Studies on a carbazole alkaloid from *Murraya koenigii* Spreng. and flavonoids from *Pongamia glabra* Vent.

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Phytochemical investigations on the root bark of *Murraya koenigii* Spreng. (Fam. Rutaceae) afforded a new carbazole alkaloid (1a), characterized as 3,6-dimethyl-1-isopentenylcarbazole, on the basis of spectral data, and its N-methyl derivative (1b) was also synthesized. In addition, two known flavonoids, pongamol (2), and karanjin (3) were isolated from the seeds of *Pongamia glabra* Vent. (Fam. Leguminosae) and these two compounds were independently characterized by us with some new structural probability of pongamol (2) on the basis of spectral evidences. All these compounds were subjected to the comparative larvicidal studies on the third instar larvae of *Culex quinquefasciatus* at 100 ppm concentration at an interval of 24 h; some activity was observed which was related to their structural features.

*Murraya koenigii* Spreng. (Fam. Rutaceae), commonly known as Indian Curry leaf plant is non-toxic and highly valued for its characteristic aroma and medicinal importance in the indigenous system of treatment\(^1\). Root bark of the plant relieves renal pain\(^1\) while leaves exhibit antioxidant\(^2\) and hypoglycemic\(^3\) actions.

*M. koenigii* Spreng. is also a rich source of carbazole alkaloids, which are known to possess significant biological activities e.g. anti-biotic, anti-cancer, anti-viral, central nervous system, anti-inflammatory, pesticidal etc.\(^4\). Carbazole alkaloids e.g. mahanimbine, murrayanol, mahanine, obtained from bioassay guided fractionation of the acetone extract of fresh leaves of *M. koenigii* Spreng. show mosquitocidal, anti-microbial activities and also exhibited topoisomerase I and II inhibition activities\(^5\). Besides these, murrayanol shows anti-inflammatory activity while mahanimbine shows anti-oxidant property\(^6\). Murrayanine, girinimbine, mahanimbine have anti-fungal property and in addition, mahanimbine also shows larvicidal activity\(^6\).

9-Formyl-3-methylcarbazole, isolated from roots of *M. koenigii* Spreng., displays weak cytotoxicity against both mouse melanoma B16 and adriamycin resistant P388 mouse leukemia cell lines\(^7\). This prompted us to further investigate the root bark of this plant for new bioactive compounds and our investigations furnished a new carbazole alkaloid, 3,6-dimethyl-1-isopentenylcarbazole (1a). Structure of this compound was elucidated on the basis of spectral data (UV, IR, \(^1\)H and \(^13\)C NMR and mass).

During previous investigations on various synthetic carbazole derivatives it was noticed that N-substituted derivatives are generally more toxic than the carbazoles, and it was particularly noticed that N-methyl carbazole is highly toxic on the larvae of *Culex quinquefasciatus*, while carbazole itself has little toxicity\(^8\). Besides that, it was observed that the antinemic activities of pure carbazole alkaloids e.g. koenimbine, koenidin, obtained from this plant increases due to N-methylation\(^9\). So, we converted the new carbazole alkaloid, 3,6-dimethyl-1-isopentenylcarbazole (1a), to its N-methyl derivative (1b) by the action of dimethylsulphate and alkali in dry acetone medium with a view to make a comparative larvicidal study and its structure was confirmed on the basis of spectral data (UV, IR, \(^1\)H and \(^13\)C NMR and mass).

*Pongamia glabra* Vent. (Fam. Leguminosae), commonly known as Karanja, is also an important medicinal plant distributed throughout India, and is non-toxic\(^10\). Various parts of this plant are extensively used in the traditional medicines\(^10\). Besides that, various parts of this plant also show a wide range of biological activities, e.g. the ethanol extract of seed coat of this plant showed significant larval mortality of *Culex quinquefasciatus* and *Aedes aegypti*\(^11\).

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Direct application of ethanol and sequential petroleum ether, chloroform, acetone and ethanol extract of seed of this plant revealed anti-inflammatory, analgesic and anti-ulcerogenic activities in rats\(^2\). Antiviral properties of the seed extract was also investigated against herpes simplex virus in vitro studies in vero-cells\(^3\). This plant is the reservoir of large number of flavonoid type of compounds\(^4\) and it is well known that flavonoids represent one of the most diverse and wide-spread groups of natural products, having a varied range of biological activities\(^14\).\(^15\). These prompted us to investigate the seeds of Pongania glabra Vent. for new bioactive compounds but we isolated two known flavonoids, pongamol (2) and karanjin (3), having similarity in their structures. Though these two compounds were known\(^6\), in this communication, we have given a special method of isolation of these two compounds from the seeds of the plant and also independently characterized (2) and (3) on the basis of spectral data (UV, IR, \(^1\)H and \(^13\)C NMR and mass). In addition to that, we also want to explicate some special features of the structure of pongamol (2) in this communication.

