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DASHAMULARISHTA: PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING AGAINST EFFECT OF ENVIRONMENTAL POLLUTION

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ABSTRACT

Pollution have a serious impact on human health and environment. The incidences of various diseases are becoming prominent with the increase in rate of population. The diseases mainly include respiratory disorders, cardiovascular disorders, throat inflammation, skin infections etc. In the present study, widely claimed crude drug Dashamularishta, have been screened for their antibacterial activity against *Shigella flexneri, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris, Enterobacter aerogenes, Aspergillus niger, Candida albicans, Trichophyton rubrum*. Phytochemical screening and antimicrobial activity of petroleum ether, chloroform, benzene, ethyl acetate and ethanol and distilled water extracts of Dashamularishta have been screened. Phytochemical screening recorded positive results for reducing sugar in all six extracts, terpinoids present in pet. ether, ethyl acetate, methanol and distilled water extract, Flavonoids present in ethyl acetate and distilled water, Tannin present in chloroform and methanol, saponins present in pet. ether, benzene, ethyl acetate and distilled water extract. The results were expressed in terms of the diameter of the inhibition zone: The maximum efficacy of ethyl acetate extract was showed against *Aspergillus niger*(18mm) and *Shigella flexneri* (25mm).

INTRODUCTION: Environmental Pollutants can cause anything and everything- i.e. diseases, including cancer, lupus, immune diseases, allergies, and asthma. Pollution leads different type of skin disorders. Air pollution can be a main cause of pre aging of skin. Dust mist may generate eczema if it comes in contact with skin. Chemicals used in various products (paints, cleaning stuffs, lacquers, adhesives, building materials etc.) can seriously pollute the air. Those agents are entered into our body through the breathing and affect our lungs, eyes, nose, and can create skin allergies. Ozone is the basic element in smog which leads skin cancer. Besides ozone the other reason of skin cancer is harmful UV radiation of sun.

Microbial diseases of the skin are usually transmitted by contact with an infected individual. Although the skin normally provides a barrier to infection, when it is penetrated by microorganisms, infection develops. Diseases of the eye are considered with the skin diseases because both occur at the surface of the body.

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Dashamularishta (DMK) is a classical Ayurvedic preparation. It contains ten plants – *Aegle marmelos* Correa., *Oroxylum indicum* (Linn.) Vent., *Stereospermum suaveolens* Linn., *Premna integrifolia* Linn., *Gmelina arborea* Linn., *Solanum xanthocarpum* Burm. f., *Solanum indicum* Linn., *Desmodium gangeticum* DC., *Uraria lagopoides* DC., *Tribulus terrestris* Linn. In the Ayurvedic system of medicine it is used as analgesic, antiarthritic, against cough, rheumatism, etc [1].

In the present study, widely claimed crude drugs of Dashamularishta used in medicinal system of science, have been screened for their antimicrobial activity against *Shigella flexneri, Staphylococcus aureus , Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris, Enterobacter aerogenes, Aspergillus niger, Candida albicans, Trichophyton rubrum*. The crude drug was the Dashamularishta used as antimicrobial drug. The antibacterial activity of this plant extract was compared with standard antibacterial drug Tetracycline.

MATERIALS AND METHODS:

Collection: Plant sample Dashamularishta was collected from various tribes living in tribal pockets of Mt. Abu, arid zone of Rajasthan, in the month of July, 2009. These plants were used by these tribes in their daily lives to cure various ailments.

Identification: These samples were authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

Sources of test organisms: Bacteria-Pure culture of all test organisms, namely *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Shigella flexneri, Proteus vulgaris, Enterobactor aerogenes* and fungi *Candida albicans, Aspergillus niger,Trichophyton rubrum* were obtained through the courtesy of Mahatma Gandhi Institute of applied Sciences (MGiaS), Jaipur, which were maintained on Nutrient broth media.Culture of test microbes: For the cultivation of bacteria, Nutrient Agar Medium (NAM) was prepared by using 20 g Agar, 5 g Peptone, 3 g beef extract and 3 g NaCl in 1 L distilled water and sterilized at 15 lbs pressure and 121°C temperature for 25-30 min. Agar test plates were prepared pouring approximately 15 ml of NAM into the Petri dishes (10 mm) under aseptic conditions. A saline solution was prepared (by mixing 0.8% NaCl) in distilled water, followed by autoclaving and the bacterial/cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24-48 h. To prepare the test plates, in bacteria, 10-15 ml of the respective medium was poured into the Petri plates and used for screening. For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown Agar slant.

