PART-III

Chemical constituents of

Justicia gendarussa
Section 1: A brief review of phytochemicals reported from different Justicia species

The genus Justicia belongs to family, Acanthaceae, which comprises about 2500 species. Most of the species of Acanthaceae are found in tropical regions of the world [1]. Justicia is the largest genus of this family, with about 600 species that are found in pantropical and tropical regions [2]. In India about 20 species are found [3]. The species of Justicia can be easily recognized by their inflorescences in spikes or panicles cimas, bilobial corolla, with a posterior lip that is generally two-lobed, an anterior lip that is three-lobed, two stamens, a capsule with four seeds, and a basal sterile portion [4, 5]. About 36 species of Justicia have been studied. The most studied species of Justicia are Justicia pectoralis, Justicia gendarussa Burm.f; Justicia anselliana (Nees) T. Anderson and Justicia procumbens L. The phytochemistry and pharmacology of other Justicia species are needed to be explored.

Several species of Justicia are widely used in folk medicine for the treatment of respiratory and gastrointestinal diseases, rheumatism and arthritis, epilepsy and other mental disorders, cancer, diabetes and HIV as well as used as sedative, depressors, analgesic and somniferous agents [6].

Different classes of chemical compounds are found in several species of Justicia. Lignans represent the major class of compounds followed by alkaloids, triterpenoids glycosides and flavonoids. Several biological activities of the isolated lignans have been reported. Most of the lignans contain an arylnaphthalide skeleton. Different classes of phytochemicals isolated from several species of Justicia are listed in Table 3.1 to highlight their structural and substitution diversities.
### Table 3.1. List of phytochemicals from different species of *Justicia*

<table>
<thead>
<tr>
<th>Str. No.</th>
<th>Name and structure of phytochemical</th>
<th>Plant source</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[A] 1</td>
<td>Helioxanthin</td>
<td><em>J. flava</em></td>
<td>[7,8]</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>J. simplex</em></td>
<td>[44]</td>
</tr>
<tr>
<td>2</td>
<td>Justicidin E</td>
<td><em>J. procumbens</em></td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>J. orbiculata</em></td>
<td>[11]</td>
</tr>
<tr>
<td>3</td>
<td>Elenoside</td>
<td><em>J. hypssopifolia</em></td>
<td>[12, 13]</td>
</tr>
<tr>
<td>4</td>
<td>Neojusticin B (=Justicidin C)</td>
<td><em>J. ciliate</em></td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>J. procumbens</em></td>
<td>[15,16,17]</td>
</tr>
</tbody>
</table>
5  Justicidinoside A  \( J. \) procumbens  [15]

6  Justicidin D  \( J. \) procumbens  [15, 17]

7  Justicidin B  \( J. \) purpurea  \( J. \) procumbens  \( J. \) hayatai var. decumbens  [18, 19, 20, 21]

8  Chinensinaphthol methyl ether  \( J. \) ciliata  [14]
9. Justicidinoside C        \( J. \text{procumbens} \) [15]

10. Podophyllotoxin        \( J. \text{flava} \) [22]

11. 1-\((2'\text{-Methoxy-}4', 5'\text{-methylenedioxy})\) 2-\(\beta\)\(-\text{hydroxy} - 6, 7\text{-methyleneedioxy} 2\text{-naphthanoic acid lactone}        \( J. \text{heterocarpa} \) [23]

12. 1, 4-Dihydrotaiwanin C  \( J. \text{neesii} \) [24]
13 Jusnesiin  

\[ \text{J. neesii} \quad [24] \]

14 Taiwanin E  

\[ \text{J. procumbens} \quad [20] \]

15 Taiwanin E methyl ether (=Neojusticin A)  

\[ \text{J. procumbens} \quad [16, 20] \]

\[ \text{J. ciliate} \quad [14] \]

\[ \text{J. purpurea} \quad [18] \]

\[ \text{[10, 16]} \]

16 Diphyllin  

\[ \text{J. extensa} \quad [25] \]

\[ \text{J. procumbens} \quad [10] \]
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Review on Different *Justicia* Species

17. **Justicidin A**
   - *J. extensa* [25]
   - *J. hayatai var. decumbens* [21]
   - *J. procumbens* [10, 26]
   - *J. cilita* [14]

18. **Jusnesiinol**
   - *J. neesii* [24]

19. **Justalakonin**
   - *J. purpurea* [18]

20. **4'-Demethyl chinensinaphthol methyl ether**
   - *J. ciliata* [14]
   - *J. procumbens* [20]
20a Justirumalin

\[
\text{OH} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{OH}
\]

\(J. \text{neesii}\) [26a]

21 Chinensinaphthol

\[
\text{OH} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{Me} \\
\text{OMe}
\]

\(J. \text{ciliate}\) [14]

\(J. \text{procumbens}\) [20]

22 Justicidinoside B

\[
\text{MeO} \\
\text{MeO} \\
\text{GlcO} \\
\text{O} \\
\text{Me}
\]

\(J. \text{procumbens}\) [15]

23 Justicidin P

\[
\text{MeO} \\
\text{MeO} \\
\text{OMe} \\
\text{OMe}
\]

\(J. \text{extensa}\) [25]
24 Justicinol  
\[
\begin{align*}
\text{MeO} & \quad \text{O} \\
\text{OH} & \quad \text{OMe} \\
\text{OMe} & \quad \text{O} \\
\end{align*}
\]
\(J.\) pateniflora  [27]

