Evaluation of anti-bacterial activity of Dashapushpam in the form of Ghritham

Sreedevy K\textsuperscript{1}, Praseetha P.K\textsuperscript{2}

\textsuperscript{1}Research Scholar, Department of Nano Technology, Noorul Islam Center for Higher Education, Kumaracoil, Thucklay, Kanyakumari - 629 180, Tamil Nadu, India
\textsuperscript{2}Department of Nano Technology, Noorul Islam Center for Higher Education, Kumaracoil, Thucklay, Kanyakumari - 629 180, Tamil Nadu, India

\textbf{ABSTRACT}

The state Kerala in India is famous for its plant resource both culturally and medicinally. There is a cluster of ten sacred medicinal plants commonly known as Dashapushpam. These herbs are of great importance in the cold rainy season. Each plant of this group possesses many medicinal values. There are many formulations using these herbs. Ancient people knew the value of using these herbs in a cluster, so they included them in their diet to improve immunity in the monsoon season. There are many Ayurvedic texts which mention the uses of these sacred herbs. Formulations that use all the members of Dashapushpam are rare. The Dasapushpagritham is one such formulation taken from the text vishavaidhya jyostnika. The present work intends to evaluate the anti-bacterial property of Dashapushpam when used in an Ayurvedic formulation known as Dasapushpa Ghritham. The bacterial strains used as the test micro-organisms for the study was \textit{Pseudomonas aeruginosa} and \textit{Bacillus cereus}. The method of the anti-bacterial evaluation was done through agar-gel diffusion technique. The samples including the plant extracts possessed a varying level of anti-bacterial activity against these two bacteria, and their values obtained were compared with standard antibiotic amoxicillin. Based on these results, it was concluded that the anti-bacterial property of the Ayurvedic drug Dasapushpa Ghritham has significant value when compared to the drug base and other individual plant extracts. Hence the present study proves the significant usage of the Dashapushpam plants in various therapeutics used as an anti-microbial agent.

\textbf{INTRODUCTION}

Pandemic diseases have always shattered the economic and healthy stability of society. Most of the pandemics are caused by microbial infections. The occurrence of such pandemics has increased in the present-day world. The prolonged usage of synthetic antibiotics helped the infectious micro-organisms to develop resistance towards them (Natarajan \textit{et al.}, 2010; Raj \textit{et al.}, 2013). This increased the efforts towards the production of Ayurvedic drugs from natural origin. These Ayurvedic formulations have fewer side effects and thus preferred as safe (Sayeed \textit{et al.}, 2006). Dashapushpam constitute a group of ten var-
ious plants namely *Emilia sonchifolia* (L) DC (Dash et al., 2015; Sophia et al., 2012), *Aerva lanata* (L) Juss (Payal et al., 2015; Indira, 2015), *Eclipta alba* (L) Hassk (Singh et al., 2014; Jaglan et al., 2013), *Cardiospermum halicacabum* (Linn.) (Stalin et al., 2013; Suresh et al., 2012; Raza, 2013), *Biophyrum sensitivum* (L) DC (Saritha and Brindha, 2015; Pawar and Vyawahare, 2014), *Evolvulus alsinoides* (Linn.) Linnv (Singh, 2008; Anbarasu et al., 2016), *Cynodon dactylon* (Pers.) (Pandey et al., 2016; Das et al., 2013), *Ipomoea sepriaria* Roxb. (Sayani et al., 2012), *Curculigo orchioides Gaertn* (Irshad et al., 2006), *Vernonia cinerea* L (Varghese et al., 2010; Prabha, 2015). They are famous for their different medicinal features such as antihelmintic, anti-diabetic, antioxidant, hepatoprotective, anti-diarrheal, antimicrobial activity, anticancer, anti-inflammatory, antitumor and immune-modulatory (Bitasta and Madan, 2016; Mini et al., 2010). The different medicinal properties of these plants can be summarised as in Table 1 and Table 2.

The current work is intended to analyse the antibacterial property of the individual aqueous extract of the plants and their combinational formulation as the drug Dasapushpa Ghritham when tested against *Bacillus cereus* and *Pseudomonas aeruginosa*. The Ayurvedic formulation of Dasapushpa Ghritham is from “visha vaidhya jyostnika” which is an old text in Ayurveda used in ancient times. The method of preparation of the drug is given in the sixth chapter of the text, which deals with the viper venom and non-healing ulcers.