Our previous investigations revealed that of the flavonoids type of compounds, like flavones and chalcones have larvicidal activity, and their activity depends on the nature and position of the substituent(s) present in these compounds, and it was also noticed that the chalcones were more active than flavones\(^17\). So we included these two compounds in comparative larvicidal study to note whether in this case the chalcone (2) is more active than the flavone (3) or not.

The advantages of the allelochemics over the synthetic pesticides are their low mammalian toxicity, easy biodegradability and minimal residual effect to the non-target animals. Moreover, the larvicidal activity of many allelochemics has not been properly studied. Considering all these facts, we were interested to make a comparative larvicidal study to note whether in this case the chalcone (2) is more active than the flavone (3) or not.

Comparison of the UV maxima with other carbazole compounds\(^18\) revealed the presence of a carbazole moiety in the compound. The \(^1\)H NMR spectrum showed signals for six protons of gem-dimethyl group at \(8\) 1.44 with a tertiary adjacent proton at \(8\) 1.63. The methyl region of the spectrum showed two aromatic methyl groups at \(8\) 2.13 (H\(_3\)C-3) and 2.34 (H\(_3\)C-6). The olefinic protons were in the form of a doublet at \(8\) 5.68 (H-10) and a triplet at \(8\) 7.18 (H-11). The signals at \(8\) 6.57 and 7.90 could be assigned to H-7 and H-8 respectively which are obtained as symmetrical doublet. The H-2 proton appeared at \(8\) 7.22 and N-H proton at \(8\) 7.81. The H-4 and H-5 protons appeared at \(8\) 7.34 and 7.65 respectively. Hence the \(^1\)H NMR spectrum of this compound showed the presence of a N-H group, two aromatic methyl groups and one CH=CH-CH(CH\(_3\))\(_2\) group. The positions of these groups in compound (1a) were further confirmed by its \(^13\)C NMR spectrum (vide Experimental). All these data confirmed the structure of the unknown carbazole alkaloid (1a) as 3,6-dimethyl-1-isopenteny1carbazole.

The presence of >N-H group in this compound was further confirmed by the synthesis of its N-methyl derivative (1b) (m.p. 142°C, yield 50.0%) by shaking compound (1a) with dimethyl sulphate and concentrated caustic potash in acetone medium, followed by recrystallisation of the crude product from ethanol.

From analysis and mass spectral measurements, the molecular formula of N-methyl derivative of the compound (1a) i.e. (1b) was obtained as C\(_{29}\)H\(_{23}\)N; M\(^+\) 277 (numbers of protons and carbon atoms were the same as it was observed in its \(^1\)H and \(^13\)C NMR). The IR spectrum of this compound showed absence of >N-H peak and the presence of >N-C-H group present in the compound is indicated by the peak at 2947 cm\(^{-1}\). Besides these, the peaks at 1635 (olefinic unsaturation, CH=CH), 1600 (aromatic residue) and 866 cm\(^{-1}\) (aromatic substitution) were obtained.

Comparison of the UV maxima with other carbazole compounds\(^18\) revealed the presence of a carbazole moiety in the compound. The \(^1\)H NMR spectrum of compound

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Results and discussion

Structure elucidations of carbazole alkaloid (1a) and its N-methyl derivative (1b):

The petroleum ether extract of dried and finely pulverized root bark of M. koenigii Spreng. (Fam. Rutaceae) afforded compound (1a), as a white crystalline solid, m.p. 168°C (yield 0.02%) (vide Experimental). Compound (1a), C\(_{19}\)H\(_{23}\)N (number of protons and carbon atoms were the same as obtained from its \(^1\)H and \(^13\)C NMR spectra), M\(^+\) 263 was found to be homogeneous by tlc and mass spectrometry. The IR spectrum (KBr) of compound (1a) showed a sharp peak at 3317 (>N-H) along with the peaks at 1641 (CH=CH), 1600 (aromatic residue), 1459 (CH\(_2\)), 1403 (C-CH\(_3\)) and 880 cm\(^{-1}\) (aromatic substitution).

