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

Sreedevy K^{*1}, Praseetha P.K²

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

²Department of Nano Technology, Noorul Islam Center for Higher Education, Kumaracoil, Thucklay, Kanyakumari - 629 180, Tamil Nadu, India

Article History:	ABSTRACT
Received on: 21 Aug 2020 Revised on: 21 Sep 2020 Accepted on: 22 Sep 2020 <i>Keywords:</i>	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
Dasapushpa Ghritham,	using these herbs in a cluster, so they included them in their diet to improve
anti-bacterial property,	immunity in the monsoon season. There are many Ayurvedic texts which men-
Dashapushpam,	tion the uses of these sacred herbs. Formulations that use all the members
Agargel diffusion	of Dashapushpam are rare. The Dasapushpagritham is one such formulation
technique	taken from the text vishavaidhya jyostnika. The present work intends to eval-
	uate 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 <i>Pseudomonas aeruginosa</i> and <i>Bacil</i> -
	<i>lus cereus.</i> 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 therapeu-
	tics used as an anti-microbial agent.

*Corresponding Author

Name: Sreedevy K Phone: +91 9496758956 Email: sreedevyk@gmail.com

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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 et al., 2010; Raj et al., 2013). This increased the efforts towards the production of Ayurvedic drugs from natural origin. These Avurvedic formulations have fewer side effects and thus preferred as safe (Sayeed et al., 2006).

Dashapushpam constitute a group of ten various 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), Biophytum sensitivum(L.) DC (Saritha and Brindha, 2015; Pawar and Vyawahare, 2014), Evolvulus alsinoides (Linn.) Linnv (Singh, 2008; Anbarasu et al., 2016), Cvnodon dactylon (Pers.) (Pandey et al., 2016; Das et al., 2013). Ipomoea sepiaria Roxb. (Savani 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 antihelminthic, anti-diabetic, antioxidant, hepatoprotective, antidiarrheal, 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 Avurvedic formulation of Dasapushpa Ghritham is from "visha vaidhva jyostnika" which is an old text in Ayurveda used in ancient times. The formulation constitutes the aqueous extract of the Dashapushpam herbs as the actual content and a drug base like ghee, turmeric, sandal etc. (Krishnapriya et al., 2018)

MATERIALS AND METHODS

The Dashapushpam herbs were collected from different parts of Malappuram district, Kerala, India. The plants were first washed in tap water and then dried in the shade and powdered.

Extraction preparation

The Dashapushpam plants were washed in tap water and shade dried. 50g of powdered sample was weighed and taken and added to 300ml distilled water. This solution was heated for 15 min and stirred continuously. The plant extract was then cooled for 24hrs at room temperature. After that, the solution was filtered through a Whatman filter paper [no.1] and vacuum pump. The filtrate obtained was concentrated at 40° c until all the solvents evaporated completely. Then each sample was dissolved in sterile distilled water separately (Parekh and Chanda, 2006).

Drug preparation

The Avurvedic formulation of Dasapushpa Ghritham is taken from the Avurvedic text Vishavaidhva Jvostnika. 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 Kalkam is prepared by using the various parts of plants.

Micro-organisms used

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

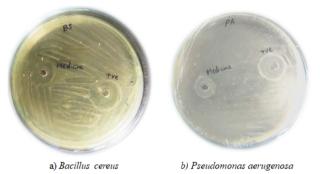
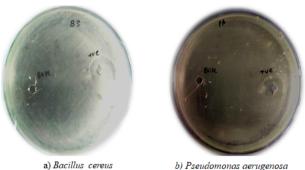


Figure 1: Inhibition zone shown by Dasapushpa Ghritham (Ayurvedic drug) and Positive control amoxilin with gram positive and gram negative bacteria



b) Pseudomonas aerugenosa

Figure 2: Inhibition zone shown by Ayurvedic drug Base and positive control amoxilin with gram positive and gram negative bacteria

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.

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 sensi- tivum (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	Evolvulus alsi- noides (Linn.) Linn.	+	+				
9	Ipomoea sepiaria Ro+b.	+					
10	Vernonia cinerea L.	+	+		+		

Table 1: Plant-based studies

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 sensi- tivum (L.) DC.	+	+		+	+
3	Cardiospermum hali- cacabum (Linn.)	+	+	+	+	
4	Curculigo orchioides Gaertn.			+	+	
5	Cynodon dactylon (Pers.)	+	+			+
6	Eclipta alba (L.) Hassk.	+		+	+	
7	Emilia sonchifolia (L.) DC.	+	+	+	+	
8	Evolvulus alsinoides (Linn.) Linn.					
9	Ipomoea sepiaria Ro+b.		+			
10	Vernonia cinerea L.		+			

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	Gm -ve	1.12cm	1.75cm	0	
	halicabum	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 dactlyon	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	Evolvulus 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	Gm -ve	1.74cm	1.75cm	0	
	Ghritham	Gm +ve	1.54cm	1.85cm	0	
13	Base	Gm -ve	0.8cm	1.75cm	0	
		Gm +ve	0.8cm	1.85cm	0	

Table 3: Individual Dashapushpam plants Vs Dashapushpa-Ghrithamin its Antibacterial activity

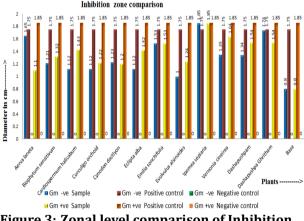


Figure 3: Zonal level comparison of Inhibition zone

The samples, positive control (Amoxicillin 30μ g/ml), negative control (sterile distilled water) was added in the first, second and third well respectively. 20 μ l 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 aeroginosa 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 *Bacilluscereus* and 1.74cm with the gram-negative bacteria *Pseudomonasaeruginosa*, 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 aeruginosa* 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 Bacilluscereus. 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 diseasecausing 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.

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