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Original Article

PHYTOCHEMICAL ANALYSIS AND SCREENING THE BIOLOGICAL ACTIVITIES OF UNRIPE PODS OF *PONGAMIA GLABRA VENT*

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ABSTRACT

Objective: *Pongamia glabra vent* is widely used in folk medicine. The chemicals found in these have gained prominence in various studies. The phytochemical constituents of the benzene extract of the unripe pods of *Pongamia Glabra Vent* were investigated qualitatively and quantitatively using standard methods.

Methods: The compounds belonging to the group of flavonoids were isolated from benzene extracts of unripe pods of *Pongamia Glabra Vent*.

Results: A total six known natural compounds were separated and purified by chromatography techniques and five compounds were isolated and identified as flavonoid derivatives such as lanceolatin B, Karanjin, Pongapin, Kanjone, Pinnatin and the other one is a furanodibenzoyl methane namely Pongamol.

Conclusion: Physical studies such as UV, IR, ^{1}H and ^{13}C NMR, Mass and Element analysis have been used to confirm the structures of the isolated compounds. In the study, all compounds exhibited strong antibacterial activity against selected bacteria and fungi. The compound of C06 showed better antioxidant performance compared to standard ascorbic acid and butylated hydroxytoluene.

Keywords: Phytochemical analysis, Flavonoids, Antimicrobial and antioxidant activity

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INTRODUCTION

Pongamia glabra vent (Family: Leguminosae; Subfamily: *Papilionaceae* (Fabaceae) is a moderately deciduous, evergreen tree, commonly found in India and also present in South China and Australia. It is growing under a variety of agro-climatic conditions. The tree is nationally known as Beech in Indian and Ponga in Tamil, they are considered to be similar to *Pongamia pinnata* and *Derris indica* [1-3].

The plants of Pongamia glabra are majorly found in the chemical molecules of furanoflavone, Karanjin, Pongapin and Kanjone. This type of plant is widely used in folk medicine because of all the parts of the tree are active against various diseases [4-6]. The parts of this plant are used in various forms in traditional medicine [7]. The plant parts of *Pongamia pinnata* are used for anti-inflammatory [8], anti-virus [9], antinociceptive [10], antioxidant [11], anti-diabetic [12], antidiarrheal [13], anti-ulcer [14], antimicrobial [15], antifungal and anti-bacterial [16].

In the present study, the phytochemical components of the benzene extract of the unripe pods of *Pongamia glabra vent* were studied using standard methods. The study found that they are present in the condensed furanoderivatives. The analysis revealed that the benzene extract of the unripe pods of *Pongamia glabra vent* is rich in simple flavones derivative and furanodibenzoylmethanes etc. Spectroscopic techniques have been used to confirm the compound structures. Antimicrobial and antioxidant properties were tested on those studied compounds.

MATERIALS AND METHODS

All chemicals were obtained in analytical quality and unpurified. The melting points were determined and improper. The original nature of the compounds was confirmed by the thin layer chromatography. The UV and IR spectra were recorded with the help of a UV1800-*Shimadzu* and *Shimadzu IR Affinity-1* spectrometers respectively. The *Bruker 400 MHz NMR* Spectrometer was used to analysis the proton and carbon environments, using the TMS as the internal standard and the $CDCl_3$ for solvent. Antimicrobial activity was tested by Kirby-Bauer disk diffusion method. Antioxidant activity was measured by the DPPH radical method.

Preparation of crude extract

The unripe pods of *Pongamia glabra vent* (fig. 1) used for this study were collected between the months of January–March 2020. The dried unripe pods (1 kg) of *Pongamia glabra* were extracted in hot benzene with the soxhlet extractor method. Solvents were filtered from the extract and the residue was collected.

Separation of the crude mixture

Column chromatography is being used to extract compounds from benzene extract. To extract this fraction, a solid phase having 60-120 mesh silica gel is employed and a fluid of petroleum ether (250 ml) coupled with silica gel (150 g) was used. The eluted started with Petroleum ether (100%) and a further mixture of solvents were used and the compounds were separated. 50 ml of the fractions were collected each time, distilled off the solvent and the homogeneity of the fractions was examined TLC silica gel plates. Table 1 shows the fractions, solvents and residues obtained.

