Abstract: Dashapushpam is a group of ten sacred plants famous for its cultural and medicinal properties and it is used by the people of Kerala, specially during the monsoon season. This study is to demonstrate the anti bacterial activity of the ten herbs when used as a drug called dashapushpa ghritham. The anti bacterial activity was evaluated against the bacterial strains pseudomonas aerugenosa and bacillus cereus by agar-gel diffusion method. All the extract showed various level of activity for this test organisms and their activity is compared with the standard antibiotic. This study shows that the drug has significant activity than the base and individual plant extract. This result encourages the studies for the significant usage of the ten herbs in various medicines used as anti-infective agent.

Keywords— Dashapushpa ghritham, antibacterial activity, agar-gel diffusion method.

I. INTRODUCTION

Microbial infections are always a threat to the mankind [1,2,3]. The frequency and the novelty of such infections have increased in the recent years worldwide. The infectious microorganisms developed resistance towards the synthetic antibiotic due to their prolonged usage [4,5,6]. This marks the significance of developing natural drugs using plant extracts. Such medicinal formulations are also free from side effects and safe to use due to their organic origin [7,8,9]. Dashapushpam are a cluster of ten different herbs namely Aerva lanata (L) Juss, Biophytum sensitivum(L.) DC, Cardiospermum halicacabum (Linn.), Curculigo orchioides Gaertn, Cynodon dactylon (Pers.), Eclipta alba (L.) Hassk., Emilia sonchifolia (L.) DC, Evolvulus alsinoides (Linn.) Linnv, Ipomoea sepriaria Roxb., Vernonia cinerea L [10,11,12,13,14]. They have various medicinal properties including anti-diabetic, anti-cancer, anti-tumor, hepatoprotective, immunomodulatory, anti-diarrheal, anti-helminthic, anti-inflammatory, antioxidant and anti-microbial activity [15,16,17,18]. The different properties of dashapushpam plants are shown in table 1 [19,20,21,22]. The present study is to investigate the anti bacterial activity of these plants extract and the drug dashapushpa ghritham when tested against gram positive and gram negative bacteria [23,24,25]. Dashapurshpa ghritham is a drug formulation from an ancient ayurvedic text “visha vaidhya jyostnika”. The drug contains the aqueous extract of ten sacred plants with the base like ghee, sandal etc [26,27,28,29,30].

II. MATERIALS AND METHODS

A. PLANT MATERIAL

The ten sacred plants dashapushpam was collected from various parts of malappuram district, Kerala, India. The plants were washed in tap water, shade dried and powdered.

B. EXTRACTION PREPARATION

The plants were cleaned and shade dried. 50g of powdered sample was added to 300ml distilled water and heated for 15 min with continuous stirring. Then the extract was cooled at room temperature for 24hrs.

Anti Bacterial Activity of Dashapushpa Ghritham

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Table 1: Plant based studies

| Sl no | Botanical name of the herb | Anti-bacterial activity | Antioxidant activity | Hepatoprotective activity | Antitumour activity | Diuretic activity | Antipyretic activity | Anti-inflammatory activity | Antifungal activity | Anti-Cancer | Anti-Diabetic | Wound Healing | Ref: |
|-------|---------------------------|-------------------------|----------------------|---------------------------|---------------------|------------------|----------------------|----------------------------|---------------------|-------------|--------------|---------------|----------|
| 1     | Aerva lanata (L) Juss.    | +                       | +                    | +                         | +                   | +                | +                    | +                          | +                   | +           | +            | +             | 4, 9, 10  |
| 2     | Biophytmum sensitivum (L) DC. | +                       | +                    | +                         | +                   | +                | +                    | +                          | +                   | +           | +            | +             | 1, 11, 12 |
| 3     | Cardiospermum halicacabum (Linn.) | +                       | +                    | +                         | +                   | +                | +                    | +                          | +                   | +           | +            | +             | 13, 14, 15 |
| 4     | Curculigo orchiodes Goerz | +                       | +                    | +                         | +                   | +                | +                    | +                          | +                   | +           | +            | +             | 16       |
| 5     | Cynodon dactylon (Pers.) | +                       | +                    | +                         | +                   | +                |                      | +                          | +                   | +           | +            | +             | 17, 18   |
| 6     | Echta alba (L) Hassk. | +                       | +                    | +                         | +                   | +                |                      | +                          | +                   | +           | +            | +             | 19, 20   |
| 7     | Emilia sonchifolia (L) DC. | +                       | +                    | +                         | +                   | +                | +                    | +                          | +                   | +           | +            | +             | 21, 22   |
| 8     | Evolvulus alnoides (Linn.) | +                       | +                    | +                         | +                   | +                | +                    | +                          | +                   | +           | +            | +             | 23, 24   |
| 9     | Ipomoea sepiumia Ro-b. | +                       | +                    | +                         | +                   | +                | +                    | +                          | +                   | +           | +            | +             | 25, 26   |
| 10    | Vernonia cinerea L.       | +                       |                      | +                         | +                   | +                |                      | +                          |                      | +           | +            | +             | 27, 28   |

The solution was filtered using whatman filter paper [no.1] using vacuum pump. The filtrate was concentrated at 40°C until solvents evaporated completely. The sample was dissolved in sterile distilled water [6].

C.DRUG PREPARATION
The method of preparation of drug is based on the ayurvedic text Vishavaidhya jyostnika in connection with viper venom and non-healing ulcers.

D.MICRO ORGANISMS USED
Gram negative bacteria used was Pseudomonas aerogenosa and gram positive was bacillus cereus.