The content of the drug is mainly classified into two parts that are the paste of plant parts known as Kalkkam and the fresh juice of ten sacred plants known as Swarasam. The paste of Kalkkam is prepared by using the various parts of plants.

**Micro-organisms used**

Test organisms used were Gram-negative bacteria as *Pseudomonas aeruginosa*, and a gram-positive bacterium was *Bacillus cereus*.

**Anti-bacterial activity**

Agar-gel diffusion technique was done to analyse the anti-bacterial property of the plant extract samples. Medium for bacterial culture was nutrient agar plates. On the 20ml solidified nutrient agar, 1ml of each bacterial suspension was inoculated by spread plate method. Each plate had 6mm diameter wells cut out in it. The samples, positive control (Amoxicillin 30μg/ml), negative control (sterile distilled water) was added in the first, second and third well respectively. 20μl of the plant extract (0.5 g/ml) was added into the first well of one gram-positive and one gram-negative plate as the sample. The Dasapushpa Ghritham (Ayurvedic drug) and the base (Ayurvedic drug without the plant extract) was added as the sample to the *Pseudomonas aeruginosa* and *Bacillus cereus* cultured nutrient agar plates with a positive and negative control. After inoculation, the incubation of the plates was done at 37°C for 2-3 days. Inhibition zone was formed, and the diameter was measured around the sample well and the positive control.

**RESULTS AND DISCUSSION**

The anti-bacterial assay results obtained from the agar-gel diffusion technique was given in Table 3. The Ayurvedic drug Dasapushpa Ghritham has a diameter of 1.54cm in inhibition zone with the gram-positive bacteria *Bacillus cereus* and 1.74cm with the gram-negative bacteria *Pseudomonas aeruginosa*, as shown in Figure 1. The Ayurvedic drug base without the Dashapushpam plant extracts also showed some anti-bacterial property. The Dasapushpa Ghritham base gave a 0.8cm diameter of inhibition zone with the two test organisms, as shown in Figure 2. The individual plants coming under the group of Dashapushpam shows the varying range of values for the anti-bacterial assay done with *Pseudomonas aerug-
inosa and Bacillus cereus, which is compared in a graphical representation in Figure 3.

The positive control used was the standard drug amoxicillin which has a diameter of 1.75cm in inhibition zone with Gram-negative bacteria Pseudomonasaeruginosa and 1.85cm diameter of inhibition zone with the Gram-positive bacteria Bacillus cereus. Negative control (distilled water) used did not give a zone of inhibition. Ipomea sepiaria from the Dashapushpam family was the sample which gave the highest value for the anti-bacterial assay with a zone of inhibition of 1.85cm diameter for both the test organisms. Thus this result is equivalent to that of the standard drug (positive control) amoxicillin. Evolvulus alsinoides was the sample that gave the least value with the test organisms with the diameter of 1cm and 1.24cm each. The rest of the members of the Dashapushpam family have the antibacterial assay results ranging between these values.

**CONCLUSION**

After performing the anti-bacterial assay, the results prove that the members of the Dashapushpam, when used in a combination as a drug, shows significant anti-bacterial activity than when used as an individual plant extract. The Ayurvedic drug shows the anti-bacterial activity as that of the positive control antibiotic amoxicillin in the case of Pseudomonas aeruginosa. More studies are required to understand the therapeutic potential of the drug Dasapushpa Ghritham. Another important aspect of this work is that it encourages the use of a combinational formulation of the ten sacred plants (Dashapushpam) for many other therapeutic drugs. Using the plant extracts as a medical remedy has always been of great interest. The present study also proves the efficiency of using Ayurvedic herbs in medicinal formulations which can be used against disease-causing micro-organisms. A natural drug for microbial infection always has an excellent acceptance in the world of modern medicine.

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**Conflict of Interest**

We declare that we have no conflict of interest for this study.

**REFERENCES**

Anbarasu, R., Selvan, G., Baskar, S., Raja, V. 2016. Pharmacological Potential of Silver Nanoparticles (AgNPs) derived from Evolvulus alsinoides. *International Journal of Recent Research and Applied Studies*, 3(5):30–38.

Bitasta, M., Madan, S. 2016. Aerva lanata: A blessing of Mother Nature. *Journal of Pharmacognosy and Phytochemistry*, 5(1):92–101.