25 Cilinaphthalide A  
\[
\begin{align*}
\text{MeO} & \quad \text{OMe} \\
\text{MeO} & \quad \text{OMe} \\
\text{MeO} & \quad \text{OMe} \\
\text{OH} & \quad \text{OMe} \\
\end{align*}
\]
\(J.\) ciliata  [14]

26 Cilinaphthalide B  
\[
\begin{align*}
\text{MeO} & \quad \text{OMe} \\
\text{MeO} & \quad \text{OMe} \\
\text{MeO} & \quad \text{OMe} \\
\end{align*}
\]
\(J.\) ciliata  [14]  
\(J.\) procumbens  [28]

27 6′-Hydroxy justicidin A (= JR 6)  
\[
\begin{align*}
\text{MeO} & \quad \text{OMe} \\
\text{MeO} & \quad \text{OMe} \\
\text{OH} & \quad \text{OMe} \\
\end{align*}
\]
\(J.\) procumbens  [29]
28 Cleistanthin A

\[
\text{MeO} \quad \text{O} \quad \text{MeO} \\
\text{O} \quad \text{MeO} \quad \text{O}
\]

\[\text{Glc} \]

\[\text{J. purpurea} \quad [30]\]

29 Neesiinoside A

\[
\text{MeO} \quad \text{O} \quad \text{MeO} \\
\text{O} \quad \text{MeO} \quad \text{O}
\]

\[\text{Glc-O-GLc} \]

\[\text{J. neesii} \quad [31]\]

30 4"-O-Acetylpentiflorin B

\[
\text{Me} \quad \text{O} \quad \text{OH} \\
\text{AcO} \quad \text{MeO} \quad \text{MeO} \\
\text{MeO} \quad \text{O}
\]

\[\text{J. patentifolia} \quad [27]\]

31 Patentiflorin A

\[
\text{HO} \quad \text{Me} \quad \text{O} \\
\text{HO} \quad \text{OH} \quad \text{O}
\]

\[\text{J. patentiflora} \quad [27]\]
32 Patentiflorin B  
\[ \text{J. patentiflora} \] [27]

33 Tuberculatin  
\[ \text{J. ciliate} \] [14]  
\[ \text{J. betonica} \] [32]  
\[ \text{J. procumbens} \] [26]

34 4"-O-Acetylmananthoside B  
\[ \text{J. patentiflora} \] [27]

35 Ciliatoside A  
\[ \text{J. ciliata} \] [33]
36  Ciliatoside B  
\[ J. ciliata \]  
\[ \text{[33]} \]

37  Procumbenoside A  
\[ J. procumbens \]  
\[ \text{[26]} \]

38  Diphyllin apioideside  
\[ J. procumbens \]  
\[ \text{[15]} \]
39 Diphyllin apioside-5-acetate

\[ \text{J. procumbens} \ [15] \]

40 Jusmicranthin

\[ \text{Justicia neesii} \ [34] \]

41 Jusmicranthin methyl ether

\[ \text{J. neesii} \ [35] \]

42 Jusmicranthin ethyl ether

\[ \text{J. neesii} \ [35] \]
43 Juspurpurin \( J. \text{purpurea} \) [18]

44 Heliobuphthalmin \( J. \text{ciliata} \) [14]

45 (-) Hibalactone \( J. \text{neesii} \) [24]

46 (+) Isolariciresinol \( J. \text{flava} \) \( J. \text{diffusa} \) [36, 8] [37]
<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Species</th>
<th>Reference</th>
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<td>Justiflorinol</td>
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<td>48</td>
<td>Carinatone</td>
<td><em>J. patentiflora</em></td>
<td>[27]</td>
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<tr>
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<td><img src="image" alt="Carinatone" /></td>
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<td></td>
</tr>
<tr>
<td>49</td>
<td>Sesamin</td>
<td><em>J. purpurea</em></td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Sesamin" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>Xanthoxylol</td>
<td><em>J. purpurea</em></td>
<td>[38]</td>
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<tr>
<td></td>
<td><em>J. orbiculata</em></td>
<td></td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Xanthoxylol" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>(+) Simplexolin</td>
<td><em>J. orbiculata</em></td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Simplexolin" /></td>
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<td></td>
</tr>
</tbody>
</table>
52  (+) Sesamolin  \( J. \ orbiculata \) [11]

53  Pinoresinol  \( J. \ diffusa \) [37]

54  (-) Medioresinol  \( J. \ diffusa \) [37]

55  Medioresinol dimethyl ether  \( J. \ diffusa \) [37]
**Review on Different *Justicia* Species**

56. **Justiciresinol**
   \[ J. glauca \] [39]

57. **(+)-Lariciresinol**
   \[ J. diffusa \] [37]

58. **(-)-Tiruneesiin**
   \[ J. neesii \] [40, 41]

[B] **Flavonoids**

59. **Apigenin**
   \[ J. gendarussa \] [42]

60. **Vitexin**
   \[ J. gendarussa \] [42]
61 Hesperidin

\[
\text{Rha (1\rightarrow 6) Glc-O} \quad \text{O} \quad \text{OH} \quad \text{O} \quad \text{OMe}
\]

\[ \text{J. spicigera} \quad [43] \]