Comparison of the UV maxima with other carbazole compounds\(^18\) revealed the presence of a carbazole moiety in the compound. The \(^1\)H NMR spectrum of this compound showed signals for six protons of gem-dimethyl group at \(8\) 1.44 with a tertiary adjacent proton at \(8\) 1.63. The methyl region of the spectrum showed two aromatic methyl groups at \(8\) 2.13 (H\(_3\)C-3) and 2.34 (H\(_3\)C-6). The olefinic protons were in the form of a doublet at \(8\) 5.68 (H-10) and a triplet at \(8\) 7.18 (H-11). The signals at \(8\) 6.57 and 7.90 could be assigned to H-7 and H-8 respectively which are obtained as symmetrical doublet. The H-2 proton appeared at \(8\) 7.22 and N-H proton at \(8\) 7.81. The H-4 and H-5 protons appeared at \(8\) 7.34 and 7.65 respectively. Hence the \(^1\)H NMR spectrum of this compound showed the presence of a N-H group, two aromatic methyl groups and one CH=CH-CH(CH\(_3\))\(_2\) group. The positions of these groups in compound (1a) were further confirmed by its \(^13\)C NMR spectrum (vide Experimental). All these data confirmed the structure of the unknown carbazole alkaloid (1a) as 3,6-dimethyl-1-isopenteny1carbazole.

The presence of >N-H group in this compound was further confirmed by the synthesis of its N-methyl derivative (1b) (m.p. 142°C, yield 50.0%) by shaking compound (1a) with dimethyl sulphate and concentrated caustic potash in acetone medium, followed by recrystallisation of the crude product from ethanol.
(1b) showed the presence of the N-methyl protons at δ 4.08. $^{13}$C NMR spectrum of this compound were also recorded which confirmed the N-methyl derivative of compound (1a) i.e. (1b) as N-methyl-3,6-dimethyl-1-isopentenylcarbazole.

From the comparison of the properties (m.p., analytical and spectral data) of compound (1a) with various literature data, it is expected that compound (1a) is a new carbazole alkaloid, 3,6-dimethyl-1-isopentenylcarbazole, isolated from the root-bark of *M. koenigii* Spreng. (Fam. Rutaceae). Hence the probable structures of compound (1a) and its N-methyl derivative (1b) are represented in the following figure as:

![Fig. 1(a). 3,6-Dimethyl-1-isopentenylcarbazole. (b) N-Methyl derivative of 3,6-dimethyl-1-isopentenylcarbazole.](image)

We have isolated two known flavonoids, pongamol (2) and karanjin (3) from the seeds of the plant *Pongamia glabra* Vent. (Fam. Leguminosae) by solvent extraction and chromatographic separation as given in the experimental part of this paper.

*Structure elucidations of compound (2) and some modifications of its structural features:*

The molecular formula of compound (2) was found to be C$_{18}$H$_{14}$O$_{4}$, from analysis and mass spectral measurements (numbers of protons and carbon atoms were the same as it was obtained in its $^1$H and $^{13}$C NMR). The IR (KBr) spectrum of this compound shows a band at 1650–1640 cm$^{-1}$ which is attributed to the α,β-unsaturated carbonyl group. The peaks at 1610 and 1560 cm$^{-1}$ are due to the aromatic residue and the IR peaks at 1170–1150 cm$^{-1}$ indicate the presence of ether linkage. The UV (EtOH) bands at 351.0–341.0 nm and 284.5–238.0 nm represent the Band-I and Band-II of chalcone type compounds. $^1$H and $^{13}$C NMR (vide Experimental) of this compound were recorded which confirmed the following structure of compound (2).

![Fig. 2. Pongamol.](image)

The comparative study of the spectral properties ($^1$H and $^{13}$C NMR) and melting point with literature data$^{20}$ shows that the compound (2), which we have isolated from the seeds of *Pongamia glabra* Vent. was identical with a known compound, pongamol, obtained from the roots of *Tephrosia purpurea* and this compound was also obtained previously from different parts of the same plant.$^{16}$

Though the enol form of pongamol was previously reported as prominent structure$^{21}$, our observations on the spectroscopic data of pongamol indicate that it is a tautomer between keto and enol forms, and the enol form becomes more prominent due to the intra-molecular hydrogen bonded chalcone type structure.