Das, M. C., Shilpi, S., Chandra, S. 2013. Overview of Cynodon Dactylon (Doob Grass) in Modern
### Table 1: Plant-based studies

| S. No | The botanical name of the herb | Anti-bacterial activity | Antioxidant activity | Hepato protective activity | Antitumor activity | Diuretic activity | Antipyretic activity |
|-------|--------------------------------|-------------------------|---------------------|---------------------------|-------------------|------------------|---------------------|
| 1     | Aerva lanata (L) Juss.        | +                       | +                   | +                         | +                 | +                | +                   |
| 2     | Biophytum sensitivum (L.) DC. | +                       | +                   | +                         | +                 | +                | +                   |
| 3     | Cardiospermum halicacabum (Linn.) | +                   | +                   |                           | +                 | +                | +                   |
| 4     | Curculigo orchioides Gaertn.  |                         |                     |                           |                   |                  |                     |
| 5     | Cynodon dactylon (Pers.)      | +                       | +                   |                           | +                 | +                | +                   |
| 6     | Eclipta alba (L.) Hassk.      | +                       | +                   |                           | +                 |                  |                     |
| 7     | Emilia sonchifolia (L.) DC.   | +                       | +                   |                           | +                 |                  | +                   |
| 8     | Evolulus alsinoides (Linn.)   |                         | +                   |                           |                   |                  |                     |
| 9     | Ipomoea sepiaria Ro+b.        | +                       |                     |                           |                   |                  | +                   |
| 10    | Vernonia cinerea L.           | +                       | +                   |                           | +                 |                  | +                   |

### Table 2: Plant-based studies

| S. No | The botanical name of the herb | Anti-inflammato activity | Antifungal activity | Anticancer | Anti-diabetic | Wound Healing |
|-------|--------------------------------|--------------------------|---------------------|------------|---------------|---------------|
| 1     | Aerva lanata (L) Juss.        | +                        | +                   | +          | +             |               |
| 2     | Biophytum sensitivum (L.) DC. | +                        | +                   | +          | +             | +             |
| 3     | Cardiospermum halicacabum (Linn.) | +            | +                   | +          | +             | +             |
| 4     | Curculigo orchioides Gaertn.  |                         | +                   | +          |               |               |
| 5     | Cynodon dactylon (Pers.)      | +                        | +                   | +          | +             | +             |
| 6     | Eclipta alba (L.) Hassk.      |                         | +                   | +          | +             |               |
| 7     | Emilia sonchifolia (L.) DC.   | +                        |                     | +          | +             | +             |
| 8     | Evolulus alsinoides (Linn.)   |                         | +                   | +          |               |               |
| 9     | Ipomoea sepiaria Ro+b.        | +                        |                     | +          | +             | +             |
| 10    | Vernonia cinerea L.           | +                        | +                   | +          | +             | +             |
### Table 3: Individual Dashapushpam plants Vs Dashapushpa-Ghritham its Antibacterial activity

| S. No | Scientific Name   | Bacteria | The diameter of the Zone of inhibition |
|-------|-------------------|----------|----------------------------------------|
|       |                   |          | Sample | Positive control | Negative control |
| 1     | Aerva laneta      | Gm -ve   | 1.65cm | 1.75cm | 0 |
|       |                   | Gm +ve   | 1.31cm | 1.85cm | 0 |
| 2     | Biophytum sensitivum | Gm -ve | 1.21cm | 1.75cm | 0 |
|       |                   | Gm +ve   | 1.32cm | 1.85cm | 0 |
| 3     | Cardiospermum halicabum | Gm -ve | 1.12cm | 1.75cm | 0 |
|       |                   | Gm +ve   | 1.43cm | 1.85cm | 0 |
| 4     | Curculigo orchoid | Gm -ve   | 1.12cm | 1.75cm | 0 |
|       |                   | Gm +ve   | 1.25cm | 1.85cm | 0 |
| 5     | Cynodon dactylon | Gm -ve   | 1.23cm | 1.75cm | 0 |
|       |                   | Gm +ve   | 1.25cm | 1.85cm | 0 |
| 6     | Eclipta alba      | Gm -ve   | 1.12cm | 1.75cm | 0 |
|       |                   | Gm +ve   | 1.42cm | 1.85cm | 0 |
| 7     | Emilia sonchifolia | Gm -ve | 1.53cm | 1.75cm | 0 |
|       |                   | Gm +ve   | 1.53cm | 1.85cm | 0 |
| 8     | Evvolulus alsinoides | Gm -ve | 1cm    | 1.75cm | 0 |
|       |                   | Gm +ve   | 1.24cm | 1.85cm | 0 |
| 9     | Ipomea sepiaria   | Gm -ve   | 1.85cm | 1.75cm | 0 |
|       |                   | Gm +ve   | 1.85cm | 1.85cm | 0 |
| 10    | Vernonia cineirea | Gm -ve   | 1.35cm | 1.75cm | 0 |
|       |                   | Gm +ve   | 1.64cm | 1.85cm | 0 |
| 11    | Dashapushpam      | Gm -ve   | 1.34cm | 1.75cm | 0 |
|       |                   | Gm +ve   | 1.54cm | 1.85cm | 0 |
| 12    | Dashapushpa Ghritham | Gm -ve | 1.74cm | 1.75cm | 0 |
|       |                   | Gm +ve   | 1.54cm | 1.85cm | 0 |
| 13    | Base              | Gm -ve   | 0.8cm  | 1.75cm | 0 |
|       |                   | Gm +ve   | 0.8cm  | 1.85cm | 0 |
bial Activities of Extracts of Launaea procumbens Roxb. (Labiateae). *African Journal of Biomedical Research*, 9:89–93.