62 Kaempferol

\[
\text{HO} \quad \text{O} \quad \text{O} \quad \text{OH} \quad \text{O} \quad \text{OH}
\]

\[ \text{J. spicigera} \quad [43] \]

63 Kaempferitrin

\[
\text{GlcO} \quad \text{OH} \quad \text{O} \quad \text{O} \quad \text{OH} \quad \text{GlcO}
\]

\[ \text{J. spicigera} \quad [44] \]

64 3', 4'- Dihydroxy flavonol

\[
\text{OH} \quad \text{OH} \quad \text{O} \quad \text{O} \quad \text{OH}
\]

\[ \text{J. cataractae} \quad [45] \]

65 Naringenin

\[
\text{HO} \quad \text{O} \quad \text{O} \quad \text{OH}
\]

\[ \text{J. spicigera} \quad [43] \]
[C] Alkaloids

66  5H, 6H-Quinindolin-11-one

\[ \text{\includegraphics[width=10cm]{5H_6H_Quinindolin_11-one.png}} \]

\( J. \text{betonica} \) [46]

67  10H-Quindoline

\[ \text{\includegraphics[width=10cm]{10H_Quindoline.png}} \]

\( J. \text{betonica} \) [46, 47]

68  Jusbetonin

\[ \text{\includegraphics[width=10cm]{Jusbetonin.png}} \]

\( J. \text{betonica} \) [46, 47]

69  6H-Quinindoline

\[ \text{\includegraphics[width=10cm]{6H_Quinindoline.png}} \]

\( J. \text{betonica} \) [46]

70  Vasicine

\[ \text{\includegraphics[width=10cm]{Vasicine.png}} \]

\( J. \text{adhatoda} \) [48, 49]

71  Vasicinone

\[ \text{\includegraphics[width=10cm]{Vasicinone.png}} \]

\( J. \text{adhatoda} \) [48, 48]

72  Vasicinol

\[ \text{\includegraphics[width=10cm]{Vasicinol.png}} \]

\( J. \text{adhatoda} \) [48, 49]
73 Allantoin

\[
\text{Allantoin} \quad J. \text{ spicigera} \quad [44]
\]

74 Lupeol

\[
\text{Lupeol} \quad J. \text{ simplex} \quad [9]
\]

75 2α-Hydroxyoleanolic acid

\[
\text{2α-Hydroxyoleanolic acid} \quad J. \text{ graciliflora} \quad [50]
\]

\[
\text{2α-Hydroxyoleanolic acid} \quad J. \text{ refractifolia} \quad [50]
\]

\[
\text{2α-Hydroxyoleanolic acid} \quad J. \text{ secunda} \quad [50]
\]

76 Justicisaponin

\[
\text{Justicisaponin} \quad J. \text{ simplex} \quad [51]
\]
<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>77</td>
<td>Justicioside A</td>
<td><em>J. betonica</em></td>
<td>[52]</td>
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<tr>
<td></td>
<td><img src="image1" alt="Justicioside A" /></td>
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<tr>
<td>78</td>
<td>Justicioside B</td>
<td><em>J. betonica</em></td>
<td>[52]</td>
</tr>
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<td></td>
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<tr>
<td>79</td>
<td>Justicioside C</td>
<td><em>J. betonica</em></td>
<td>[52]</td>
</tr>
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<td><img src="image3" alt="Justicioside C" /></td>
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<td></td>
</tr>
<tr>
<td>80</td>
<td>Justicioside D</td>
<td><em>J. betonica</em></td>
<td>[52]</td>
</tr>
<tr>
<td></td>
<td><img src="image4" alt="Justicioside D" /></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
81  Justicioside E  \( J. \) betonica  [53]

82  Justicioside F  \( J. \) betonica  [53]

83  Justicioside G  \( J. \) betonica  [53]
[E] Steroids

84 β – Sitosterol
   \( J. \) heterocarpa [23]
   \( J. \) simplex [9]

85 β – Sitosterol glucoside
   \( J. \) simplex [9]
   \( J. \) flava

86 Stigmasterol
   \( J. \) heterocarpa [23]

[F] Coumarin

87 Umbelliferone
   \( J. \) pectoralis [50]
[G] Miscellaneous

88 Justiciamide \( J. ghiesbrechtiana \) [54]

89 Hexahydrofarnesylacetone \( J. heterocarpa \) [23]

90 Farnesylacetone \( J. heterocarpa \) [23]

91 Farnesyl acetate \( J. heterocarpa \) [23]

92 Phytol \( J. heterocarpa \) [23]
Pharmacological activities of crude extracts and pure isolated chemicals from different Justicia species

Several Justicia species such as Justicia gendarussa, J. ciliata, J. patentiflora, J. purpurea, J. neesii, J. procumbens, J. pectoralis, J. prostrata, J. adhatoda, J. flava, J. betonica, J. spicigera etc are popularly used in different countries for treatment of several diseases. To substantiate the traditional claims, several pharmacological activities of crude extracts and their pure isolates from different Justicia species have been reported. Some of these important pharmacological activities are discussed briefly.