*Evidence for the enol form (hydrogen bonding in pongamol (2) (Fig. 2a)):*

In pongamol, a band at 2700 cm$^{-1}$ was obtained which is the characteristic of chelated hydroxyl group in enolic type of compounds$^{21}$. So we may expect following type of intra-molecular hydrogen bonding in pongamol.

![Fig. 2a. Hydrogen bonding in pongamol.](image)

*Evidence for the keto form (keto-enol tautomerism in pongamol (2)):*

The presence of keto form of pongamol is supported by the following facts:

(a) IR : β-Diketones$^{21}$, which partly exist in the enol form, show carbonyl absorption in region 1640–1540 cm$^{-1}$. This compound also gave IR peaks in the region 1650–1560 cm$^{-1}$.

(b) $^{13}$C NMR : Chalcon-β,β'-dione form can be readily differentiated from the enolic form due to the presence of an aliphatic methylene group (C-α) resonating at 55.3$^{22}$. There was a prominent $^{13}$C NMR signal at δ 54.3 for compound (2); which can not be explained on the basis of only enolic structure of pongamol.

From these facts, it may be stated that pongamol is a keto-enol tautomer (2b), of which the enol form is stabilised by the intra-molecular hydrogen bonding.

![Fig. 2b. Keto-enol tautomerism in pongamol.](image)
Structure elucidation of compound (3):

The molecular formula of compound (3) was found to be C_{18}H_{11}O_{4} from analysis and mass spectral measurements (numbers of protons and carbon atoms were the same as it was obtained in its {^1}H and {^{13}}C NMR).

The IR (KBr) spectrum of this compound reveals the presence of \( \alpha,\beta \)-unsaturated carbonyl band at 1640-1620 cm\(^{-1}\). The peaks at 1580-1550 cm\(^{-1}\) are due to the aromatic residue and peaks at 1160-1150 cm\(^{-1}\) confirm the presence of ether linkage. The UV (EtOH) bands at 303.5 nm and 259.5-280.5 nm represent the Band-I and Band-II of flavone type compounds respectively. {^1}H and {^{13}}C NMR of this compound were recorded which confirmed the following structure of compound (3).

![Fig. 3. Karanjin.](image)

The comparative study of the spectral properties (mainly the {^{13}}C NMR)\(^{23}\) and melting point\(^{16}\) with literature data shows that compound (3), which we have isolated from the seeds of *Pongamia glabra* Vent. was identical with a known compound, karanjin, obtained from different parts of the same plant\(^{16}\).

Bioassay of compounds (1a-b, 2 and 3):

On the basis of toxicity tests, it was noticed that N-methyl derivative of compound (1a) i.e. compound (1b) showed highest toxicity (with a % mortality value of 68.95 at 100 ppm concentration after 24 h at 30 ± 2°C). So variations of % mortality of this compound at different concentrations were measured and LC\(_{50}\) was determined by Probit Analysis and obtained as 83.01 ppm.

![Fig. 4. Probit analysis of 1b.](image)

Compounds (1a-b, 2 and 3) showed variation of % mortality at 100 ppm concentration after 24 h at 30 ± 2°C. So Analysis of Variance (ANOVA) calculations were done for these compounds and their toxicities are represented in the following Anova-table.

| Table. Variation of mortality of mosquito larvae at 100 ppm after 24 h at 30 ± 2°C |
|-----------------------------------------------|
| Treatment                  | Average % mortality |
| 3.6-Dimethyl-1-isopentenylcarbazole (1a)    | 47.59 (43.60)*     |
| *N-Methyl derivative of 3.6-dimethyl-1-isopentenylcarbazole (1b) | 68.95 (56.21) |
| Pongamol (2)                 | 27.50 (30.94)      |
| Karanjin (3)                 | 46.56 (43.01)      |
| F value                     | 14.50              |
| CD at 1%                     | 11.20              |
| CD at 5%                     | 8.13               |

*Figures in the parentheses represent the angular transformation.

From the ANOVA-table, CD (critical difference) values were calculated for these compounds; CD at 1% level was 11.20 and at 5% level was 8.13. From the CD values, the order of toxicity of these compounds can be determined. On the basis of CD, a suitable larvicide may be selected depending upon the availability and economy.