Pawar, A. T., Vyawahare, N. S. 2014. phytochemical and pharmacological profile of biophytum sensitivum (L) dc. *Int J Pharm Pharm Sci*, 6(11):18–22.

Payal, C., Gurlaganjeet, K., et al. 2015. A Review on Phytochemistry and Biological Activities of Aerva. *Medicinal & Aromatic Plants*, 04(02):1–4.

Prabha, L. 2015. Therapeutic Uses of Vernonia cinerea - A Short Review. *International Journal of Pharmaceutical and Clinical Research*, 7(4):323–325.

Raj, A., Shailaja, G. R., U, Prasanna, R., Ajayan, N., S 2013. The therapeutic potential of ten sacred plants (dashapushpam) of kerala state of southern india”. *Journal of Ayurveda and Holistic Medicine*, 1(3):1–15.

Raza, A. 2013. Review of beneficial and remedial aspects of Cardiospermum halicacabum L. *African Journal of Pharmacy and Pharmacology*, 7(48):3026–3033.

Saritha, P. B., Brindha 2015. Wound healing potential of Biophytum sensitivum (L.) DC.: An ayurvedic drug. *Journal of Chemical and Pharmaceutical Research*, 7(3):87–94.

Sayani, M., Ashok, B. K., Nishteswar, K. 2012. phytochemical and antifungal studies on root of ipomoea sepiaria koenig ex. Roxb. *Global Journal of Research on Medicinal Plants & Indigenous Medicine (GJRMI)*, 1(8):372–380.

Sayeed, M. A., Al-Bari, M. A. A., Rahman, M. 2006. Characterization and antimicrobial activities of extracts in a phenolic acid derivative produced by Streptomyces bangladeshiensis, a novel species collected in Bangladesh”. Res. *Research Journal of Medicine and Medical Sciences*, 1(2):77–81.

Singh, A. 2008. Review of Ethnomedicinal Uses and Pharmacology of Evolvulus alsinoides Linn. *Ethnobotanical Leaflets*, 12:734–740.

Singh, A., Singh, A., Dwivedi, V. 2014. Antidiabetic effect of Eclipta alba. *International Journal Of Scientific & Engineering Research*, 5(2):1462–1466.

Sophia, D., Kanniaapan, V., Ragavendran, P., Raj, C. A., Gopalakrishnan 2012. Antioxidant properties of Emilia sonchifolia(L): An in vitro study. *Journal of pharmacy research*, 5(2):1162–1164.

Stalin, C., Vivekanandan, Bhavya, E. 2013. In Vitro Antidiabetic Activity of Cardiospermum Halicacabum leaves Extracts. *Global Journal of Medical Research*, 13(7):41–43.

Suresh, S. N., Rathishkumar, S., Rajeshwari, V, Sagadevan, P, Gayathri, S., Eswari, D. V. 2012. Phytochemical analysis and antibacterial potential of Cardiospermum halicacabum Linn. *Int. J. of Pharm. & Life Sci. (IJPLS)*, 3(12):2209–2212.

Varghese, J., Anila, K. J., Nagalekshmi, R., Sonu, J., Resiya, S. 2010. Dasapushpam: The Traditional Uses And The Therapeutic Potential Of Ten Sacred Plants Of Kerala State In India. *International Journal of Pharmaceutical Sciences and Research*, 1(10):50–59.