ANNEXURE
I. Research Papers Published


II. Research Papers Communicated


2. S. Gopalakrishnan, K. Saroja and J. Dulcy Elizabeth. “GC-MS analysis of the methanolic extract of the leaves of *Dipteracanthus patulus* (Jacq.) Nees.” *Reports on Natural Products*. 
III. Research Papers Presented in National / International Conference / Seminar / Symposium


2. K. Saroja, J. Dulcy Elizabeth and S. Gopalakrishnan, “*Medicinal plants used by traditional healers in Tirunelveli District of Tamil Nadu to treat cuts and wounds*”. National Conference on ”The role of Indian Medicinal Plants and Indian systems of Medicine on Rural health, organized by A.V.V.M. Sri Pushpam College (Autonomous), Poondi – 613 503.


3. K. Saroja, J. Dulcy Elizabeth and S. Gopalakrishnan, “*Studies on Pharmacognostic profiles of the leaves of Dipteracanthus patulus (Jacq.) Nees.*” UGC sponsored State level seminar on “Plant Medicine: Present Scenario” organized by Dept. of Plant Biology and Plant Biotechnology, St. Mary’s College, Thoothukudi – 628 001.


7. K. Saroja, J. Dulcy Elizabeth and S. Gopalakrishnan, “Ethnomedicinal plants used for poisonous bites in Courtallam region of Tirunelveli District of Tamil Nadu”. International Conference on “Recent trends in Biotechnology - (RBT-10)” organized by Holy Cross College (Autonomous), Nagarcoil.

WOUND HEALING ACTIVITY OF THE LEAVES OF
DIPTERACANTHUS PATULUS (JACQ.) NEES.

K.Saroja, J.Dulcy Elizabeth and S.Gopalakrishnan

*Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University,
Tirunelveli-627 012, Tamil Nadu, India.

Department of Botany, Sri Parasakthi College for Women (Autonomous),
Courtallam-627 802, Tamil Nadu, India.

Department of Botany, St. Mary’s College, Tuticorin-628 001, Tamil Nadu, India.

Corresponding author: E-mail: sgkmsu@yahoo.co.in

Summary

The methanolic extract of the leaves of Dipteracanthus patulus (Jacq.) Nees was investigated for its wound healing activity in excision and dead space wound models. The extract significantly increased the rate of wound contraction, weight of granulation tissue, tensile strength and collagen formation when compared with the control.

Keywords: Dipteracanthus patulus, wound healing, excision wound model, dead space wound model
Introduction

Wounds are physical injuries that result in an opening or break of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. Current estimates indicate that nearly 6 million people suffer from chronic wounds worldwide. Community based epidemiological study of wounds in India revealed the prevalence of acute and chronic wounds as 10.55 and 4.48 per 1000 population respectively (1). Healing of chronic lower extremity wounds is a global problem (2). Research on wound healing agents is one of the developing areas in modern biomedical sciences. Many traditional practitioners across the world particularly in countries like India and China with age old traditional practices have valuable information of many lesser-known hither to unknown wild plants used for treating wounds and burns. Some of these plants have been screened scientifically for the evaluation of their wound healing activity in different pharmacological models but the potential of most of the plants remain unexplored (3).

*Dipteracanthus patulus* (Jacq.) Nees. (Syn. *Ruellia patula* Jacq). (Acanthaceae) commonly known as Kiranthinayagam or Kayappacchilai in Tamil is a medicinal herb traditionally used in the treatment of wounds in the rural areas. The leaves are ground into a paste and applied on fresh wounds. The plant is commonly distributed on wastelands in Tamil Nadu. The leaves are used for treating itches, insect bites, paranychia, venereal diseases, sores, tumours, rheumatic complaints and eye diseases (4-6). Pharmacological and phytochemical studies indicated that it is a cardiotonic (7) and it contained two lignan glycosides namely 5, 5'-dimethoxy-lariciresinol-9-O-β-D-glucopyranoside and lyoniresinol-9-O-β-D-glucopyranoside (8). The wound healing properties of the leaves of *Dipteracanthus patulus* has not been scientifically evaluated so far. Hence the present study was undertaken to evaluate the wound healing activity of the leaves of *Dipteracanthus patulus*.