Identification of compounds (1-6)

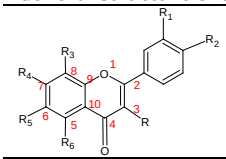
Compound 1 was obtained as a brownish yellow solid from the column fraction eluted with 90-10% petroleum ether in benzene in Conc. H_2SO_4. The Compound 1 produces blood red colour with alc. $FeSO_4$. It also react with Con. Sulphuric acid [17] to give a yellow solution. The Compound 2 crystallized from ethyl acetate-petroleum ether mixture as colourless needles, the column fraction eluted with 75-25% petroleum ether in benzene. It presented an orange yellow colour with the Shinoda test [18]. The Compound 3 was colourless and crystallized from alcohol as rectangular plates and eluted with 30-70 % petroleum ether in benzene. It produces a yellow solution when it interacts with concentrated sulphuric acid. When heated to 100 °C the yellow solution turns red and finally green [19]. It gave orange yellow colour with Shinoda test. Compound 4 crystallized from methanol as pale yellow needles and eluted with 100 % benzene. It gave a yellow colour with Shinoda test. Compound 5 crystallised from methanol as colourless substance and eluted with 90-10 % benzene in ethyl acetate. It gave a brown colour with Shinoda test. Compound 6 was pale yellow in colour and crystalline needles

from chloroform-petroleum ether and eluted with 60-70% Benzene in ethyl acetate and gave a yellow colouration to Shinoda test. All the compounds were purified by PTLC. The Elemental analysis and spectroscopic methods such as UV, IR, NMR, and Mass spectroscopy were used to confirm the structures (table 2) and the spectrum data was compared to existing literature values.

Table 1: The fractions, the eluting solvents and the quantities of residue obtained

Fractions	Eluting solvent	Residue weight and compounds
1-16	Petroleum ether (60-80 °c)	0.2 g: low melting waxy residue
17-35	Petroleum ether-benzene(90:10)	0.4 g: brownish yellow solid (Compound 1)
36-70	Petroleum ether-benzene(75:25)	1.1 g: colourless solid (Compound 2)
71-90	Petroleum ether-benzene(50:50)	Negligible residue
91-120	Petroleum ether-benzene(30:70)	0.4 g: colourless solid (compound 3)
121-150	Pure benzene	0.3 g: pale yellow solid (compound 4)
151-180	Benzene-ethyl acetate(90:10)	0.2 g: colourless solid (compound 5)
181-210	Benzene-ethyl acetate(60:40)	0.25g: pale yellow solid(compound 6)

Table 2: General structure of compounds 2-6

General structure of compds2-6	Componds number	Residue (R, R_1-R_6)	
	2	R=H; R_1=R_2=R_5=R_6=H	R_3+R_4=-O-CH=CH
	3	R=OCH_3; R_1=R_2=R_5=R_6=H	R_3+R_4=-O-CH=CH
	4	R=OCH_3; R_1=R_2=-O-CH_2-O-; R_5=R_6=H	R_3+R_4=-O-CH=CH
	5	R=H; R_1=R_2=R_6=H; R_5=OCH_3	R_3+R_4=-O-CH=CH
	6	R-H; R_1=R_2=H; R_4+R_5=-CH_2-CH-O-; R_6=OCH_3	R_3=H

Antimircobial activity

The Kirby-Bauer disc diffusion method [20] of *in vitro* antibacterial activity was used to evaluate all the isolated compounds. Bacteria such as B. subtilis, S. aureus, S. typhi and E. coli being used to test the compounds anti-bacterial activity. *Candida albicans* was employed to evaluate antifungal activity. *Ciprofloxacin* and *fluconazole* were acting as standards for this activity. The inhibition zone of isolated compounds was compared with standard drugs. Table 3 shows the antimicrobial activity values of the compounds.

Antioxidant activity

All isolated compounds for antioxidant activity were screened using the DPPH evaluation method [21]. Table 4 shows the antioxidant results for all the compounds. DPPH method is a reduction principle of the purple DPPH (free radical) reduced and changes to yellow coloured diphenylpicrylhydrazine. The remaining purple coloured DPPH exhibited maximum absorption of 517 nm. The 2 ml of different concentrations of isolated chemicals or standards were mixed with 2 ml of DPPH solution (0.1 mmol) and stored in the dark. The solution absorbance was measured at 517 nm after attaining a temperature of 37 °C for 20 min. AA and BHA were used as positive controls. The following formula was used to calculate the percentage of inhibition. Inhibition (%) = (blank OD–sample OD/blank OD)×100.

