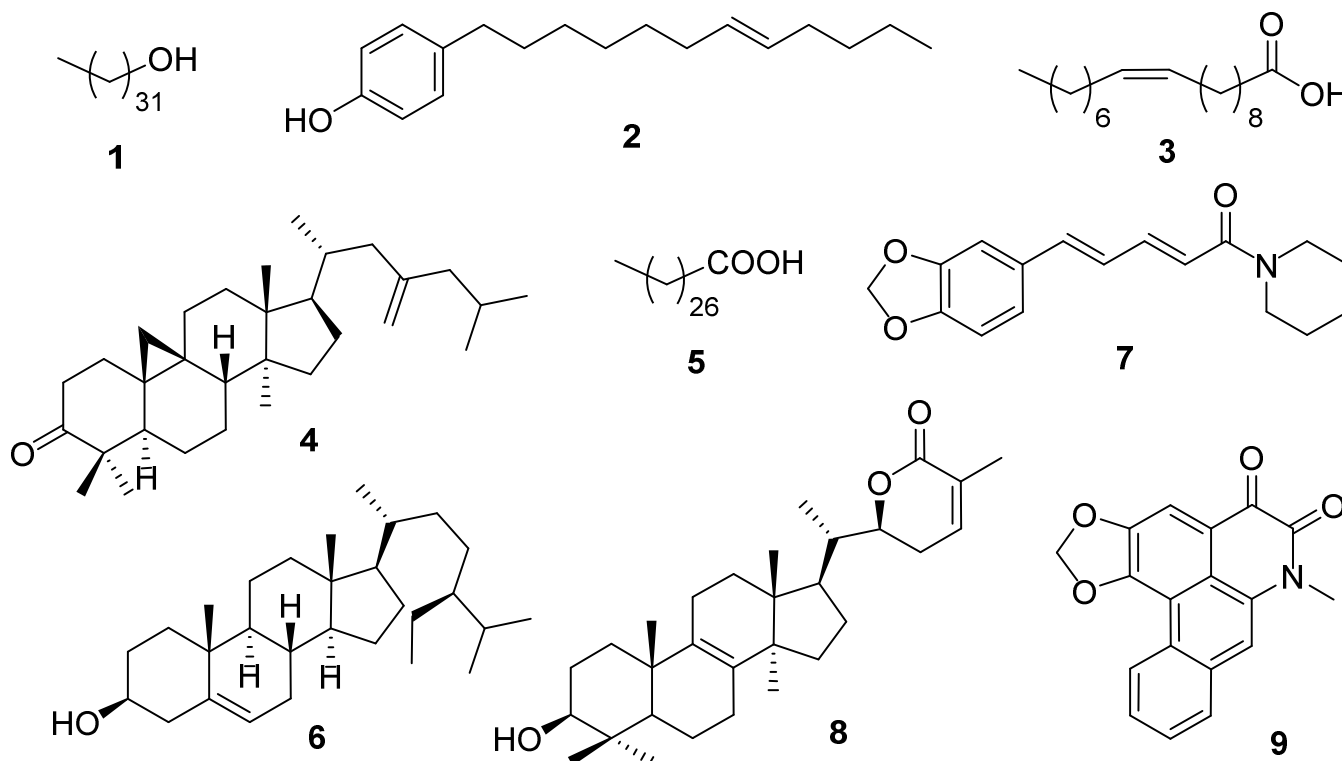


Figure-1
Structures of compounds 1-9

Table-1
¹H and ¹³C NMR and HMBC spectral data for compounds 4 and 8

C	4			8		
	C	HMBCC	¹ H	C	HMBCC	¹ H
1	33.2	2, 5, 19	1.52 (m), 1.81 (m)	34.4	2,3, 5, 19	1.71 (m), 1.26 (m)
2	37.5	1	2.68 (dt, J = 14.0, 6.2 Hz), 2.35 (ddd, J = 13.9, 4.3, 2.3 Hz)	30.7	1, 3	1.50 (m), 1.68 (m)
3	216.5	-	-	79.3	1, 2, 5, 29, 30	3.23 (dd, J = 11.3, 4.5 Hz)
4	50.6	-	-	37.8	-	-
5	49.1	1, 6, 7, 19	1.66 (m)	49.2	1, 6, 7, 19, 29, 30	1.64 (m)
6	22.3	5, 7, 8	1.01 (m)	18.0	5, 7	1.97 (m), 1.32 (m)
7	22.5	5, 6, 8	1.25 (m)	25.3	5, 6	2.15 (m), 1.35 (m)
8	43.1	6, 7, 11, 15, 19, 31	1.58 (m)	134.9	-	-
9	36.7	-	-	134.4	-	-
10	36.0	-	-	35.9	-	-
11	34.4	8, 12, 19	1.39 (m)	19.9	12	2.26 (m), 1.53 (m)
12	27.1	11, 17, 18	1.34 (m)	29.7	11, 18	1.47 (m)
13	45.7	-	-	43.4	-	-
14	46.9	-	-	48.8	-	-
15	33.8	16, 17, 31	1.61 (m)	29.8	16, 17	1.58 (m)
16	34.2	15, 17, 20	2.02 (m)	26.6	15, 17, 20	2.04 (m)
17	50.6	15, 16, 18, 20, 21, 22	1.34 (m)	44.7	12, 15, 16, 18, 20, 21, 22	1.66 (m)
18	18.4	12, 17	0.85 (s)	14.3	12, 17	0.70 (s)
19	29.9	1, 5, 11	0.78 (d, J = 4.2 Hz), 0.58 (d, J = 4.2 Hz)	17.1	1, 5	0.91 (s)
20	48.2	16, 17, 21, 22	1.46 (m)	39.3	16, 17, 21, 22, 23	1.88 (m)
21	18.6	17, 20, 22	0.93 (d, J = 6.7 Hz)	12.2	17, 20, 22	1.04 (d, J = 6.6 Hz)
22	31.6	17, 20, 21, 25	2.20 (m)	80.7	17, 20, 21, 24	4.47 (m)
23	157.2	-	-	28.6	20, 22, 24	2.02 (m), 2.56 (m)
24	106.3	22, 25	4.67 (d, J = 15.4 Hz)	139.9	22, 23, 27	6.59 (dd, J = 6.4, 3.2 Hz)
25	30.1	22, 24, 26, 27, 28	1.95 (m)	128.6	-	-
26	37.8	25, 27, 28	1.42 (m)	166.9	-	-
27	26.2	25, 26, 28	1.10 (d, J = 5.8 Hz)	16.0	24	1.91 (s)
28	26.2	25, 26, 27	1.10 (d, J = 5.8 Hz)	14.5	15	0.98 (s)
29	21.1	5, 30	0.98 (s)	23.3	3, 5, 30	0.81 (s)
30	22.2	5, 29	1.04 (s)	26.8	3, 5, 29	1.00 (s)
31	19.6	8, 15	0.95 (s)	-	-	-