Materials and methods

Preparation of plant extract: Leaves of *Dipteracanthus patulus* were collected from Courtallam region, Tamil Nadu, India. The plants were identified and the voucher specimen (SMCH-3009) of the plant is deposited in the Department of Botany, St.Mary’s College, Tuticorin, Tamil Nadu. The leaves were dried in shade and powdered.
The leaf powder (1Kg) was subjected to hot extraction with solvents of increasing polarity starting from Petroleum ether (40-60°C), Benzene, Chloroform and Methanol. The methanolic extract was concentrated under reduced pressure in a rotary evaporator (Buchi, USA). Preliminary qualitative phytochemical analysis of the methanolic extract was carried out (9). The methanolic extract showed the presence of flavonoids, phenols, saponins, steroids and tannins. The methanolic extract was used for evaluating wound healing activity. Extract was incorporated into a simple ointment base BP. Two formulations of extract ointment 5% (w/w) and 10% (w/w) were prepared by incorporating 5g and 10g of extract in 100g of simple ointment base BP, respectively.

**Animals:** Wistar albino rats weighing (150-200g) were used. Animals were fed with standard diet and water *ad libitum* and maintained under standard laboratory conditions. Ethical clearance for the animal study was obtained (IAEC-265/CPCSEA).

**Wound healing activity:** Excision and dead space wound models were used to evaluate the wound healing activity of the methanolic extract of leaves of *Dipteracanthus patulus*.

**Excision wound model:** Excision wound was inflicted by cutting away approximately 500 mm², full thickness of skin from the depilated area on the back under light ether anaesthesia and were placed in their individual cages. The animals were divided into four groups (n=6). Group 1 was the control group that received simple ointment base, group 2 was treated with reference standard (0.2 % w/w nitrofurazone ointment), group 3 received 5% (w/w) extract ointment and group 4 received 10% (w/w) extract ointment. The ointments (0.5g each) were applied topically with a fine brush once daily till the wound was completely healed. Wound contraction rate was monitored by planimetric measurement of the wound by tracing the wound margin on a graph paper every alternate day (10). Wound contraction was calculated as percentage of original wound size.

Dead space wound model: Three groups of rats (n=6) were used. Dead space wounds were made by implanting subcutaneously a polypropylene tube (2.5 x 0.5 cm) beneath the dorsal paravertebral lumbar skin. Group 1 was the control group that received 2 ml of 1% carboxymethyl cellulose, group 2 received extract (100 mg /kg) and group 3 received extract (200 mg/kg) orally, once daily for 10 days. On the 11th day, the granulation tissue formed on the implanted tubes was dissected out carefully. The wet weight of the granulation tissue was noted. Tensile strength of the granulation tissue was measured by the method of Lee (11).

The granulation tissue was dried in an oven at 60°C for 12 hours and the dry weight was recorded. Histopathological studies of granulation tissue were done by staining with haemotoxylin and eosin so as to enable the assessment of fibroblast population and collagen content under a light microscope.

Statistical analysis: Data are expressed as mean ± SE and subjected to Student’s t test by comparing with the control.

Results

In the excision wound model, wound contraction progressed faster in extract ointment treatment, when compared to control and standard drug. Significant increase in the rate of wound contraction has been observed on day 16 (98.49 %, P < 0.001) on the 10% extract treated animals. On 18th day of post wounding, 100% wound closure was observed in 10% (w/w) extract ointment treated animals whereas in 5 % (w/w) extract ointment treated animals and in nitrofurazone ointment treated animals 100% wound closure was observed on 20th day of post wounding (Table 1 and Figure 1).

In dead space wound model, significant increase in the weight of the granulation tissue and its tensile strength were observed in the animals treated with leaf extract (Table 2). The histological profiles of the granulation tissue of extract treated animals showed increased collagen formation when compared to control (Figure 2).
Table 1: Effect of leaf extract of *Dipteracanthus patulus* on wound contraction in Excision model