The carbazole alkaloid (1a) and the flavone type compound (3) have moderate toxicity which is almost the same. But the N-methyl derivative of (1a) i.e. compound (1b) is more toxic than the flavonoids (2 and 3) and it is also more toxic than the carbazole alkaloid (1a). This supports our earlier findings that due to N-substitution by methyl group, the toxicity of carbazole compound enhances. The isolated chalcone, pongamol (2), has lowest toxicity in these cases.

Our previous study\(^{17}\) showed that chalcones are generally more active than flavones. But in the present study, it was noticed that the isolated flavone type compound, karanjin (3) has moderate toxicity and it was more toxic than the isolated chalcone type compound, pongamol (2). This may be due to the complexity of their structures. From previous study\(^{17}\), it was evident that activity of flavone type compounds depends on the nature and positions of the substituents present in the compound and it was noticed that absence of substituents at 3, 7 and 8 positions and presence of substituent at 6 position enhanced the activity of the flavone type compounds. Karanjin (3) contains a furan ring fused at 7, 8 positions and it contains a methoxy group at 3 position. These may be the probable cause for its moderate activity. We also noticed earlier that, the toxicity of chalcone type compounds decreased due to the introduction of different groups in the aromatic ring, adjacent to carbonyl group of chalcone\(^{17}\). Moreover, when the carbonyl group of chalcone type compounds was blocked by derivatisation except in oxime derivative of benzalacetone, the toxicity of the resultant compound also decreased (unpublished results). In the case of pongamol (2), a furan ring is attached at 3′ and 4′ positions and it has also substit-
These plant materials were dried under shade in June, and seeds of *Spreng.* were identified and dried. They were collected locally (during the month of July, 1999). The dried seeds were crushed, and the meal was extracted with petroleum ether. After the complete removal of the solvent, the meal was reduced pressure for 24 h. Melting points of all the samples were taken in sulphuric acid bath in open capillaries and were uncorrected. The petroleum ether, b.p. (60–80°C) was used, unless otherwise stated. Reagents were used were Sigma, Aldrich, Fluka, E. Merck, B.D.H., S.D. or S.R.L. Column chromatography was carried out with silica gel (100–200 mesh) of Sisco product and homogeneity of all the samples was tested by thin layer chromatography (TLC) using silica gel G coated plate of size 7.5 × 2.5 cm² with different solvents as developer. FT-IR (KBr) and UV spectra were recorded on Finnigan MAT mass spectrometer. ¹H NMR spectra are in δ-scale. Electron impact mass spectra were recorded on Finnigan MAT 8230 mass spectrometer.

Root-barks of *Murraya koenigii* Spreng. (Fam. Rutaceae) and seeds of *Pongamia glabra* Vent. (Fam. Leguminosae) were collected locally (during the month of July, 1999 and June, 1998, respectively) and these plants were further identified by the Department of Botany of our University. These plant materials were dried under shade and pulverized in a mechanical grinder.

3,6-Dimethyl-1-isopentenylcarbazole (1a). 1 kg of dried and powdered root-bark of *Murraya koenigii* Spreng. was extracted with petroleum ether in a soxhlet for two weeks at room temperature. Solvent was removed from the petroleum ether extract by cautious distillation under reduced pressure. After the complete removal of the solvent, a non-homogeneous, concentrated mass was obtained. This was subjected to column chromatography over silica gel and the column was eluted with petroleum ether, petroleum ether and benzene mixture in different proportions, and benzene. 100 ml of eluent was collected in each fraction. The fractions [12–19], eluted by petroleum ether and benzene (3 : 2) mixture, furnished a white residue in pale yellow oil. This was washed with petroleum ether and benzene mixture [4 : 1] and repeatedly crystallized in petroleum ether and benzene mixture and finally from acetone, whereby compound (1a) was obtained as white, crystalline, homogeneous solid: (1a) (0.020%) m.p. 168°C (Found : C, 86.77; H, 8.08; N, 5.35. C₁₉H₂₃N requires : C, 86.65; H, 8.04; N, 5.32%); ₁H NMR δ (CDCl₃) 1.44 (6H, d, J 14.01 Hz, H-13/14), 1.63 (1H, m, H-12), 2.13 (3H, s, H-15), 2.34 (3H, s, H-16), 5.65 (1H, d, J 9.76 Hz, H-8), 7.18 (1H, t, J 6.87 Hz, H-11), 7.22 (1H, s, H-2), 6.57 (1H, d, J 9.76 Hz, H-7), 7.90 (1H, d, J 9.76 Hz, H-5), 7.81 (1H, s, H-5/N); ¹³C NMR δ (CDCl₃) δ 116.93 (C-1), 117.27 (C-2), 135.00 (C-3), 119.35 (C-4), 124.30 (C-4a), 104.52 (C-4b), 119.58 (C-5), 139.67 (C-6), 118.67 (C-7), 110.46 (C-8), 124.10 (C-8a), 129.40 (C-9a), 121.22 (C-10), 149.94 (C-11), 30.97 (C-12), 27.69 (C-13/14) and 16.13 (C-15/16); νₑₒₚ 263.