Compound 2, obtained as oil, gave a molecular ion peak [M⁺] at m/z 260 in its EIMS and coupling this information with C and H count in its NMR suggested it to have molecular formula C₁₈H₂₈O. In its ¹³C NMR spectrum, the appearance of resonances for eight sp² hybridized carbons suggested one aromatic ring and one carbon-carbon double bond. Only twenty-seven hydrogens could be characterized through the ¹H and DEPT spectra, which indicated that left out one hydrogen could be in the form of a hydroxyl group. The initial assignments of two partial skeletons were done on the bases of ¹H NMR and ¹H-¹H COSY spectral data. Two aromatic resonances at δ 6.74 (d, 2H, J = 8.5 Hz) and δ 7.03 (d, 2H, J = 8.5 Hz) suggested that a distinctive 1,4-substitution pattern is present in the aromatic ring. The one substituent in the aromatic ring was in the form of

a phenolic hydroxyl group as was indicated by its ¹H NMR which displayed an exchangeable signal with D₂O at δ 5.1 and also supported by its ¹³C NMR showed a peak at δ 153.5, characteristic for a carbon bearing hydroxyl group in the benzene ring. ¹H NMR when coupled with its mass spectrum indicated that second substituent to be an alkenyl chain attached to the 4th position of the aromatic ring. The two proton multiplet at δ 5.42 suggested a disubstituted trans double bond in the side chain which was confirmed by its ¹³C NMR spectrum in which sp² carbons appeared at δ 131.3 and 131.4 and also by its IR spectrum as it exhibited a band at 930 cm⁻¹. Double bond position in the chain was assigned by mass fragmentation, in which two allylic cleavages were observed as a result of a peak at m/z 203 after the loss of C₄H₉ group and at m/z 177 after the

loss of C_6H_{11} . This suggested that double bond placed at C-7 in the alkenyl chain. The peak at m/z 167 confirmed twelve carbon atoms in the side chain. Hence compound **2** was characterized as (E)-4-(dodec-7-en-1-yl)phenol.