Anticancer activity

The EtOAc extracts of leaves and stems of Justicia patentiflora exhibited significant cytotoxic effect against KB cells producing 64 % and 76 % inhibition of cell growth at 0.1 μg / ml. Six lignans were isolated from these extracts. Among them, two lignans, patentiflorin B (32) and 4"-O-acetyl patentiflorin B (30) showed most significant cytotoxicity against KB, HCT 116, MCF7-S and MCF7-R tumor cells with IC50 values of 0.004, 0.012, 0.003 and 0.040 μM / L; and 0.024, 0.030, 0.012 and 0.10 μM / L, respectively. The study of the mechanism of cell growth arrest indicated that cell growth was arrested in the G0 / G1 phase of the cell cycle [27].

Elenoside (3) isolated from Justicia hyssopifolia showed significant cytotoxicity against leukemia cell lines, CCRF-CEM, K-526, MOLT-4 and RPM1-8226 with 79-97% growth inhibition at a concentration of 10^-4 M. It also showed antiproliferative effect at a concentration of 10^-4 M, against melanoma cell lines M19-MEL (81 % growth inhibition) and SK-MEL-2 (84% growth inhibition), a CNS cancer cell line, S NB-19 (81% inhibition), a renal cancer cell line, UO-31 (80% inhibition), and a colon cancer cell line, HCC-2998 (87% inhibition) [13].
Justicidine A (17) isolated from *Justicia ciliata* whole plant exhibited *in-vitro* potent cytotoxic effects against cancer cells, T-24, Ca Ski, SiHa, HT-3, PLC / PRF / 5 and 212 cells with ED$_{50}$ in the range, $1.8 \times 10^{-3} - 22.7 \times 10^{-3}$ µg / ml, which were comparable to that of positive control, actinomycin D having ED$_{50}$ values in $8.1 \times 10^{-4} - 1.4 \times 10^{-3}$ µg / ml, in MTT assay [14].

Lignans, justicidin A (17), diphyllin (16) and tuberculatin (33) isolated from the whole plant of *Justicia procumbens* showed *in-vitro* potent cytotoxic effects against cancer cells, 212, Ca Ski, Hep 3B, Si Ha, Hep G2, HT-29, HCT116, MCF-7 and MCF-7-ras with ED$_{50}$ values in range $7.4 \times 10^{-3}-1.2 \times 10^{-1}$ g / ML in MTT assay [26].

Justicidin A (17) isolated from *Justicia procumbens* suppressed the viability of human hepatocellular carcinoma (HCC)-Hep 3B and Hep 2G cells with IC$_{50}$ at day 6 was $0.048 \pm 0.020$ and $0.052 \pm 0.50$ µM, respectively *in-vitro* MTT assay. Justicidin A also suppressed the viability of non-malignant Chang liver cells with IC$_{50}$ of $0.95 \pm 0.12$ µM. The study of the mechanism of apoptosis of HCC cells revealed that justicidin A induced both intrinsic and extensive apoptotic pathways by activation caspase-8 and mitochondria. (to activate caspase- 9 and capase-3). *In-vivo*, justicidin A also suppressed the growth of Hep 3B implanted in NOD – SC1D mice by feeding them justicidin A (20 mg / kg / d for 60 consecutive days) before cancer cell treatment. This results scientifically justified the traditional use of *J. procumbens* in cancer by Taiwanese [55].

Justicidin B (7) isolated from *Justicia pectoralis* exhibited *in-vitro* antiproliferative effect against murine leukemia P-388 (9PS) with ED$_{50}$ of 3.3 µg / ml and against bronchial epidermoid carcinoma cell, NSCLCN6 with IC$_{50}$ of 28 µg / ml [56].

6'-Hydroxyjusticidine A (27) isolated from *Justicia procumbens* exhibited significant cytotoxicity against human bladder cancer EJ cells with IC$_{50}$ value of 57.1 µM in MTT assay and 69.8 µM in SRM assay after
treatment for 48 h. The compound induced apoptosis of the cancer cells by increasing the content of reactive oxygen species (ROS) and activation of caspase-8, caspase-9 and subsequent of caspase-3 inducing both intrinsic and extrinsic apoptosis pathways. It also disrupted the mitochondrial membrane potential (Δψm) and unregulated the base and p53 expressions in EJ cells. These results support the potential use of \textit{J. procumbens} in treatment of bladder cancer [29].

Elenoside (3) isolated from \textit{Justicia hyssopifolia} showed cytotoxic effect against several tumor cells of US-NCI such as NCI-H522, NCI-H226, NCI-H23, LXFL 529, colon cancer (HCC-2998, HCT-116, HCT-15, HT-29, KM12), renal cancer (AC HN, CAKI, RXF-393, SN12C, VO-31) and ovarian cancer (OV-CAR-3, OVACR-4, OVCAR-8) cells in the concentration range of $10^{-5} – 10^{-4}$ M [57].

Justicidins A (17) and B (7), diphyllin (16), diphyllin apioe (38) and diphyllin apioe-acetate (    ) exhibited mild cytotoxicity against cultered rabbit lung cells, RL-33 with MTC (minimum cytotoxic concentration) values in the range 31 - 63 μg / ml [15].

\textit{Hepatoprotective activity}

The methanolic extract of \textit{Justicia schimperiana} exhibited significant hepatoprotective activity against CCl$_4$ – induced hepatotoxicity in Swiss albino rats [58].