N-Methyl derivative of 3,6-dimethyl-1-isopentenylcarbazole (1b). No. 0.0004 mol of 3,6-dimethyl-1-isopentenylcarbazole was dissolved in 15 ml of dry acetone and this solution was shaken with 15 ml of dimethylsulphate and caustic potash (0.5 g/ml) for 1 h at room temperature. On standing for 48 h at room temperature, it furnished a crystalline mass. This was recrystallised from ethanol, whereby the product was obtained as homogeneous, crystalline, white solid: (1b) (50.0%) m.p. 142°C (Found : C, 86.63; H, 8.41; N, 5.07. C₂₀H₂₅N requires : C, 86.59; H, 8.35; N, 5.05%); ₁H NMR δ (CDCl₃) 1.45 (6H, d, J 14.01 Hz, H-13/14), 1.63 (1H, m, H-12), 2.13 (3H, s, H-15), 2.34 (3H, s, H-16), 5.65 (1H, d, J 9.76 Hz, H-8), 7.18 (1H, t, J 6.87 Hz, H-11), 7.22 (1H, s, H-2), 6.57 (1H, d, J 9.76 Hz, H-7), 7.90 (1H, d, J 9.76 Hz, H-5), 7.81 (1H, s, H-5/N); ¹³C NMR δ (CDCl₃) δ 116.93 (C-1), 117.27 (C-2), 135.00 (C-3), 119.35 (C-4), 124.30 (C-4a), 104.52 (C-4b), 119.58 (C-5), 139.67 (C-6), 118.67 (C-7), 110.46 (C-8), 124.10 (C-8a), 129.40 (C-9a), 121.22 (C-10), 149.94 (C-11), 30.97 (C-12), 27.69 (C-13/14) and 16.13 (C-15/16); νₑₒₚ 263.

Isolation of pongamol (2) and karanjin (3). 1 kg of...
dried and crushed seeds of *Pongamia glabra* Vent. (Fam. Leguminosae) were extracted in a soxhlet with alcohol for two weeks at room temperature. The solvent was removed from the extract by cautious distillation under reduced pressure, whereby a gummy mass was obtained. This was dissolved in 40% alcohol and fractionated by three solvents, viz. n-hexane, methylenechloride and ethyl acetate, one after another. The methylenechloride fraction was made free from the solvent by evaporation under reduced pressure, whereby a gummy mass was obtained. This gummy mass was dissolved in methanol and partitioned between petroleum ether (40–60°C) and methanol (90%).

The epi-phase i.e. the petroleum ether (40–60°C) layer furnished no appreciable result after complete removal of the solvent and repeated crystallizations in different solvent mediums. The hypo-phase i.e. the methanol layer, after complete removal of the solvent under reduced pressure, gave a gummy mass which was subjected to column chromatography over silica gel, 60–120 mesh, and the column was eluted by different solvents and their mixtures in different proportions, viz. petroleum ether, petroleum ether and benzene mixture, benzene, benzene. 100 ml of eluent was collected in each fraction.