<table>
<thead>
<tr>
<th>Post wounding days</th>
<th>Wound Area (mm²) and percentage of wound contraction</th>
<th>Simple ointment (control)</th>
<th>Nitrofurazone ointment (0.2%)</th>
<th>Extract ointment (5% w/w)</th>
<th>Extract ointment (10% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>522±36.8 (0.0%)</td>
<td>518±25.9 (0.0%)</td>
<td>528±31.6 (0.0%)</td>
<td>533±35.2 (0.0%)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>507±28.2 (2.87%)</td>
<td>473±37.1 (8.68%)</td>
<td>461±21.3 (12.68%)</td>
<td>412±29.6 (22.70%)</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>480±23.6 (8.04%)</td>
<td>341±32.5* (34.16%)</td>
<td>341±19.6 (35.41%)</td>
<td>339±23.9* (36.91%)</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>421±19.2 (19.34%)</td>
<td>72±20.8* (47.49%)</td>
<td>269±17.2* (49.05%)</td>
<td>212±16.2** (60.22%)</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>379±17.4 (27.3%)</td>
<td>181±16.2** (65.05%)</td>
<td>200±13.8** (62.12%)</td>
<td>107±12.5** (79.92%)</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>315±13.7 (39.65%)</td>
<td>118±9.4*** (77.22%)</td>
<td>112±9.2** (78.78%)</td>
<td>56±9.8** (89.49%)</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>281±11.3 (46.16%)</td>
<td>69±5.1** (86.67%)</td>
<td>75±5.9** (85.79%)</td>
<td>36±8.9** (93.24%)</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>242±10.5 (53.63%)</td>
<td>48±3.7** (90.73%)</td>
<td>57±4.7** (89.20%)</td>
<td>19±4.7** (96.43%)</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>201±9.7 (61.49%)</td>
<td>37±2.6** (92.85%)</td>
<td>41±3.5** (92.23%)</td>
<td>8±1.4** (98.49%)</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>156±7.4 (70.11%)</td>
<td>11±1.2** (97.87%)</td>
<td>15±1.1** (97.15%)</td>
<td>0.0±0.0** (100%)</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>128±9.4 (75.47%)</td>
<td>0±0.0** (100%)</td>
<td>0.0±0.0** (100%)</td>
<td>0.0±0.0** (100%)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ±SE; n = 6.
P values versus respective control by student’s t-test.
* *P* < 0.01, ** *P* < 0.001
Figure 1. Effect of leaf extract on wound contraction

Figure 2. Comparative histopathological study of control and treated group (a) 100 mg/Kg, (b) 200 mg/Kg extract treated groups show increase in collagen deposition in comparison of (c) control group.

Table 2: Effect of leaf extract of *Dipteracanthus patulus* in dead space wound model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wet weight of granulation tissue (mg)</th>
<th>Dry weight of granulation tissue (mg)</th>
<th>Tensile strength (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% CMC, 2ml, P.O)</td>
<td>223.73±13.6</td>
<td>32.8±2.4</td>
<td>328±21.6</td>
</tr>
<tr>
<td>Extract (100 mg/kg, P.O)</td>
<td>442.6±27.3*</td>
<td>83.2±7.3*</td>
<td>537.3±41.5*</td>
</tr>
<tr>
<td>Extract (20 mg/kg, P.O)</td>
<td>505.8±39.2*</td>
<td>112.6±9.5*</td>
<td>626±47.4*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ±SE, n = 6; *P* < 0.001 Vs control by student’s t test.
Discussion

Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state. Wound healing involves different phases such as contraction, epithelization, granulation and collagenation. Dead space wound provides an opportunity to study the effect on granulation and collagenation of the healing process (12). In the present study, the methanolic extract has promoted wound healing activity by increasing cellular proliferation and formation of granulation tissue. The granulation tissue of the wound is primarily composed of fibroblast, collagen and new blood vessels. Increase in granulation tissue weight in the extract treated animals suggests increase in collagen synthesis (13). The collagen is the major component of extracellular tissue which gives support and strength. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands. In the present study increase in the rate of wound contraction in extract treated groups may be due to the enhanced activity of fibroblasts. Lignan glycosides namely, 5, 5’-dimethoxy-lariciresinol-9-O-β-D-glucopyranoside and lyoniresinol-9-O-β-D-glucopyranoside were isolated from the methanol extract of the entire plant of *Dipteracanthus patulus* (8). The lignans, lignan glycosides and triterpenoids isolated from *Alocasia odora* showed a stimulation of cell proliferation thus contributing to wound healing activity of that plant (14). The wound healing potential of the methanolic extract of the leaves of *Dipteracanthus patulus* may be attributed to the phytochemical constituents such as flavonoids, saponins, steroids, phenols, tannins and lignan glycosides present in it. Pharmacological activity of many herbal medicines may often be the result of many secondary metabolites acting synergistically (15). This study provides the scientific basis to the traditional uses of *Dipteracanthus patulus* for wound healing. This herb is a promising wound healing promoter worthy of further studies and clinical evaluation.