\textit{Anti-inflammatory activity}

The aqueous and alcoholic extracts of \textit{Justicia prostrata} showed significant anti-inflammatory activity in carrageenan – induced acute inflammation and cotton pellet induced granuloma (subacute inflammation), respectively in rats. At the dose of 500 mg / kg bw, p.o., both the extracts were found to exhibit maximum inhibition (51-39 % and 62.5 %, respectively) of paw edema volume at the first hour of carrageenan – induced acute
inflammation. In the cotton pellet granuloma assay, both aqueous and alcoholic extracts at the dose of 500 mg / kg, p.o., suppressed the transudative, exudative and proliferative phases of chronic inflammation. These extracts reduced lipid peroxide content of exudates of liver and normalized the increased activity of acid and alkaline phosphatases in serum and liver of cotton pellet granulomatous rats. The inflammatory effects produced by the extracts at the dose of 500 mg / kg, p.o. was comparable with the reference drug, diclofenac sodium (5 mg / kg, p.o.) [59].

The MeOH extract of Justicia tranquabariensis aerial parts exhibited anti-arthritic activity in Freund’s adjuvant induced arthritis model in rats. The extract at a dose of 200 mg/ kg bw/d for 14 d in complete Freund’s adjuvant (1 %, 0.1 ml) induced rats significantly showed anti-arthritic activity by preventing the paw edema volume and normalizing the levels of SGOT, SGPT and ALP as compared to arthritis control group of rats. The effect was comparable to that of standard reference drug indomethacin ( 3 mg/kg bw/d). These findings justified the traditional use of the plant in treatment of inflammation and arthritis [60].

The ethanolic leaf extract of Justicia gendarussa showed significant in-vivo anti-arthritic activity in Freund’s adjuvant-induced and collagen-induced arthritic rat models. The leaf extract at a dose of 100 mg/kg bw/d for 20 d of treatment in Freund’s complete adjuvant (FCA) (0.5 ml/rat) treated rats showed paw edema inhibition of 43%, compared to 26% inhibition of aspirin (300 mg / kg) treated rats.

The ethanolic leaf extract in the same dose for same period in collagen emulsified incomplete Freund’s adjuvant (IFA) induced-rats exhibited inhibition of rat paw edema by 47 % compared to 38 % inhibition in aspirin treated rats. The study of haematological parameters such as Hb, RBC count, serum copper levels and C-reactive protein levels for both normal and experimental rats showed a significant increase in the levels of RBC and Hb near normal levels, increase in WBC count, ESR, serum C-reactive protein
level and decrease in serum copper level in the extract-administered arthritic group. These results were opposite in arthritic rats [61]. In Indian and Chinese traditional medicine, the leaf of the plant is recommended to treat rheumatism, arthritis and respiratory disorders etc [62, 63]. Therefore, the anti-arthritic potential results of leaf extract justified the traditional claim of this plant in treatment of arthritis.

The hydro alcoholic extract of Justicia pectoralis showed significant analgesic and antioedema activities on acetic acid induced writhing in mice and on carrageenan induced paw oedema in rats. The isolated coumarin, umbelliferone from this extract also exhibited analgesic and anti-inflammatory effects in the same assay models. Pretreatment with naloxone did not reverse the antinociception indicating that opioid system is not involved, while pretreatment with L-arginine reversed the antinociception caused by umbelliferone, suggesting the involvement of the nitric oxide system. One unidentified coumarin isolated from this extract decreased the rat paw volume in dextran model [64].

Two lignin glycosides, ciliatosides A (35) and B (36) isolated from the whole plant of Justicia ciliata exhibited in-vitro potent anti-inflammatory effect by inhibiting the accumulation of NO2- in lipopolysaccharide-stimulated RAW 264.7 cells in a concentration-dependent manner with IC50 values of 27.1 ± 1.6 and 29.4 ± 1.4 μM, respectively [33].

Lignans justicidin A (17) and tuberculatin (33) isolated from Justicia procumbens showed strong proinflammatory effect by enhancement of tumor-necrosis factor-α (TNF-α) generation from LPS-induced mouse macrophage, RAW 264.7 cells [26].

Justirumalin (209) isolated from J. neesii exhibited anti-inflammatory activity by inhibiting the activity of pro-inflammatory enzymes COX-2 and COX 1 by 67.2 and 59.9 %, respectively at 25 mg / ml [26a].
Wound healing activity

The methanolic leaf extract of *Justicia flava* exhibited significant wound healing potential in excision wound model in rats. The extract at a dose of 7.5 % w / w showed wound closure of 99 % \((P < 0.01)\) on 9\textsuperscript{th} day compared to untreated wounds. The extract significantly increased the tensile strength of wounds, angiogenesis, collagenation, and reepithelialisation compared to the untreated wound tissues. This wound healing property justifies the folklore use of *J. flava* in treatment of wounds [65].

Anti- anxiety activity

The ethanolic extract of the aerial part of *Justicia gendarussa* at a dose of 250 and 500 mg / kg / d for 21 d, p.o. showed anti-anxiety effect in elevated plus-maze and light-dark models in mice. The higher dose was more effective. The treatment of extract (500 mg / kg) in mice caused the time spent in the open arms \((151.3 \pm 18.05 \text{ sec})\) and number of entries into open arms \((10.5 \pm 0.76)\), which were comparable to those of standard reference drug diazepam \((2 \text{ mg / kg bw / d for 21 d})\) having almost similar values of \(154.83 \pm 22.1 \text{ sec and 10.6 \pm 1.05, respectively}\) [66].