Compound **4**, obtained as a white amorphous solid, was assigned the chemical formula using elemental analysis as $C_{31}H_{50}O$, which was supported by its molecular ion peak m/z M^+438 in its EIMS. It passes *Liebermann Burchard* test and yellow colour with tetranitromethane, characteristic for a tetracyclic triterpenoid. Its 1H NMR showed two doublets at δ 0.58 ($J = 4.2$ Hz) and δ 0.78 ($J = 4.2$ Hz) and characterized for C-9& C-10 cyclopropyl methylene group of a cycloart-3-one triterpenoid¹³. The HMBC correlation studies confirmed that the protons at δ 0.58 and 0.78 were cyclopropyl methylene protons and attached to carbons at δ 36.7 (q) and 36.0 (q) and the carbon at δ 36.7 was also attached with the carbons at δ 33.2 ($-CH_2$) and 37.5 (CH_2).

Absorption band in its IR spectrum displayed at 1710 cm^{-1} suggested the presence of a carbonyl group, which was confirmed by its ^{13}C NMR spectrum which displayed a peak at δ 216.5. The HMBC correlation of $-C=O$ group with carbons at δ 33.2 ($-CH_2$), 37.5 ($-CH_2$), 50.6 (q) and 49.1 ($>CH-$) confirmed the presence of carbonyl group at C-3, which was also akin to the basic unit of cycloart-3-one^{13,14}.

The mass fragmentation displayed a prominent peak at m/z 313 ($M^+-C_9H_{17}$) due to the loss of 125 amu thereby showing one site of unsaturation in the side chain. Further its ^{13}C NMR and DEPT spectrum showed two sp^2 carbons at δ 157.2 (q) and 106.3 ($-CH_2$) and indicated the presence of an exocyclic methylene group which was confirmed by its 1H NMR spectrum as a characteristic doublet appeared at δ 4.67 ($J = 15.4$ Hz). This exocyclic double bond was fixed at C-23 because this was the only best suited position available in the side chain which was also supported by its *McLafferty-type fragmentation*¹⁵ (figure-2), in the absence of other olefinic proton and the vinylic methyl in the molecule.

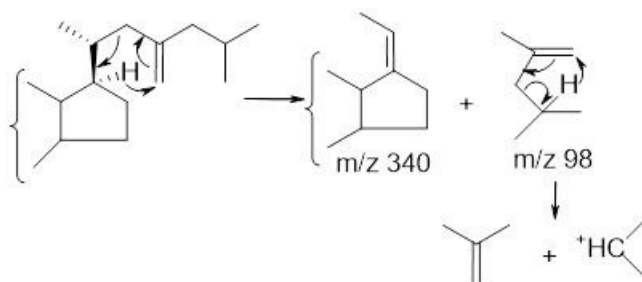


Figure-2
McLafferty-type rearrangement of the side chain of compound 4

The 1H NMR spectral pattern as well as the mass fragmentation of **4** was compared to those reported for cycloart-25-en-3-one

isolated from *Polypodium formosanum* fern¹⁶. The HMBC spectrum correlations (figure-2) were deduced to cycloart-23-en-3-one, the constitution assigned for compound **4**. HMBC spectra finally assisted in the assignment of the protonated carbons (table-1). On the basis of spectral discussion, compound **4** was defined as (2aR, 3R, 5aS, 5bS, 7aR, 11aR, 12aS)-2a, 5a, 8, 8-tetramethyl-3-((R)-6-methyl-4-methyleneheptan-2-yl)tetradecahydro-9H,12H-cyclopenta [a] cyclopropano [e] phenanthren-9-one (which shortly termed as cycloart-23-en-3-one).