Acknowledgements

One of the authors (K.S.) wishes to thank the Management, Sri Parasakthi College for Women (Autonomous), Courtallam–627 802, Tamil Nadu and University Grants Commission, New Delhi for financial assistance under Faculty Improvement Programme during the Tenth Plan period and Dr.A.Jaswanth, Department of Pharmacology, Periyar College of Pharmacy, Trichy for providing facilities to carryout this work.
References

Excision wound model:

Excision wound was inflicted by cutting away approximately 500 mm², full thickness of skin from the depilated area on the back under light ether anaesthesia and were placed in their individual cages. The animals were divided into four groups (n=6). Group 1 was the control group that received simple ointment base. Group 2 was treated with standard drug (0.2% w/w nitrofurazone). Group 3 received 5% (w/w) extract ointment. Group 4 received 10% (w/w) extract ointment. The ointments (0.5 g each) were applied topically with a fine brush once daily till the wound was completely healed. Wound contraction rate was monitored by planimetric measurement of the wound by tracing the wound margin on a graph paper every alternate day. Wound contraction was calculated as percentage of original wound size.

Dead space wound model:

Three groups of rats (n=6) were used. Dead space wounds were made by implanting subcutaneously a polypropylene tube (2.5 x 0.5 cm) beneath the dorsal paravertebral lumbar skin. Group 1 was the control group that received 2 ml of 1% carbamazepine solution. Group 2 received ethanolic extract (100 mg kg bw) and group 3 received extract (200 mg kg bw) orally, once daily for 10 days. On the 11th day, the granulation tissue formed on the implanted tubes was dissected out carefully. The wet weight of the granulation tissue was noted. Tensile strength of the granulation tissue was measured by the method of Lee. The granulation tissue was dried in a hot air oven at 60°C for 12 hours and the dry weight was recorded.

Statistical analysis:

Data are expressed as mean ± SEM and subjected to Student’s ’t’ test by comparing with the control.

RESULTS

Phytochemical screening:

Qualitative analysis of phytochemicals of the Ethanolic extract of A. fruticosa showed the presence of flavonoids, phenols, saponins, steroids and tannins.

Table 1. Effect of extract of Acalypha fruticosa on Excision wound contraction

<table>
<thead>
<tr>
<th>Post-wounding days</th>
<th>Wound Area (mm²) (%)</th>
<th>Simple ointment (control)</th>
<th>Nitrofurazone ointment</th>
<th>Extract ointment (5%)</th>
<th>Extract ointment (10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>529±31.6(0%)</td>
<td>529±31.6(0%)</td>
<td>531±26.2(0%)</td>
<td>529±30.0(0%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>309±14.2(60.0%)</td>
<td>418±14.5(60.0%)</td>
<td>384±16.2(60.0%)</td>
<td>256±13.9(60.0%)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>291±13.4(60.0%)</td>
<td>384±16.2(60.0%)</td>
<td>265±13.9(60.0%)</td>
<td>202±12.6(60.0%)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>345±14.2(70.0%)</td>
<td>384±16.2(60.0%)</td>
<td>314±13.9(60.0%)</td>
<td>356±13.9(60.0%)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>378±18.6(77.70%)</td>
<td>384±16.2(60.0%)</td>
<td>378±18.6(77.70%)</td>
<td>356±13.9(60.0%)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>432±26.4(80.0%)</td>
<td>384±16.2(60.0%)</td>
<td>432±26.4(80.0%)</td>
<td>356±13.9(60.0%)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>406±13.9(80.0%)</td>
<td>384±16.2(60.0%)</td>
<td>406±13.9(80.0%)</td>
<td>356±13.9(60.0%)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>378±18.6(77.70%)</td>
<td>384±16.2(60.0%)</td>
<td>378±18.6(77.70%)</td>
<td>356±13.9(60.0%)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>353±14.2(70.0%)</td>
<td>384±16.2(60.0%)</td>
<td>353±14.2(70.0%)</td>
<td>356±13.9(60.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. n=6; p values versus respective control by Student’s t-test. *p<0.01, **p<0.001
its antioxidant properties. Open wounds are particularly prone to infection by bacteria. Infected wounds heal less rapidly. Extracts of aerial parts of A. fruticosa were active against Staphylococcus aureus and multiresistant Staphylococcus epidermidis. The synergistic effect of both antimicrobial and antioxidant properties may accelerate the wound healing process.

The results of this study seem to confirm the traditional use of A. fruticosa for the treatment of wounds.

CONCLUSION

The results of the present study provide pharmacological evidence on the folklore use of Acalypha fruticosa for the treatment of wounds. Efforts are being made to isolate the phytochemicals and subject them for wound healing activities.