*Pongamol* (2) : Fractions [9–17] were collected by elution with petroleum ether which furnished an orange solid in yellow oil. After washing with petroleum ether and on repeated crystallization from petroleum ether and benzene mixture, and finally from acetone, afforded orange, homogeneous, crystalline compound (2) (0.045%), m.p. 126°C (lit.23) (Found: C, 73.46; H, 4.79; O, 21.74%); \( \lambda_{\text{max}} \) (log \( e \)) 351.0 (4.34). 341.5 (4.38), 339.0 (4.35). 284.5 (3.78), 238.0 (4.42). 221.8 (4.28). 214.5 (4.29) nm; \( \nu_{\text{max}} \) 2700 (hydrogen bonded enol) 1650–1640 (\( \alpha,\beta \)-unsaturated carbonyl group), 1610–1560 (aromatic residue), 1170–1150 cm\(^{-1}\) (ether linkage); \(^1\)H NMR \( \delta \) (CDCl\(_3\)) 7.98 (2H, m, Ar-H, C\(_2\)-H, C\(_6\)-H), 7.50 (3H, m, C\(_1\)-H, C\(_2\)-H, C\(_3\)-H), 7.88 (1H, d, J 8.6 Hz, C\(_5\)-H), 7.30 (1H, d, J 8.6 Hz, C\(_5\)-H), 7.17 (1H, s, C\(_8\)-H), 7.61 (1H, d, J 2.1 Hz, C\(_8\)-H), 6.99 (1H, d, J 2.4 Hz, C\(_7\)-H), 4.13 (3H, s, OCH\(_3\)), 16.95 (br, s, OH); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 135.9 (C-1), 128.6 (C-2, C-6), 127.1 (C-3, C-5), 132.1 (2-C), 184.3 (C-7), 97.9 (C-8), 186.1 (C-9), 119.8 (C-1'), 155.8 (C-2'), 122.5 (C-3'), 153.6 (C-4'), 105.3 (C-5'), 126.5 (C-6'), 107.1 (C-7'), 144.9 (C-8'), 55.3 (CH\(_3\)); \( m/z \) 293.

*Karanjin* (3) : Fractions [27–35], collected by elution with petroleum ether and benzene (1:1) mixture gave a white solid in pale yellow oil, which on washing with petroleum ether and benzene (2:1) mixture furnished a white crystalline solid. This was repeatedly crystallized from petroleum ether and benzene mixture, and finally from acetone, whereby compound (3) was obtained as white, homogeneous, crystalline, solid. Compound (3) (0.048%) m.p. 157°C (lit.26) (Found : C, 74.00; H, 4.22; O, 21.78. C\(_{18}H_{12}O_4\) requires : C, 73.96; H, 4.14; O, 21.89%); \( \lambda_{\text{max}} \) (log \( e \)) 303.5 (4.20). 280.5 (4.02). 259.5 (4.37), 236.0 (4.15). 219.0 (4.10); \( \nu_{\text{max}} \) 1640–1620 (\( \alpha,\beta \)-unsaturated carbyl \( \epsilon \)-oup), 1580–1550 (aromatic residue), 1160–1150 cm\(^{-1}\) (ether linkage); \(^1\)H NMR \( \delta \) (CDCl\(_3\)) 8.15 (3H, m, Ar-H, C\(_1\)-H, C\(_2\)-H, C\(_3\)-H), 7.58 (2H, m, Ar-H, C\(_2\)-H, C\(_6\)-H, C\(_5\)-H), 8.20 (1H, d, J 8.0 Hz, C\(_3\)-H), 7.55 (1H, d, J 8.2 Hz, C\(_3\)-H), 7.76 (1H, d, J 2.0 Hz, C\(_1\)-H), 7.18 (1H, d, J 2.0 Hz, C\(_2\)-H), 3.93 (3H, s, OCH\(_3\)), \(^13\)C NMR (75 MHz, CDCl\(_3\)) \( \delta \) 153.2 (C-2), 141.9 (C-3), 174.9 (C-4), 121.7 (C-5), 109.8 (C-6), 154.7 (C-7), 118.9 (C-8), 149.9 (C-9), 119.8 (C-10), 131.1 (C-1'), 128.3 (C-2'), 128.6 (C-3'), 130.5 (C-4'), 128.6 (C-5'), 128.3 (C-6'), 145.7 (C-11), 104.1 (C-12); \( m/z \) 292.

**Method of bio-assay**:

Toxicity tests of the pure compounds (1a-b, 2 and 3) were performed on the 3rd instar larvae of *Culex quinquefasciatus* in water medium. 0.5% of the alcoholic solution of each compound was added in thin steam with gentle stirring into the beaker containing 25 third instar mosquito larvae in water, so that the final concentration of the compound became 100 ppm at 30 ± 2°C. In the similar fashion, same amount of alcohol was added to the control. Few drops of emulsifier were also added to maintain the uniformity of the concentration of the compound in the solution and similar addition was also done in control. Five replications were performed for each set. Percent mortality was calculated after 24 h. No mortality was observed in control. Analysis of Variance (ANOVA) calculations were performed for those compounds, which were apparently found not to be equivalent. The order of toxicity was determined by Critical Difference (CD) values.

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