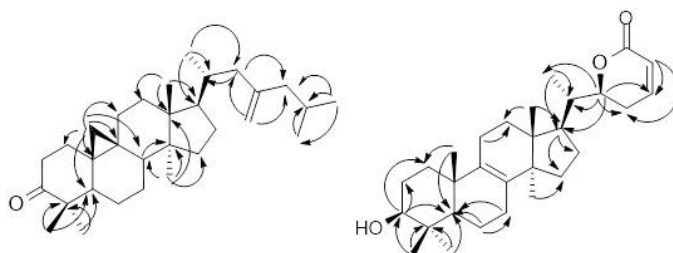


Figure-2
HMBC (H→C) correlation of compounds 4 and 8

Compound **8** was purified as a white amorphous solid and its chemical formula was confirmed as $C_{30}H_{46}O_3$ by its elemental analysis and EIMS. Its DEPT and 1H - ^{13}C COSY spectrum showed signals for seven methyl, nine methylene and five methine groups. The 1H NMR spectrum displayed among other resonances, the two low field protons attached to carbons bearing oxygen at δ 3.23 (dd, $J = 5.1$ Hz and 11.3 Hz) and at δ 4.47 (m). The presence of 3β -hydroxy was confirmed by a peak at δ 79.3 for C-3 in ^{13}C NMR and also by a characteristic double doublet at δ 3.23 for 3α -hydrogen. The IR absorption at 1707 cm^{-1} and UV absorption at 252 nm indicated the presence of a six membered α, β -unsaturated δ -lactone ring which was supported by its ^{13}C NMR spectrum which displayed characteristic resonances at δ 167.13, 140.29, 127.96 and 80.51. The HMBC correlation (figure 2 and table-1) with respect to $-CH_3$ group at δ 1.91 (s) showed correlation at δ 140.29 (q), 167.13 (q) and 127.96 ($=CH-$) carbons suggesting it attached to lactone ring at C-25 position. HMBC correlation suggested that $-CH-O-$ group at δ 4.47 (m) correlations with δ 167.13 ($>C=O$), 127.96 ($=CH-$), 39.3 ($>CH-$), 28.6 ($>CH_2$), 12.2 ($-CH_3$) carbons clearly suggested that it is C-22 carbon from lactone ring and confirmed the presence of six-membered α, β -unsaturated δ -lactone ring and is attached at δ 39.3 (C-20) carbon with lanostane unit. The ^{13}C NMR spectra also revealed tetrasubstituted olefinic carbons at δ 134.1 and 134.4 positioned between C-8 and C-9. By comparison of these spectral features with steroids containing α, β -unsaturated δ -lactone indicated that **8** has lanostane skeleton which was further confirmed by the HMBC spectrum (figure-2) and its Mass fragmentation pattern. Also δ -lactone carbon resonances of **8** was identified with those reported for steroids containing δ -lactones^{17,18}. Based on that we have assigned the stereochemistry as 22S. On the basis of above spectral analysis and also comparing with reported data¹⁹,

compound **8** was assigned as 3 β -22-dihydroxylanosta-8,24-dien-26-oicacid- δ -lactone.

Conclusion

We have performed a phytochemical exploration of *Piper thomsoni* and isolated two novel secondary metabolites of (E)-4-(dodec-7-en-1-yl) phenol (**2**) and cycloart-23-en-3-one (**4**). However, 3 β -22-dihydroxylanosta-8, 24-dien-26-oicacid- δ -lactone (**8**) was first time reported from the *Piper* genus.