ACKNOWLEDGEMENT

One of the authors (K.S.) wishes to thank the Management, Sri Parakkath College for Women (Autonomous), Courtlallam—627 802, Tamil Nadu and University Grants Commission, New Delhi for financial assistance under Faculty Improvement Programme during the Tenth Plan period and Dr.A.Jaswanth, Department of Pharmacology, SRM College of Pharmacy, Trichy for providing facilities to carryout this work.

REFERENCES

5. Moragamadoula KG, Materia Medica (Vegetable Part) Section 1, 6th edition Directorate of Sylode System of Medicine, Madras, 1983, 361.
11. Durgapriya V, Ayusaran M, Ignacimuthu S, Antimicrobial activity of some ethnomedicinal plants used by Palliyur tribe from Tamil Nadu India, BINC Complementary and Alternative Medicine, 6, 2006, 35 pp 11.1056/j.1478-6822.6-34.

Source of support: Nil, Conflict of interest: None Declared

Table 2. Effect of extract of Acalypha fruticosa on Dead Space Wound in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wet weight of granulation tissue (mg)</th>
<th>Dry weight of granulation tissue (mg)</th>
<th>Tensile strength (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.3±6.13 ±6</td>
<td>32.8±2.4</td>
<td>302±21.6</td>
</tr>
<tr>
<td>Ethanolic extract (100mg/ kg bw)</td>
<td>45.8±4.0*</td>
<td>51.8±2.5*</td>
<td>340±23.5*</td>
</tr>
<tr>
<td>Ethanolic extract (200mg/ kg bw)</td>
<td>51.2±7.46*</td>
<td>51.8±2.5*</td>
<td>649±34.9*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ±SEM, n=6. Values versus control by Student's t-test. *p<0.001.

Excision wound
The progress of wound healing induced by the ethanolic extract ointments (5% and 10% w/w), simple ointment (control) and nitrofurazone (standard drug) in animals are shown in Table 1. Wound contraction progressed faster in the ethanolic extract treated groups than that of the control. The 10% (w/w) ethanolic extract ointment treated groups showed significant increase in wound healing from the fourth day onwards. The wound closure time was lesser with 10% (w/w) ethanolic extract ointment (18 days for 100% closure). Five percent extract ointment treatment resulted in 100% closure on the 20th day which was similar to that of the nitrofurazone treatment.

Dead space wound
In the dead space wound model, significant increase in wet and dry weight and tensile strength of the granulation tissue were observed in the animals treated with the ethanolic extract (Table 2). Increases in wet and dry weight and tensile strength of the granulation tissue were found to be dose dependent and a dose of 200 mg/kg bw was more effective.

DISCUSSION

Wounds are physical injuries that result in an opening or break of the skin. Proper healing of wounds is essential for the restoration of anatomical continuity and disturbed functional status of the skin. Wound healing involves different phases such as contraction, epithelization, granulation and collagenation.

Wound contraction is a factor, which indicates the rate of reduction of unhealed area during the course of treatment. Greater the reduction better is the efficacy of medication. In other words, the wound will close at fast rate if the medication is more effective. In the present study, the ethanolic extract ointment treatment of excision wounds resulted in significant increase in the rate of wound contraction. Dead space wound provides an opportunity to study the effect on granulation and collagenation of the healing process. The granulation tissue of the wound is primarily composed of fibroblast, collagen and new blood vessels. The significant increase in the weight of the granulation tissue in the extract treated animals suggests an increase in collagen synthesis. Tensile strength is the strength of a healing wound and it is measured experimentally by the amount of force required to disrupt it. Generally wound healing agents have the properties to enhance the proliferation of fibroblasts. Fibroblasts secrete collagen. Deposition of collagen provides strength to the tissue. Tensile strength increases rapidly as collagen deposition increases and cross-linkages are formed between the collagen fibers. In the present study, the ethanolic extract treatment resulted in significant increase in the tensile strength of the granulation tissue which may be due to the collagen deposition. The wound healing activity of the ethanolic extract of A. fruticosa may be attributed to the synergistic action of phytochemical constituents such as flavonoids, saponins, steroids, phenols and tannins present in it. Flavonoids and phenols have been reported to have antioxidant activity. Excess of proteases and reactive oxygen species (ROS) are often formed by neutrophils and phytochemical constituents such as flavonoids, saponins, steroids, phenols and tannins present in it. Flavonoids and phenols have been reported to have antioxidant activity. Excess of proteases and reactive oxygen species (ROS) are often formed by neutrophils accumulated in the wound area. Fibroblasts and other cells may be killed by excess ROS. Antioxidants counter the excess proteases and reactive oxygen species (ROS) and protect these enzymes from oxidative damage. Because of these factors, the overall antioxidant effects appear to be important in the successful treatment of wounds. Studies on Acalypha fruticosa have revealed its antioxidant properties. Open wounds are particularly prone to infection by bacteria. Infected wounds heal less rapidly. Extracts of aerial parts of A. fruticosa were active against Staphylococcus aureus and multiresistant Staphylococcus epidermidis. The synergistic effect of both antimicrobial and antioxidant properties may accelerate the wound healing process.