Preparation of Test Extracts: Crushed powders of species were successively soxhlet extracted. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were cooled individually. Each filtrate was concentrated to dryness in vitro and re dissolved in respective solvents, were stored at 4°C in a refrigerator, until screened for antibacterial activity.

Bactericidal Assay: For both, bactericidal in vitro Disc diffusion method was adopted [2], because of reproducibility and precision. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whattman No. 1 paper (6 mm in diameter), which were containing three different concentration, its control (of the respective solvent) and tetracycline as reference drugs (standard disk) separately.

Such treated discs were air-dried at room temperature to remove any residual solvent, which might interfere with the determination, sterilized and inoculated. These plates were initially placed at low temperature for 1 h so as to allow the maximum diffusion of the compounds from the test disc into the agar plate and later, incubated at 37°C for 24 h in case of bacteria, after which the zones of inhibition could be easily observed. Five replicates of each test extract were examined and the mean values were then referred.

The Inhibition Zone (IZ) in each case were recorded and the Activity Index (AI) was calculated as compared with those of their respective standard reference drugs (AI = Inhibition Zone of test sample/Inhibition zone of standard).

RESULTS AND DISCUSSION:

Phytochemical Screening: Phytochemical analysis for Dashamularishta extract was performed and the phyto constituents reported in the **Table 1**. This reveals moderate concentration of reducing sugar, flavonoids, saponins, terpenoids and tannin in different extraction solvents, some of which chemical compounds have been associated to antimicrobial activities and thus have curative properties against selected bacteria and fungi. Standard method were used for preliminary phytochemical screening of the extract was performed to know the phyto-constituents in the extract and it was found that petroleum extract contains reducing sugar, terpenoids and saponins, benzene extract contains reducing sugar, tannin and saponins, chloroform extract contain reducing sugar and tannin, ethyl acetate extract contains reducing sugar, terpenoids, flavonoids and saponins, methanol extract contains reducing sugar, terpenoids and tannin and distilled water extract contains redusing sugar, terpenoids, flavonoids and saponins.

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF THE PLANT DASHAMULARISHTA

PLANTS	Reducing sugar	Terpenoides	Flavonoids	Tannin	Saponin
Extracts					
Pet.ether	+ve	+ve	-ve	-ve	+ve
Benzene	+ve	-ve	-ve	+ve	+ve
Chloroform	+ve	-ve	-ve	+ve	-ve
Ethyl acetate	+ve	+ve	+ve	-ve	+ve
Methanol	+ve	+ve	-ve	+ve	-ve
Distilled water	+ve	+ve	+ve	-ve	+ve

(+: present) (- : absent)

Antimicrobial activity: Antimicrobial activity of extracts from Dashamularishta was tested against *Shigella flexneri, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris, Enterobacter aerogenes, Aspergillus niger, Candida albicans, Trichophyton rubrum* by using the disc diffusion method. The plates containing the micro organisms were then perforated and the disc were placed with 50mg/20disc, 100mg/20disc, 150mg/ 20disc, 200mg/20disc, 250mg/20disc concentrations. Microbial growth was determined by measuring the diameter of zone of inhibition (**table 2**).

TABLE 2: ANTI MICROBIAL ACTIVITY OF THE PETROLEUM ETHER, CHLOROFORM, BENZENE, ETHYL ACETATE AND ETHANOL AND DISTILLED WATER EXTRACTS OF DASHAMULARISHTA