RESULTS AND DISCUSSION

Spectra of compound 1: 3-Hydroxy-1-(4-methoxy-1-benzofuran-5-yl)-3-phenyl-2-propen-1-one; $C_{18}H_{14}O_4$; Yellowish white solid crystals, m. p 136 °C; UV (EtOH, $\lambda_{max,}$ nm): 240, 351; IR (KBr, ν_{max}, cm^{-1}): 3051 (Aromatic C-H str), 2953, 2852 (Aliphatic C-H str), 1736 (C=O str) 1639 (Aromatic C-C str) and 1456 (Aliphatic C-H str); ^{1}H NMR (400 MHz, CDCl3, δ (ppm)): 16.94 (1H,s, OH), 7.98–8.02 (2H, m), 7.90 (1H, d, *J* = 8.77 Hz), 7.64 (d, 1H, *j*=2.3Hz), 7.54-7.48 (m,3H), 7.32 (d, 1H, *J*=8.7Hz), 7.18 (s, 1H), 7.01 (d, 1H, J=2.3Hz) and 4.17 (3H, s);[13]C NMR (100 MHz, CDCl3, δ (ppm)): 186.12 (C_1=O), 184.31 (C_3=O), 158.71 (C-7a), 153.77 (C-4), 144.84 (C-2), 135.61 (C-1′), 132.18 (C-4′), 128.67 (C-3′ and C-5′), 126.98 (C-2′ and C-6′), 126.49 (C-8),122.19 (C-5), 119.62 (C-3a), 107.10 (C-7), 105.11 (C-3), 97.92 (C-2), 61.19 (OMe); MS(EI): m/z 294 [M+]; Elemental analysis-calcd: C, 73.46; H, 4.76 (%); found: C, 73.46; H, 4.79 (%) and was confirmed by comparing its spectral data in published literature [17,22].

Spectra of compound 2: 2-Phenyl-4*H*-furo[2,3-*h*]chromen-4-one; $C_{17}H_{12}O_3$; Colourless needles, m. p.141-142 °C; UV (EtOH, $\lambda_{max,}$ nm): 250, 330; IR (KBr, ν_{max}, cm^{-1}): 3053 (Aromatic C-H str), 2951 (Aliphatic C-H str), 1644 (C=O str) 1580 (Aromatic C-C str) and 1405 (Aliphatic C-H str); ^{1}H NMR (400 MHz, CDCl3, δ (ppm)): 6.88 (s, 1H, C-3), 7.21 (d, *J*=4Hz, 1H, C3′′-H), 7.55-7.60 (m, 4H, C3′ -H, C4′-H, C5′-H, and C6-H), 7.79 (d, 1H, J=4Hz, C2′′-H), 7.96 (m, 2H, C2′ -H and C6′-H) and 8.20 (d, *J* = 8.1 Hz, 1H, CH-5);[13]C NMR (100 MHz, CDCl3, δ (ppm)): 95.28 (C-2), 59.61 (C-3), 178.21 (C-4), 121.81 (C-5), 110.21 (C-6), 158.41 (C-7), 117.21 (C-8), 150.91 (C-9), 119.10 (C-10), 126.21 (C-2' and C-6'), 129.11 (C-3' and C-5'), 131.52 (C-4'), 145.81 (C-2'') and 105.01 (C-3''); MS (EI): m/z 264 [M+]; Elemental analysis-calcd: C, 77.27; H, 4.54 (%); found: C, 77.24; H, 4.58 (%). Which was found to be similar with the reported values for lanceolatin B [23-25].