E.ANTI BACTERIAL ACTIVITY
Anti-bacterial activity of the plant extract was evaluated by agar-gel diffusion method. Nutrient agar plates were prepared and 1 ml of each bacterial suspension was evenly spread on the solidified
20ml nutrient agar plate. Plate had three well of 6mm diameter cut out in it. Sample was added in the first well, positive control (Amoxillin 30µg/ml) in the second and negative control (sterile distilled water) in the third. 20µl of 0.5g/ml plant extract was added into one gram positive and one gram negative plate. The dashapushpa ghritham was also added to a well on both gram positive and gram negative bacterial culture plate with positive and negative control. The base (drug excluding the plant extract) was also tested in the similar way for its anti-bacterial activity. The plates were incubated at 37°C for 2-3 days. Zone of inhibition was observed around the sample well and the positive control. Diameter of the zone was measured.

III. RESULTS AND DISCUSSION

The results for the antimicrobial activity by agar-gel diffusion method are presented in table 2. All the members of dashapushpam showed various range of antibacterial activity with pseudomonas aerugenosa and bacillus cereus. Positive control amoxillin gave 1.7 cm diameter of inhibition zone with pseudomonas and 1.8 cm diameter of inhibition zone with bacillus cereus. Negative control distilled water does not have any zone of inhibition. Among the individual plant extracts ipomea sepiaria showed maximum anti bacterial activity with zone of inhibition of 1.8cm diameter for both gram negative and gram positive organisms. This value is same as that of the positive control amoxillin. Least activity was observed in evolvulus alsinoides with a diameter of 0.9 cm and 1.2 cm with gram negative and gram positive bacteria respectively. All other plant extracts ranges between these values. The drug dashapushpa ghritham showed 1.5 cm diameter zone of inhibition with gram positive and 1.7 cm with gram negative bacteria. The base of the drug without the plant extract also had some antibacterial activity. The base had 0.7 cm diameter of zone of inhibition with both gram positive and negative organisms.
Anti Bacterial Activity of Dashapushpa Ghritham

Table .2 Antibacterial activity of dashapushpa Ghritham Vs individual

| Sl no | Scientific Name       | Bacteria | Diameter of inhibition Zone |
|-------|-----------------------|----------|----------------------------|
|       |                       |          | Sample | Positive control | Negative control |
| 1     | Aerva laneta          | Gm -ve   | 1.6cm  | 1.7cm            | 0                |
|       |                       | Gm +ve   | 1cm    | 1.8cm            | 0                |
| 2     | Biophytum sensitivum  | Gm -ve   | 1.2cm  | 1.7cm            | 0                |
|       |                       | Gm +ve   | 1.3cm  | 1.8cm            | 0                |
| 3     | Cardiospermum halicabum| Gm -ve | 1.1cm  | 1.7cm            | 0                |
|       |                       | Gm +ve   | 1.4cm  | 1.8cm            | 0                |
| 4     | Curculigo orchid      | Gm -ve   | 1.1cm  | 1.7cm            | 0                |
|       |                       | Gm +ve   | 1.2cm  | 1.8cm            | 0                |
| 5     | Cynodon dactylon      | Gm -ve   | 1.2cm  | 1.7cm            | 0                |
|       |                       | Gm +ve   | 1cm    | 1.8cm            | 0                |
| 6     | Eclipta alba          | Gm -ve   | 1.1cm  | 1.7cm            | 0                |
|       |                       | Gm +ve   | 1.4cm  | 1.8cm            | 0                |
| 7     | Emilia sonchifolia    | Gm -ve   | 1.5cm  | 1.7cm            | 0                |
|       |                       | Gm +ve   | 1.5cm  | 1.8cm            | 0                |
| 8     | Evolvulus alsinoides  | Gm -ve   | 0.9cm  | 1.7cm            | 0                |
|       |                       | Gm +ve   | 1.2cm  | 1.8cm            | 0                |
| 9     | Ipomea sepiaria       | Gm -ve   | 1.8cm  | 1.7cm            | 0                |
|       |                       | Gm +ve   | 1.8cm  | 1.8cm            | 0                |
| 10    | Vernonia cineirea     | Gm -ve   | 1.3cm  | 1.7cm            | 0                |
|       |                       | Gm +ve   | 1.6cm  | 1.8cm            | 0                |
| 11    | Dashapushpam          | Gm -ve   | 1.3cm  | 1.7cm            | 0                |
|       |                       | Gm +ve   | 1.5cm  | 1.8cm            | 0                |
| 12    | Dashapushpa Ghritham  | Gm -ve   | 1.7cm  | 1.7cm            | 0                |
|       |                       | Gm +ve   | 1.5cm  | 1.8cm            | 0                |
| 13    | Base                  | Gm -ve   | 0.7cm  | 1.7cm            | 0                |
|       |                       | Gm +ve   | 0.7cm  | 1.8cm            | 0                |

IV. CONCLUSION

From the above results, it is observed that the ten plant extracts when used together in a drug has significant antibacterial property than most of the other individual plant extracts. In the case of gram negative organisms the drug has similar activity of the positive control antibiotic amoxilin. Further investigations can highlight the potentiality of the drug. Phytochemical studies are required to isolate the type of compound responsible for the antimicrobial activity. This study also encourages the use of dashapushpam for different medicinal formulations. The investigation ensures the use of herbal extracts as therapeutic agents against pathogenic microorganisms. The discovery of natural remedy for bacterial infection would be a great achievement for the modern medicine.
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