S. No				*Sf*	*Sa*	*St*	*Pv*	*Kp*	*Pa*	*Ea*	*An*	*Ca*	*Tr*
1	**Pet. Ether**	A1	I.Z.	7	7	0	0	0	7	7	7	0	6
			A.I.	.20	.35	0	0	0	.43	.17	.29	0	.21
		A2	I.Z.	8	6	7	0	0	6	7	8	7	7
			A.I.	.22	.30	.23	0	0	.37	.17	.33	.18	.25
2	**Benzene**	A1	I.Z.	7	7	6	0	6	8	12	7	6	12
			A.I.	.20	.35	.20	0	.15	.50	.29	.29	.16	.42
		A2	I.Z.	8	8	8	8	0	8	0	7	9	9
			A.I.	.22	.40	.21	.18	0	.50	0	.29	.24	.32
3	**Chloroform**	A1	I.Z.	12	0	0	9	9	10	9	0	13	9
			A.I.	.34	0	0	.20	.22	.62	.21	0	.35	.32
		A2	I.Z.	10	10	9	12	11	10	0	0	10	10
			A.I.	.28	.50	.30	.27	.27	.62	0	0	.27	.35
		A3	I.Z.	10	0	9	0	11	11	10	0	12	9
			A.I.	.28	0	.30	0	.27	.68	.29	0	.32	.32
4	**Ethyl acetate**	A1	I.Z.	14	14	0	0	13	8	12.66	18	7	0
			A.I.	.40	.70	0	0	.32	.50	.30	.75	.18	0
		A2	I.Z.	25	15.33	0	0	11	18	16	11	12	6
			A.I.	.71	.76	0	0	.25	.12	.39	.45	.32	.21
5	**Eethanol**	A1	I.Z.	9	6	7	0	6	10	7	7	9	7
			A.I.	.25	.30	.23	0	.15	.62	.17	.29	.24	.25

		A2	I.Z.	8	5	6	0	0	0	0	6	7	0
			A.I.	.22	.25	.20	0	0	0	0	.25	.18	0
		A3	I.Z.	8	6	6	0	7	12	8	7	7	8
			A.I	.22	.30	.20	0	.17	.75	.19	.29	.18	.28
		A4	I.Z.	13	7	6	0	7	10	9	7	8	0
			A.I.	.37	.35	.20	0	.17	.62	.21	.29	.21	0
6	**D. Water**	A1	I.Z.	0	0	9	0	0	8	0	8	10	8
			A.I.	0	0	.30	0	0	.50	0	.33	.27	.28
		A2	I.Z.	0	7	9	0	0	8	0	8	10	10
			A.I.	0	.35	.30	0	0	.50	0	.33	.27	.35
		A3	I.Z.	0	0	9	0	0	8	8	8	8	8
			A.I	0	0	.30	0	0	.50	.19	.33	.21	.28
		A4	I.Z.	10	7	9	0	0	8	9	8	11	11
			A.I.	.28	.35	.30	0	0	.50	.21	.33	.29	.39
		A5	I.Z.	0	7	8	0	0	8	0	9	10	12
			A.I.	0	.35	.26	0	0	.50	0	.37	.27	.42

Sf-*Shigella flexneri*, Sa- *Staphylococcus aureus*, St-*Salmonella typhi*, Pv- *Proteus vulgaris*, Kp- *Klebsiella pneumoniae*, Pa- *Pseudomonas aeruginosa*, Ee-*Enterobacter aerogenes*, An- *Aspergillus niger*, Ca-*Candida albicans*, Tr- *Trichophyton rubrum* ; 0 - no inhibition zone.
A1-50 mg/20 disc, A2-100 mg/20 disc, A3-150mg/20 disc, A4-200mg/20 disc, A5-250mg/20 disc. I.Z. - Inhibition zone, I.A. – Activity index.