Spectra of compound 3: 3-Methoxy-2-phenylfuro-(2,3-*h*-chrome-4-ol); $C_{18}H_{14}O_4$, Colorless crystalline solid. m. p.162-163 °C; UV (EtOH, $\lambda_{max,}$ nm): 270 and 305; IR (KBr, ν_{max}, cm^{-1}): 3052 (Aromatic C-H str), 2929 (Aliphatic C-H str), 1624 (C=O),1526 (Aromatic C-C str), 1460 (Aliphatic C-H str);^{1}H NMR (400 MHz, CDCl3, δ (ppm)): 3.89 (s, 3H, OCH_3), 7.78 (d, J=2.0Hz, 1H, C-2''), 7.21 (d, J=2.0Hz, 1H, C-3''), 8.22 (d, J=8.4Hz, 1H, C-5), 7.57 (d, J=8.4Hz, 1H, C-6), 7.45-7.55 (m, 3H,C-3',C-4' and C-5'), 7.81–8.05 (m,2H,C-2' and C-6'); [13]C NMR (100 MHz, $CDCl_3$, δ (ppm)): 145.69 (C-2''), 104.23 (C-3''), 61.23 (OCH_3), 154.87 (C-2'), 142.03 (C-3), 178.13 (C-4), 122.13 (C-5), 110.02 (C-6), 158.17 (C-7), 117.03 (C-8), 150.03 (C-9), 131.03 (C-10), 119.71 (C-1'), 128.42 (C-2' and C-6'), 128.73 (C-3' and C-5') and 131.04 (C-4'); MS (EI): m/z 294 [M+]; Elemental analysis-calcd: C, 73.48; H, 4.75 (%); found: C, 73.42; H, 4.79 (%). On this basis, the compound 3 is a 3-methoxy-2-phenylflavone whose spectral data correspond to those indicated for the Karanjin [26-28].

Spectra of compound 4: 2-(1,3-Benzodioxol-5-yl)-3-methoxyfuro [2,3-*H*]chromen-4-one**;** $C_{19}H_{12}O_6$; Pale yellow needles, m. p 191-192 °C. UV (EtOH, $\lambda_{max,}$ nm): 250, 330; IR (KBr, ν_{max}, cm^{-1}): 3049 (Aromatic C-H str), 2982 (Aliphatic C-H str), 1640 (C=O),1570 (Aromatic C-C Str), 1485 (Aliphatic C-C); ^{1}H NMR (400 MHz, CDCl3, δ (ppm)): 3.95 (s, 3H,OCH_3), 6.09 (s, 2H,OCH_2O, C-4'), 7.05 (d,1H, *J*=8.4 Hz,C-6), 7.25 (d, 1H, *J*=2.0Hz, C-3''), 7.60 (d, 1H, J=8.4Hz, C-5), 7.70 (d, 1H, *J*=2.0Hz C-2''), 7.75-7.85 (m, 2H, C-2', C-7') 8.23 (d, J= 8.2Hz, 1H,C-6'); [13]C NMR (100 MHz, $CDCl_3$, δ (ppm)): 146.92 (C-2''), 104.24 (C-3''), 59.47 (OCH_3), 153.74 (C-2), 142.53 (C-3), 174.09 (C-4), 121.07 (C-5), 109.94 (C-6), 157.57 (C-7), 116.73 (C-8), 149.18 (C-9), 119.13 (C-10), 124.12 (C-1'), 107.96 (C-2') 123.24(C-7'), 147.64 (C-9') 149.38 (C-8'), 108.23 (C-6'), and 101.69 (C-4'); MS (EI): m/z 336 [M+]; Elemental analysis-calcd: C, 67.86; H, 3.57 (%); found: C,

67.86; H, 3.59 (%). Thus, its identity was further confirmed by comparing the data of compound 4 with the published values [26].

Spectra of compound 5: 6-Methoxy-2-phenylfuro[2,3-*h*]chromen-4-one; $C_{18}H_{12}O_4$; Colourless cubes, m. p. 190–191 °C; UV (EtOH, λ_{max},nm): 270 and 305; IR (KBr, ν_{max}, cm^{-1}): 3052 (Aromatic C-H str), 2974 (Aliphatic C-H str), 1632 (C=O),1573 (Aromatic C-C Str), 1483 (Aliphatic C-C); ^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm)): 4.10 (s, 3H, C-6, OCH_3) 6.81 (s, 1H, C-3), 7.25 (d, 1H, *J*=2Hz, C-3''), 7.45-7.55 (m, 3H, C-3', C-4' and C-5'), 7.81 (d, 1H, *J*=2Hz, C-2''), 7.91-8.10 (m, 2H, C-2' and C-6'), 8.31 (s, 1H, C-5); NMR (100 MHz, CDCl3, δ (ppm)): [13]C NMR: δ 63.05 (OMe), 104.79 (C-3''), 105.23 (C-3), 105.71 (C-5), 114.73 (C-8), 119.32 (C-10), 125.75 (C-2' and C-6'),, 127.83 (C-4'), 128.41 (C-3' and C-5'), 130.32 (C-1'), 144.75 (C-6), 145.09 (C-7), 145.67 (C-2''), 150.91 (C-9), 163.53 (C-2), 177.51 (C-4); MS (EI): m/z 292 [M+]; Elemental analysis-calcd: C, 73.98; H, 4.13 (%); found: C, 73.97; H, 4.12 (%). On the basis of above spectral data, the structure of compound 5 was established as 6-Methoxy-2-phenylfuro[2,3-h]chromen-4-one, also known as Kanjone, which was found to be similar with the reported values [23, 25].