The results of this study seem to confirm the traditional use of A. fruticosa for the treatment of wounds. The synergistic effect of both antimicrobial and antioxidant properties may accelerate the wound healing process. The results of this study seem to confirm the traditional use of A. fruticosa for the treatment of wounds.
Chemical investigation of aerial parts of Acalypha fruticosa forssk

Subbarayan Gopalakrishnan1*, Krishnasami Saroja2 and Jeyaseelan Dulcy Elizabeth3

1*Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India
2Department of Botany, Sri Parasakthi College for Women, Courtallam, Tamil Nadu, India
3Department of Botany, St. Mary’s College, Tuticorin, Tamil Nadu, India

ABSTRACT

Acalypha fruticosa Forssk. [Family Euphorbiaceae] commonly known as ‘Chinnichedi’ and ‘Birch-leaved Acalypha’ is a strong smelling bushy shrub traditionally used to treat dyspepsia, stomachache, skin diseases, wounds and poisonous bites. In the present study the aerial parts of Acalypha fruticosa were analysed for phytochemicals and minerals. Qualitative analysis of phytochemicals of the various extracts of the aerial parts of Acalypha fruticosa indicated the presence of triterpenoids, steroids, saponins, tannins, phenols, flavonoids, alkaloids, anthraquinones and sugars. Quantitative estimation of phytoconstituents in the powdered samples of Acalypha fruticosa showed that flavonoids were present in high amount when compared to alkaloids, tannins, phenols and steroids. 1, 2- Benzenedicarboxylic acid diisooctyl ester, n-Hexadecanoic acid, 9, 12-octadecadienoic acid [z, z], α-D-glucopyranoside and eicosyltrichlorosilane were identified by Gas Chromatogram-Mass spectrometry [GC-MS] analysis of the extracts. Potassium, sodium, calcium, magnesium, sulphur, zinc, copper, iron, manganese, boron and molybdenum were estimated using atomic absorption spectrophotometer. Phytochemicals and minerals analysed in the present study may account for the medicinal properties of Acalypha fruticosa.

Keywords: Acalypha fruticosa, phytochemicals, minerals, GC-MS, atomic absorption spectrophotometer

INTRODUCTION

Acalypha fruticosa Forssk. [Family Euphorbiaceae] commonly known as ‘Chinnichedi’ and ‘Birch-leaved Acalypha’ is a strong smelling bushy shrub. Acalypha fruticosa is used to treat dyspepsia, stomachache, skin diseases, wounds and poisonous bites. [1-7]. In Yemen, leaf and stem have been used to treat skin diseases, malaria and wound [8]. In Tanzania, it is used to treat fungal infections and a leaf decoction is drunk to treat epilepsy. A leaf infusion is taken to treat stomach problems and swellings of the body. Leaf maceration is used in eye infections. Leaf sap is used as nose drops to treat cough and chest problems. Leaf paste is applied to scabies and
sores. Stems ground in water are applied to wounds of animals [9]. Several pharmacological studies have revealed its antidiarrhoeal [10], antioxidant, anti-inflammatory [11], anticancer [12], antiplasmodial [13], wound healing [14] and cytotoxic properties [15]. However there are no reports on the detailed chemical investigation of this potent medicinal plant. The present study was undertaken to analyze the phytochemicals and minerals present in the aerial parts of *Acalypha fruticosa*.

**MATERIALS AND METHODS**

**Plant material**
Aerial parts of *Acalypha fruticosa* were collected from Courtallam hills, Western Ghats of South India, Tamil Nadu. The plant was identified by Dr. V. Chelladurai, Research officer (Botany), Survey of Medicinal and Aromatic Plants Unit–Siddha, CCRAS, Palayamkottai, Tirunelveli District, Tamil Nadu, India. A voucher specimen (MSU-38) has been kept in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

**Preparation of powder and extract**
The aerial parts were shade-dried and pulverized to powder in a mechanical grinder. The powder (1kg) was successively extracted with various solvents such as petroleum ether (40°-60°C), chloroform, ethanol and water. The extracts were concentrated under reduced pressure in a rotary evaporator (Buchi, USA). The powder and extracts of the plant were used for phytochemical studies.