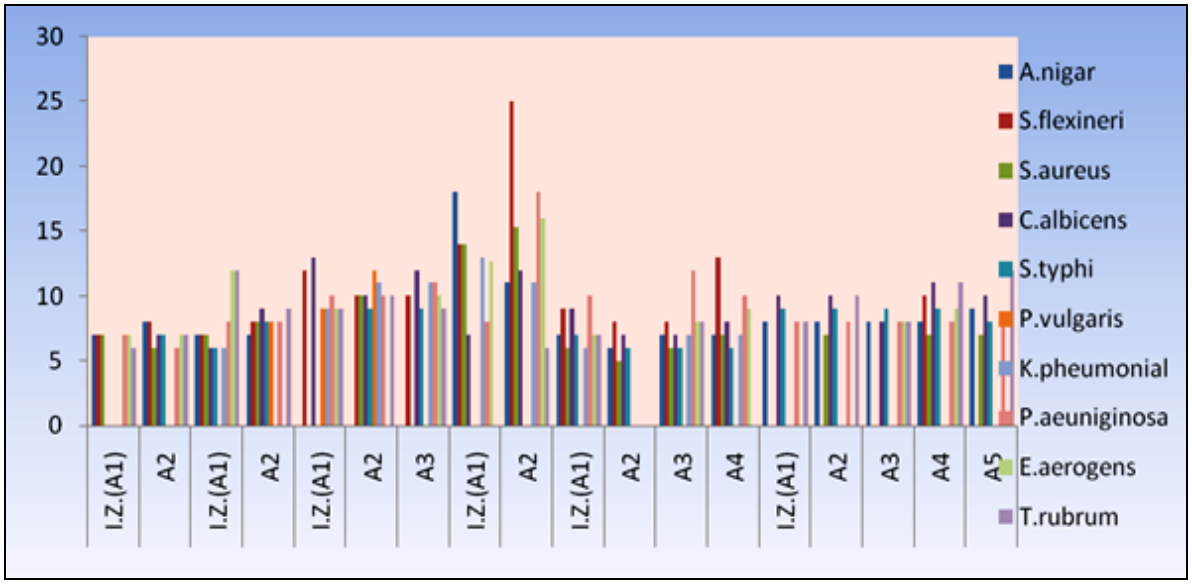

FIG. 1: ANTIBACTERIAL ACTIVITY OF PETROLEUM ETHER, BENZENE, CHLOROFORM, ETHYL ACETATE, ETHANOL AND DISTILLED WATER EXTRACT OF DASHAMULARISHTA.

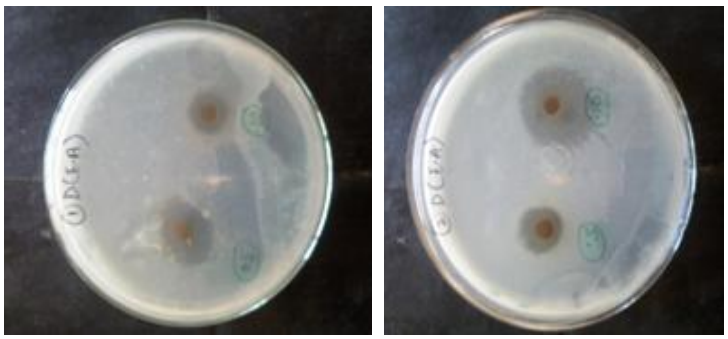

FIG. 2: (A) DASHAMULARISHTA ETHYL ACETATE EXTRACT; (B) DASHAMULARISHTA ETHYL ACETATE EXTRACT ACTIVITY AGAINST *ASPERGILLUS NIGER* ACTIVITY AGAINST *SHIGELLA FLEXNERI*.

The results were expressed in terms of the diameter of the inhibition zone: Antimicrobial efficacy of Dashamularishta extracts was screened against selected test microorganisms. All six extracts was showing appreciable inhibition against all selected test microorganisms (bacteria and fungi). Ethyl acetate extract showed the higest inhibition zone against test microorganisms. The maximum efficacy of ethyl acetate extract (A2-100mg concentration) was against *Shigella flexneri* (I.Z.-25mm), *Pseudomonas aeruginosa* (I.Z.-18mm), and concentration A1 (50mg) was also active against *Aspergillus niger* (I.Z.- 18mm) have been screened.

Brine shrimp (*Artemia salina* Leach) larvae (nauplii) have been used for over thirty years in toxicological studies, and international symposia have been held discussing their biology and potential usefulness [3].The brine shrimp bioassay is also useful in the science of food toxicology [4]. The eggs of brine shrimp, *Artemia salina* Leach, are readily available at low cost in pet shops as a food for tropical fish, and they remain viable for years in the dry state.

Upon being placed in a brine solution, the eggs hatch within 48 hours, providing large numbers of larvae (nauplii). Brine shrimp have been previously utilized in various bioassay systems [5].This bioassay determines the lethalities of materials toward brine shrimp larvae and in doing so predicts the ability to kill cancer cells in cell cultures, kill insects, and exert a wide range of pharmacological effects [3].

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