Spectra of compound 6: 4-Methoxy-7-phenyl-5*H*-furo[3,2-g]chromen-5-one; $C_{18}H_{12}O_4$; pale yellow needles, m. p. 176–177 °C; UV (EtOH, $\lambda_{max,}$ nm): 271 and 305; IR (KBr, ν_{max}, cm^{-1}): 3101 (Aromatic C-H str), 2983 (Aliphatic C-H str), 1632 (C=O),1586 (Aromatic C-C Str), 1468 (Aliphatic C-C); ^{1}H NMR (400 MHz, CDCl3, δ (ppm)): 7.98-7.88 (m, 2H, C-2' and C-6'), 7.63 (d, 1H, *J*=2.2Hz, C-2''), 7.56-7.48 (m, 3H, C-3', C-4' and C-5'), 7.39 (s, 1H, C-8), 7.05 (d, 1H, *J*=2.1Hz, C-3''), 6.72 (s, lH, C-3), 4.18 (3H, s, C-5, OCH_3); [13]C-NMR (100 MHz, CDCl3, δ (ppm)): δ 177.69 (C-4), 163.86 (C-2), 157.86 (C-7), 155.07 (C-9), 152.96 (C-5), 146.74 (C-2''), 132.86 (C-1'), 131.84 (C-4'), 128.54 (C-3' and C-5'), 126.87 (C-2' and C-6'), 114.75 (C-8), 116.05 (C-6), 112.95 (C-10), 105.68 (C-3), 105.65 (C-3''), 62.96 (OMe); MS (EI): m/z 292 [M+]; Elemental analysis-calcd: C, 73.97; H, 4.13 (%); found: C, 73.97; H, 4.11 (%). This structural features were compared with the reported values for Pinnatin and found to be similar with it [29].

General Spectral characterization of isolated compounds

Based on the identification, chemical reactions and spectral data of the isolated compounds they were known as Pongamol (compound 1), lanceolatin B (compound 2), Karanjin (compound 3), Pongapin (compound 4), Kanjone (compound 5) and Pinnatin (compound 6). Compound 1 is furanodibenzoyl methane i.e. pongamol and it is a substituted benzofuran derivative and other compounds of 2-6 are commonly referred to as benzofuran flavonoid derivatives. All the structure of the compounds is given in fig. 1