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References

1. Kirtikar K.R. and Basu B.D., Indian Medicinal Plants, **III**, 2128 (1933)
2. The Wealth of India, Raw Materials, CSIR, New Delhi, **VIII**, 83 (1969)
3. Il-Kwon P., Sang-Gil L., Sang-Chul S., Ji-Doo P. and Young-Joon A., Larvicidal activity of isobutylamides identified in *Piper nigrum* fruits against three mosquito species, *Journal of Agricultural and Food Chemistry*, **50(7)**, 1866-1870 (2002)
4. Dominguez X.A. and Alcorn J.B., Screening of medicinal plants used by Huastec Mayans of Northeastern Mexico, *J Ethnopharmacol.*, **13**, 139-156 (1985)
5. Geran R.I., Greenberg N.H., Macdonald M. M., Schumacher A. M. and Abbott B.J., *Cancer Chemotherapy Reports*, **3**, 59-61 (1972)
6. Chahal J., Ohlyan R., Kandale A., Walia A. and Puri S., Introduction, Phytochemistry, traditional uses and biological activity of genus *Piper*: A review, *International Journal of Current Pharmaceutical Review and Research*, **2(2)**, 130-144 (2011)
7. Kumar S., Kamboj J., Suman and Sharma S., Overview for Various Aspects of the Health Benefits of *Piper Longum* Linn, Fruit, *Journal of Acupuncture and Meridian Studies*, **4(2)**, 134-140 (2011)
8. Young-Cheol Y., Sang-Guei L., Hee-Kwon L., Moo-Key K., Sang-Hyun L., and Hoi-Seon L., A piperidine amide extracted from *Piper longum* L. Fruit shows activity against *Aedes aegypti* mosquito larvae, *Journal of Agricultural and Food Chemistry*, **50(13)**, 3765 - 3767 (2002)
9. Chia-Ying L., Wei-Jern T., Damu A. G., E-Jian L., Tian-Shung W., Dung N. X., Thang T. D. and Thanh L., Isolation and Identification of Antiplatelet Aggregatory Principles from the Leaves of *Piper lolot*; *Journal of Agricultural and Food Chemistry*, **55(23)**, 9436-9442 (2007)
10. Steven T.S. L., Dragull K., Chung-Shih T., Bittenbender H. C., Efirid J. T. and Nerurkar P. V., Effects of Kava Alkaloid, Pipermethystine, and Kavalactones on Oxidative Stress and Cytochrome P450 in F-344 Rats, *Toxicological Sciences*, **97(1)**, 214-221 (2007)
11. Parmar V. S., Jain S. C., Bisht K. S., Jain R., Taneja P., Jha A., Tyagi O. D., Prasad A. K., Wengel J., Olsen C. E. and Boll P. M., Phytochemistry of the genus *Piper*, *Phytochemistry*, **46(4)**, 597-673 (1997)
12. Parmar V. S., Jain S. C., Gupta S., Talwar S., Rajwanshi V. K., Kumar R., Azim A., Malhotra S., Kumar N., Jain R., Sharma N. K., Tyagi O. D., Lawrie S. J., Errington W., Howarth O. W., Olsen C. E., Singh S. K. and Wengel J., Polyphenols and alkaloids from *Piper* species, *Phytochemistry*, **49(4)**, 1069-1078 (1998)
13. Furlan M., Roque N. F. and Filho W. W., Cycloartane derivatives from *Guarea trichilioides*, *Phytochemistry*, **32(6)**, 1519-1522 (1993)
14. Majumder P. L. and Pal S., A steroidal ester from *Coelogyne uniflora*, *Phytochemistry*, **29(8)**, 2717-2720 (1990)
15. McLafferty F. W., Mass Spectrometric Analysis. Molecular Rearrangements, *Anal. Chem.*, **31(1)**, 82-87 (1959)
16. Ageta H. and Arai Y., Fern constituents: Cycloartanetriterpenoids and allied compounds from *Polypodium formosanum* and *P. niponicum*, *Phytochemistry*, **23(12)**, 2875-2884 (1984)
17. Choi Y., Kinghorn A. D., Shi X., Zhang H. and Teo B. K., Abrusoside A: a new type of highly sweet triterpene glycoside, *J. Chem. Soc. Soc., Chem. Commun.*, **13**, 887 (1989)
18. Ino C., Hirotani M. and Furuya T., Two perenniporiol derivatives, lanostane-type triterpenoids, from the cultured mycelia of *Perenniporia ochroleuca*, *Phytochemistry*, **23(12)**, 2885-2888 (1984)
19. Dine E., Salah R., Halawany E., Mahmoud A., Norio N., Chao-Mei M. and Masao H., New lanostanetriterpene lactones from the Vietnamese mushroom *Ganoderma colossum* (FR.) C. F. BAKER., *Chem. Pharm. Bull.*, **56**, 642-646 (2008)