Anti- oxidant activity

The petroleum ether, CHCl\textsubscript{3}, EtOAc and MeOH extracts of the aerial parts of *Justicia beddomei* showed in-vitro antioxidant activities in DPPH, hydroxyl radical, superoxide anion radical scavenging assays and in \(\beta\)-carotene-linoleic acid and reducing power ability assays. Among the extracts, the MeOH extract showed most significant activity in all these assays. In \(\beta\)-carotene-linoleate model MeOH extract showed potent \((P < 0.05)\) antioxidant activity with IC\textsubscript{50} value of \(86.11 \pm 0.236 \text{ \mu g / ml}\), which was comparable to that of standard BHT \((\text{IC}_{50} 70.58 \pm 0.530 \text{ \mu g / ml})\) [67].
Antihypertensive activity

The CHCl₃ extract of the aerial parts of *J. spicigera* exhibited significant antihypertensive effect in L-NAME (N-nitro-L-arginine methyl ester) – induced hypertensive rats. The extract at a single oral dose of 150 mg / kg bw, lowered the blood pressure from the values of 180 / 164 ± 1.7 / 3.2 mm Hg to 149 / 133 ± 4.0 / 3.7 mm Hg (Systolic / diastolic) after 3h, similar to those of the normotensive rats. The CHCl₃ extract yielded three major flavonoids, hesperidin, naringenin and kaempferol, that could be responsible to this antihypertensive effect of the extract [68].

Antimicrobial activity

The antimicrobial activity of ethanolic and aqueous extracts of stem and leaves of *Justicia gendarussa* were evaluated against 12 human pathogens in both disc diffusion and broth dilution methods. The aqueous extract of the stem showed maximum inhibitory activity against *Shigella flexneri*, *Proteus mirabilis*, *Escherichia coli* and *Bacillus subtilis* with MIC values of 128, 256, 512, 512 µg / ml, while the aqueous extract of leaves showed significant antimicrobial activity against *Staphylococcus aureus* with MIC value of 256 µg / ml. The ethanolic extracts of stem and leaves showed weak to moderate antimicrobial activity against the tested pathogens [26a, 71].

The MeOH extract of leaves of *Justicia flava* showed anti microbial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli* and *Pseudomonas aeruginosa* with MIC values of 5, 7.5, 5, 7.5 and 7.5 mg/ml, respectively [53].

Antiplatelet activity

Lignan, Taiwan E methyl ether (15) isolated from *Justicia procumbens* showed strong antiplatelet effect in human platelet rich plasma by inhibiting
the platelet aggregation induced by adrenaline in a concentration dependent manner, with an IC$_{50}$ value of about 27.6 µM [28]

Justicidin B (7), taiwanin E (14) and its methyl ether (15) isolated from Justicia procumbens exhibited significant antiplatelet activity by inhibiting arachidonic acid-induced platelet aggregation in rabbit platelet suspension [20].

Justicidines B (7) and D (6) isolated from Justicia procumbens showed potent antiplatelet effects on AA induced rabbit platelet aggregation with IC$_{50}$ values of 8.0 ± 1.2 and 1.7 ± 0.3 µM, respectively, while showed weak activity on adrenaline induced aggregation in human platelet-rich plasma (PRP) with IC$_{50}$ values of 104.8 ± 25.3 and 106 ± 39.4 µM, respectively [69].

**Antiviral activity**

Helioxanthin (1) isolated from different Justicia species showed antiviral activity by suppressing Hepatic B virus (HBV) gene expression and replication in HCC cells in luciferase assay. It selectively suppressed surface antigen promoter II (SPII) and core protomer present in liver cells [70].

Justicidins A (17) and B (7), diphyllin (16), diphyllin apioside (38) and diphyllin apioside acetate (39) isolated from Justicia procumbens showed strong antiviral activity against vesicular stomatitis virus (VSV) with MIC values of less than 0.25 µg / ml [15].

**Antibronchitis activity**

The alkaloids vasicine (70), vasicinone (71) and vasicinol (72) isolated from Justicia adhatoda (syn. Adhatoda vasica) leaves exhibited significant bronchodilator activity. Hence the traditional use of the plant in treatment of bronchitis is justified [48, 49]
Fish-killing activity

Justicidins A (17) and B (7) exhibited strong toxicity against *Oryzias latipes* with TL$_{50}$ values of 0.049 and 0.028 ppm after 24 h respectively, which were comparable to that of rotenone and 10 times stronger than that of pentachlorophenol [21].
Section 2: A brief history, taxonomical description and classification of

*Justicia gendarussa*

Plate-3. Photographs of *Justicia gendarussa* (family: Acanthaceae)
Justicia gendarussa Burm. f. syn. Justicia gendarussa Blanco, Gendarussa vulgaris Nees, Adhatoda subserrata Nees (local name: Jagat Madan) is grown in tropical and subtropical regions in Asian countries from India to Malaysia [Plate 3]. It is an undershrub with linear-lanceolate leaves of 5 – 12.5 × 0.5 – 1 cm, purple-white flowers in terminal spikes and capsule of about 1.2 cm long, glabrous. In flowers, calyx are of 5 cm long, corolla about 1.2 cm long, 2 lipped, two hairy stamens and 2 celled anthers are present [3].

The systematic taxonomic position of Justicia gendarussa as per Bentham & Hooker System of classification [72].