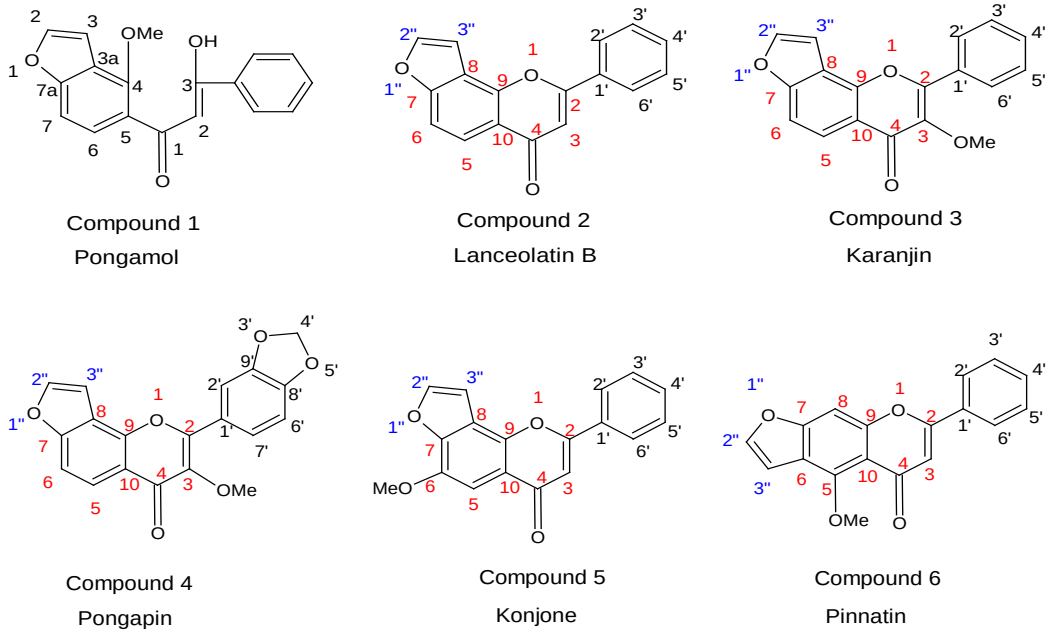


Fig. 1: Structure of compounds

The melting points of the isolated compounds correspond to the literary value. The structure of 2-6 compounds based on IR, 1H and ^{13}CNMR spectral results are all closely related to each other. This is because of the above mentioned compounds contains benzofuron carbon skeleton. Based on UV, IR, and ^{13}C-NMR spectral techniques, the values of the C=O group of the isolated compounds (1-6) conform to reported values. Methoxy group is found in compound 1 and compounds 3-6 and the values of the methoxy group are determined based on the IR, ^{1}H and ^{13}C NMR spectra and these values correspond to the reported values. The functional groups of aliphatic and aromatic C-H valves of all the compounds explained in based of IR, 1H and ^{13}C NMR spectral methods and the observed valves are matched with reported values. The molecular mass (m/z) of the isolated compounds 1-6 was confirmed based on mass spectrometric methods. The elemental composition values of the above compounds confirmed using the elemental analysis method and the founded valves are matched the calculated valves. The detailed spectral data of the compounds are given in entitled 3.1. Spectral Properties of Compounds (1-6).

Biological activities of isolated compounds

Antimicrobial activity

For *in vitro* antimicrobial activity, all compounds were examined using the Kirby-Bauer disc diffusion technique. The inhibition zone was measured and compared to the standards. Table 3 shows the antibacterial and antifungal activity results. The compounds showed significant activity against selected microorganism.

Antioxidant activity

The results of antioxidant activity of the above compounds at different concentrations and calculated IC_{50} values are shown in table 4. The most powerful compound in the test compound was C06, which had an IC50 value of 22.83g/ml, whereas the standard compounds AA and BHA had values of 6.2g/ml and 7.85g/ml respectively.

Table 3: Antimicrobial activity

Sample code	Zone of inhibition (mm)																			
	Antibacterial activity																Antifugal activity			
	Bacillus subtilis				*Candida albicans*				*Salmonella typhi*				*Escherichia coli*				*Candida albicans*			
	100 mcg	50 mcg	25 mcg	Std	100 mcg	50 mcg	25 mcg	Std	100 mcg	50 mcg	25 mcg	Std	100 mcg	50 mcg	25 mcg	Std	100 mcg	50 mcg	25 mcg	Std
C01	10	6	4	16	9	4	2	13	9	4	2	15	12	9	6	17	8	5	4	24
C02	12	7	2	21	8	6	4	15	8	5	2	17	13	7	4	21	11	9	5	19
C03	18	6	4	22	9	6	5	16	7	5	3	16	12	8	6	18	8	7	3	17
C04	9	7	6	16	12	9	4	18	9	8	4	21	11	6	5	21	13	8	6	15
C05	10	6	4	19	9	7	4	13	6	4	2	14	12	8	6	19	15	8	4	18
C06	10	7	4	17	11	9	5	13	6	4	2	19	17	8	6	19	12	9	7	24

Table 4: Antioxidant activity

Sample code	Concentration (µg/ml)					IC_{50} (µg/ml)*
	20	40	60	80	100	
C01	75.49	71.83	69.54	64.73	59.23	31.71
C02	86.1	81.69	76.22	69.49	62.64	25.38
C03	79.52	77.48	73.17	68.8	63.35	49.56
C04	78.35	70.82	65.27	53.13	45.76	29.44
C05	80.17	77.6	73.6	68.92	64.19	76.27
C06	79.48	72.22	66.94	55.64	48.6	22.83
BHT	94.13	81.91	71.76	64.81	57.88	6.2
AA	98.11	87.82	76.94	64.61	57.82	7.85

*Average of three independent determinations

CONCLUSION

From the unripe pods of *Pongamia glabra vent*, compounds (1-6) are isolated. These are furanoflavonoid derivative compounds. The structures of the compounds have been confirmed using elemental and spectroscopic techniquie. Furthermore, these compounds have significant antimicrobial activity against selective bacteria and fungi. Finally, the C06 compound has good antioxidant activity.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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