**Qualitative phytochemical analysis**
The qualitative phytochemical tests for steroids, reducing sugars, triterpenoids, alkaloids, phenolic compounds, flavonoids, saponins, tannins and anthraquinones were carried out on the concentrated extracts using the standard procedures to identify the constituents as described by Brinda *et al.* [16].

**Quantitative estimation of phytoconstituents**
Quantitative estimation of phytoconstituents like alkaloids [17], flavonoids [18], tannins and phenols [19], saponins [20] and steroids [17] were carried out in the powdered samples of *Acalypha fruticosa*.

**Isolation and characterization of chemical compounds by GC-MS analysis**
The fraction of the extract of petroleum ether (40°-60°C) and the ethanolic extract of *Acalypha fruticosa* were subjected to Gas Chromatogram- Mass spectrometry (GC-MS) analysis.

GC-MS analysis of the extracts was carried out on a GC-MS Clarus 500 Perkin Elmer system comprising a AOC- 20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30mm x 0.25mm ID x 1 μMdf, composed of 100 % Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99. 999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 μl was employed (split ratio of 10:1); injector temperature 250 °C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5 °C / min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da. Interpretation on mass spectra of GC-MS was conducted using the database of National Institute of Standards and Technology (NIST). The mass spectrum of the

www.scholarsresearchlibrary.com
unknown component was compared with that of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

**Estimation of mineral elements**

The amount of potassium, sodium, calcium, magnesium, sulphur, zinc, copper, iron, manganese, boron and molybdenum in the powdered sample was quantitatively estimated using atomic absorption spectrophotometer (Solaar AA series, Atomic Absorption Spectrophotometer).

**RESULTS AND DISCUSSION**

The qualitative analysis of the phytochemicals of the various extracts of the aerial parts of *Acalypha fruticosa* indicated the presence of triterpenoids, steroids, saponins, tannins, phenols, alkaloids, flavonoids, anthraquinones and sugars in *Acalypha fruticosa* (Table 1). Quantitative estimation of phytoconstituents present in the powdered samples of *Acalypha fruticosa* showed that flavonoids were present in high amount when compared to alkaloids, tannins, phenols and steroids (Table 2). These phytoconstituents are known to show medicinal activity [21]. Soladoye et al. [22] reported the presence of alkaloids, tannins, saponins, and cardenolides in *A. fimbriata, A. hispida, A. ornata, A. racemosa* and *A. wilkesiana*. The presence of terpenoids and flavonoids in *A. fruticosa* is confirmed with the reports of Mothana et al. [12].

GC-MS chromatogram of the ethanolic extract of *A. fruticosa* (Figure-1) showed three peaks indicating the presence of three compounds. The chemical compounds in the ethanolic extract of *Acalypha fruticosa* are presented in Table 3, with their retention time (RT), molecular formula, molecular weight and peak area (%). The compounds identified were 1, 2-Benzene dicarboxylic acid diisooctyl ester, n-Hexadecanoic acid and 9, 12-Octadecadienoic acid [a, z]. GC-MS chromatogram of the fraction of the extract of petroleum ether (40°-60°C) showed 8 peaks (Figure-2), of which, two peaks (peak-6 and peak-8) were prominent. When the mass spectra of these two peaks/compounds were compared with those of the compiled data for known compounds, peak with Retention time 12.29 was found to be identical with α-D-glucopyranoside and the peak with Retention time 13.85 was identified as Eicosyltrichlorosilane. Presence of the anti-oxidant compounds like n-Hexadecanoic acid and 9, 12-Octadecadienoic acid [23] may possibly play a role in curing skin diseases.