<table>
<thead>
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<tr>
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<tr>
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<td>Bicarpillatae</td>
</tr>
<tr>
<td>Cohort</td>
<td>Personales</td>
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<td>Acanthaceae</td>
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<td>Justicia</td>
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<tr>
<td>Species</td>
<td>Justicia gendarussa Burm. f.</td>
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</table>
Section 3: Isolation and structure elucidation of lupeol

Isolation:

Lupeol (73) was isolated as colourless needles, mp 214-215\(^0\)C from CH\(_2\)Cl\(_2\) soluble fraction of MeOH extract of *Justicia gendarussa* whole plants by silica gel column chromatography from elution with 5% EtOAc in petroleum ether (b.p. 60-80\(^0\)) solvent mixture. It was homogeneous on TLC in different solvent systems [Silica gel G, \(R_f = 0.52\) in PhH-CHCl\(_3\), 5 : 1].

Structure elucidation:

a) The Molecular formula:

The molecular formula of the compound was determined as C\(_{30}\)H\(_{50}\)O from its quasi-molecular mass ion at \(m/z\) 427.3944 [M + H]\(^+\) (Calcd for C\(_{30}\)H\(_{51}\)O : 427.3940) in HR-FAB-MS and analysis of its \(^{13}\)C-/DEPT NMR data.

b) The colour reaction:

The compound showed positive Liebermann Burchard test (red colouration) for triterpenoids.

c) The IR spectrum:

The IR spectrum of the compound in KBr (Fig. 3.1) showed the absorption bands for hydroxyl (3433 cm\(^{-1}\)) and olefinic (1640 cm\(^{-1}\)) functions.

d) The \(^1\)H-NMR spectrum:

The \(^1\)H-NMR spectrum of the compound in CDCl\(_3\) (Fig. 3.2) (Table-3.2) displayed signals for six tertiary methyls [\(\delta_H\) 0.74, 0.76, 0.82, 0.94, 0.96 and 1.02 (each 3H, s)], one isopropylene moiety [\(\delta_H\) 4.57, 4.68 (each 1H, m) and 1.67 (3H, s)], one hydroxymethine proton [\(\delta_H\) 3.18 (1H, dd, \(J = 11.2\) and 5.2 Hz)] and one typical lupene H-19 proton [\(\delta_H\) 2.37 (1H, dt, \(J = 10.9\) and 5.6 Hz)] suggesting its lupane class of triterpenoid structure [73].
e) The $^{13}$C-NMR spectrum:

The $^{13}$C-NMR spectrum of the compound in CDCl$_3$ (Figs. 3.3, 3.3a, 3.3b, 3.3c) (Table-3.2) recorded 30 carbon signals, which on DEPT experiments (Figs. 3.3d-3.3f) indicated for 7 methyl, 11 methylene, 6 methine and 6 quaternary carbons. The carbon resonances at $\delta$C 109.3 (CH$_2$) and 151.0 (C) confirmed its $\Delta^{20(29)}$ functionality in its lupane structure [74]. The carbinol carbon resonance at $\delta$C 79.0 and other carbon resonances could be interpreted by considering its lupeol structure [75].

f) The $^1$H-$^1$H-COSY NMR spectrum:

The $^1$H-$^1$H-COSY NMR spectrum of the compound (Fig. 3.4) showed correlation between H-30 and H-29, between H-3 and H-2 and between H-19 and H-18.

g) The EI-MS:

The EI-MS of the compound (Fig. 3.5) recorded mass ions at $m/z$ 426[M]$^+$, 411, 315, 218, 207 (base) and 189. The formation of these mass ions could be interpreted by considering its lupeol structure (Scheme 3.1) [76].
Scheme 3.1. Plausible EI-MS Fragmentation of 73
h) Conclusion:

The spectral data of the compound were almost identical with those reported for lupeol [56].

On the basis of all these evidence, the structure of the compound was assigned as 3β-hydroxylup-20(29)-ene or lupeol (92) (Fig. 3.6).

It is a known natural product, isolated from other Justicia species and plants of other genus, but its isolation from this plant is reported for the first time.

![Fig. 3.6. Structure of lupeol (73)](image-url)
Table 3.2. $^1$H- (400 MHz) and $^{13}$C-(100 MHz) NMR spectral data of compound 73 in CDCl$_3$ (δ, ppm)

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<th>( \delta_H )</th>
</tr>
</thead>
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<td>79.0 (CH)</td>
<td>3.18 dd (11.2, 5.2)</td>
</tr>
<tr>
<td>4</td>
<td>38.8 (C)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>55.3 (CH$_2$)</td>
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</tr>
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<td>18.3 (CH$_2$)</td>
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</tr>
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<td>7</td>
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</tr>
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<td>8</td>
<td>40.8 (C)</td>
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</tr>
<tr>
<td>9</td>
<td>50.4 (CH)</td>
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<td>10</td>
<td>37.2 (C)</td>
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<td>38.0 (CH)</td>
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<td>15</td>
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<td>35.6 (CH$_2$)</td>
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<td>20</td>
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<td>23</td>
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<td>29</td>
<td>109.3 (CH$_2$)</td>
<td>4.57 m, 4.68 m</td>
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<td>30</td>
<td>19.3 (CH$_3$)</td>
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</table>
Fig. 3.1. IR spectrum of lupeol (73) in KBr.
Figure 3.1: NMR spectrum of compound (1) in CDCl₃.
Fig. 3.3: 13C-NMR spectrum (expanded) of liguol (C73) in CDCl3.
Fig. 3.2c: 1D-1H-NMR spectrum of isolated (P)-indolylcine in CDCl₃.
Figure 3.5: 13C-NMR spectrum (expanded) of Indolol (7a) in CDCl₃.
Fig. 3.3d. $^{13}$C-DEPT NMR spectrum of lupeol (73) in CDCl$_3$
Fig. 3.3E: 1H-DEPT NMR spectrum of lupisol (73) in CDCl₃
Fig. 3.4: H-H COSY NMR spectrum of limoed (72) in CDCl₃.
Fig. 3.5. El-MS of Jupeol (73)
Section 4: Isolation and structure elucidation of ursolic acid