Table 4 shows the results of quantitative estimation of minerals in the dried powder of *Acalypha fruticosa*. The concentration of macro elements (K, Na, Ca, Mg and S) ranged from 0.01% to 4.23% and that of the microelements (Zn, Cu, Fe, Mn, Bo and Mo) ranged from 0.02 ppm to 87.62 ppm. Of the macro elements analyzed, calcium was present in high amount followed by magnesium and potassium. Among the minor elements, iron and manganese were present in higher concentrations. Minerals are essential for the normal functioning of muscles, heart, nerves and in the maintenance of body fluid composition. Therapeutic role of certain medicinal plant materials has been correlated with the presence of specific elements in their composition. Pereira and Felcman [24] analyzed the concentration of five minerals, viz. silicon, manganese, iron, copper and zinc in sixteen medicinal plants which were used in wound healing to study their possible role in the healing processes. Mineral composition of *Acalypha wilkesiana* was investigated by Ikewuchi and Ikewuchi [25]. Topical zinc-containing treatments, have improved healing of wounds [26]. Magnesium is a cofactor for many enzymatic reactions including collagen synthesis. Copper is a cofactor in protein synthesis and is essential for wound healing. Iron is required for hydroxylation of proline and lysine, both the amino acids are essential for
collagen synthesis [27]. The presence of magnesium, copper, iron, manganese and zinc in *A. fruticosa* may be responsible for its wound healing activity.

CONCLUSION

In the present study the result on the analysis of phytochemicals showed the presence of bioactive compounds. 1, 2-Benzenedicarboxylic acid diisoctyl ester, n-Hexadecanoic acid, 9, 12-Octadecadienoic acid (z, z), α-D-glucopyranoside and Eicosytrichlorosilane were identified by Gas Chromatogram-Mass spectrometry (GC-MS) analysis of the extracts. Substantial amount of macroelements and microelements were present in the aerial parts of *Acalypha fruticosa*.

Acknowledgement

One of the authors (K.S.) wishes to thank the Management, Sri Parasakthi College for Women (Autonomous), Courtallam–627 802, Tamil Nadu and University Grants Commission, New Delhi for financial assistance under Faculty Improvement Programme during the Tenth Plan period.

Table 1: Qualitative phytochemical analysis of the extracts of *Acalypha fruticosa*

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Phytochemicals</th>
<th>Petroleum ether (40-60°C)</th>
<th>Chloroform</th>
<th>Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Triterpenoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Steroids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Anthraquinones</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Sugars</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2: Quantitative analysis of phytoconstituents in the powder of aerial parts of *Acalypha fruticosa*

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Name of the phytoconstituents</th>
<th>Amount (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>0.36</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>1.19</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>0.56</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>0.30</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>0.06</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Table 3: Chemical constituents of the ethanolic extract of *Acalypha fruticosa* [GC-MS]

<table>
<thead>
<tr>
<th>Compound number</th>
<th>Retention Time</th>
<th>Name of the Compound</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Peak Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.16</td>
<td>n-Hexadecanoic acid</td>
<td>C_{16}H_{32}O_2</td>
<td>256</td>
<td>5.45</td>
</tr>
<tr>
<td>2</td>
<td>18.74</td>
<td>9,12-Octadecadienoic acid (z,z)</td>
<td>C_{18}H_{32}O_2</td>
<td>280</td>
<td>3.33</td>
</tr>
<tr>
<td>3</td>
<td>24.70</td>
<td>1,2-Benzene dicarboxylic acid, diisooyctyl ester</td>
<td>C_{24}H_{38}O_4</td>
<td>390</td>
<td>91.23</td>
</tr>
</tbody>
</table>

Table 4: Estimation of minerals in the aerial parts of *Acalypha fruticosa*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the minerals</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Potassium</td>
<td>2.87%</td>
</tr>
<tr>
<td>2</td>
<td>Sodium</td>
<td>0.01%</td>
</tr>
<tr>
<td>3</td>
<td>Calcium</td>
<td>4.23%</td>
</tr>
<tr>
<td>4</td>
<td>Magnesium</td>
<td>3.16%</td>
</tr>
<tr>
<td>5</td>
<td>Sulphur</td>
<td>0.59%</td>
</tr>
<tr>
<td>6</td>
<td>Zinc</td>
<td>7.65ppm</td>
</tr>
<tr>
<td>7</td>
<td>Copper</td>
<td>0.46 ppm</td>
</tr>
<tr>
<td>8</td>
<td>Iron</td>
<td>87.62 ppm</td>
</tr>
<tr>
<td>9</td>
<td>Manganese</td>
<td>59.16 ppm</td>
</tr>
<tr>
<td>10</td>
<td>Boron</td>
<td>0.92 ppm</td>
</tr>
<tr>
<td>11</td>
<td>Molybdenum</td>
<td>0.02 ppm</td>
</tr>
</tbody>
</table>

Figure 1. GC-MS Chromatogram of the ethanolic extract of aerial parts of *Acalypha fruticosa*
Figure 2. GC-MS Chromatogram of the fraction of petroleum ether extract of Acalypha fruticosa

REFERENCES