Isolation:

Ursolic acid (92) was isolated as white amorphous solid, mp 265-267°C from EtOAc soluble fraction of the MeOH extract of Justicia gendarussa whole plants by repeated column chromatography over silica gel. It was homogeneous on TLC in different solvent systems [Silica gel G, Rf =0.58 in CHCl3-MeOH, 9 : 1].

Structure elucidation:

a) The Molecular formula:

The molecular formula of the compound was assigned as C₃₀H₄₈O₃ from its molecular ion at m/z 456.3601 [M]⁺ (Calcd for C₃₀H₄₈O₃ : 456.3604) in HR-EI-MS and analysis of its ¹³C-/DEPT NMR data.

b) The colour reaction:

The compound exhibited positive response (red colouration) Liebermann Burchard test characteristic of triterpenoids.

c) The IR spectrum:

The IR spectrum of the compound in KBr (Fig. 3.7) showed the absorption bands for hydroxyl (3433 cm⁻¹), carboxyl (1709 cm⁻¹) and olefinic (1650 cm⁻¹) functions.

d) The ¹H-NMR spectrum:

The ¹H-NMR spectrum of the compound in CD₃OD (Fig. 3.8) (Table-3.3) displayed signals for five tertiary methyls \([δ_H 0.78, 0.85, 0.96, 1.12 \text{ and } 1.29 \text{ (each 3H, s)}]\), two secondary methyls \([δ_H 0.88 \text{ (3H, d, } J= 6.6 \text{ Hz) and } 0.97 \text{ (3H, d, } J= 6.6 \text{ Hz)}]\), one olefinic proton \([δ_H 5.23 \text{ (1H, t, } J=3.0 \text{ Hz)}]\), one carbinol methine proton \([δ_H 3.15 \text{ (1H, dd, } J= 11.4 \text{ and } 4.8 \text{ Hz)}]\) and one
typical ursane H-18 methine proton [$\delta_H$ 2.20 (1H, d, $J = 10.8$ Hz) suggesting its $\Delta^{12}$-ursane like structure [77].

e) The $^{13}$C-NMR spectrum:

The $^{13}$C-NMR spectrum of the compound in CD$_3$OD (Fig. 3.9) (Table-3.3) recorded 30 carbon signals, which on DEPT experiments indicated for 7 methyl, 9 methylene, 7 methine and 7 quaternary carbons. Two olefinic carbon resonances at $\delta_C$ 126.9 and 139.7 were characteristic of $\Delta^{12}$-ursane skeletal structure [78]. The quaternary carbon resonance at $\delta_C$ 181.8 was assigned to a free carboxyl group. Seven methyl carbon resonances at $\delta_C$ 16.0, 16.4, 17.6, 18.0, 21.6, 24.1 and 28.8 coupled with a carbinol methine carbon resonance at $\delta_C$ 79.8 and other carbon resonances of the compound could be assigned by considering its ursolic acid structure [79].

f) The FAB-MS:

The FAB-MS of the compound (Fig. 3.10) showed mass ions at $m/z$ 457 [$M + H]^+$, 441, 438, 248 (base), 203, 207 and 189. The formation of which could be rationalized by considering its ursolic acid structure (Scheme 3.2) [80]

Scheme 3.2. FAB-MS Fragmentation of 92
g) **Conclusion:**

The physical constant and spectral data of the compound were almost identical with those reported for ursolic acid [81].

Therefore, based on all these evidence, the structure of the compound was assigned as ursolic acid or 3β-hydroxyurs-12-en-28-oic acid (92) (Fig. 3.11).

It is a known compound, but its isolation from this plant is reported for the first time.

![Fig. 3.11. Structure of ursolic acid (92)](image)
Table 3.3. The $^1$H- and $^{13}$C-NMR spectral data$^a$ of ursolic acid (92) (in CD$_3$OD)

<table>
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<td>30</td>
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</table>

$^a$ 400 MHz for $^1$H- and 100 MHz for $^{13}$C-NMR spectra.
$^b$ J value in Hz in parentheses.
Fig. 3.7. IR spectrum of ursolic acid (92) in KBr
Fig. 2. 1H-NMR spectrum (expanded) of mucic acid (O2) in CD3OD.
Fig. 3.9: 1H-\text{NMR} spectrum of usonic acid (92) in CD₃OD
Fig. 3.9a. 1H-NMR spectrum (expanded) of unknown acid (02 in CD3OD)
Fig. 3.10. FAB-MS of ursolic acid (92)
References


[34] Rajasekhar D. And Subbaraju G. V., Fitoterapia, 2000, 71, 598.


