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RESEARCH ARTICLE

CLINICAL EVALUATION OF VELLARUGU CHOORANAM (*ENICOSTEMA AXILLARE* LAM.) ON HUMAN SKIN INFECTIONS.

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ABSTRACT

Superficial fungal infections of skin affect millions of people throughout the world. The information of medicinal plants has been accumulated in the course of several centuries based on various medicinal systems such as Ayurveda, Homeopathy, Naturopathy, Modern, Siddha and Unani. *Enicostema axillare* is a common Indian medicinal plant belonging to the family Gentinaceae was selected and screened for the phyto chemical compounds, antifungal activity and clinical evaluations. Samples collected from the skin infected patients and the patients were treated with plant drug Vellarugu chooranam (*Enicostema axillare).* The anti fungal activity was studied only on the predominant fungal pathogens *Trichopyton rubrum* and *Trichophyton mentagraphytes*.

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INTRODUCTION

Medicinal plants play a key role in human health care. It has been estimated that 80% world population depend upon herbal based medicines. Even in modern medicine, 25% of the drugs are plant based (Farnsworth *et al.,* 1985). India is rich in natural resourses with varied climatic conditions. It is estimated that about 8000 medicinal plants are used in traditional and folk medicinal practitioners in India (Rajasekaran and Tulsi, 2002). Indian system of medicine is based on herbs and has several hundred herbal remedies; for certain diseases allopathy cannot compete. Skin infections are commonly affects people of all age groups. More over it spread rapidly due to poor hygienic conditions and over populations as well as increasing level of environment pollution. Skin infections may be caused by bacteria, fungi or viruses. Micro organisms break the integrity and enter into dermis, frequently causing skin infections. Plant derived antimicrobial have received considerable attention in recent years. Several plants are indicated folk and other traditional systems of medicines as aseptic agents. The numerous reports have appeared on the antimicrobial activity of plants and their secondary metabolites (Jain and Pathak, 1970; Saxena and Vyas, 1996; Ratha *et al.,* 2003). *Enicostema axillare* Lam. Raynal is an important medicinal plant belonging to the Family Gentianaceae.

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It it hightly valued in Indian system of medicine. Vaithya Mooligai Agarathi compiled by the 18 Siddhars states, Vellarugu (*Enicostema axillare*) used to cure Hernia, Gastritis, Leucorrhoea, Rhematism and Skin diseases (Kandasamy Mudaliyar, 1976). Indian Materia Medica stated that the whole plant is stomachic, bitter tonic, laxative and carminative. The dried plant powder is given with honey in rheumatism, abdominal ulcers, hernia, swellings, itches and insect poisoning (Nadkarni 1954). The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances for other sources including plants (Erdogrul, 2002). The present investigation was undertaken to evaluate the plant drug *Enicostema axillare* Lam. act against superficial skin infections through microbiological and clinical studies.

MATERIALS AND METHODS

Collection of plants: The plant *Enicostema axillare* (Lam) was collected from Orathanadu of Thanjavur District, Tamilnadu, India and the collected plants were carefully examined and identified with the help of Regional Floras (Gamble, 1935; Mathew, 1983). Specimens were further confirmed in the reference to Herbarium sheets available in the Rabinat Herbarium, St. Joseph College, Thiruchirappalli.

Preparation of plant extract: Ethanol extract of the plant was prepared according to the methodology of Indian

pharmacopoeia (Anonymous, 1966). The dried whole plants were powdered and subjected to soxhlet extraction. The extract was concentrated to dryness in flash evaporator under reduced pressure and controlled temperature. The extract was weighed and preserved aseptically at 5ºc. The extract involved in phyto chemical screening of compounds and microbiological studies.

Preparation of chooranam-the test drug: The purified whole plants were shade dried and made into a powder form. The chooranam (fine powder) was prepared as per the standard specification (filtered by white cloth called Vasthirakayam) given in the Siddha literature and stored in air tight container for the clinical study. The chooranam was administered as a single drug.

Phytochemical Screening: The Preliminary phytochemical analysis of the plant extract was performed to screen for the presence of bio-active components present in the plants using the methods described by Kokate *et al.,* (1995).

Collection of Clinical Specimen: The infected skin samples were collected from 41 patients who came to the Out Patient Unit, Department of Siddha Medicine, The Tamil University, Thanjavur. The patients had no prior treatments and taking no drug for their infections. The samples were collected in the month of November 2008 to May 2009. The collected specimens were placed in a sterile container and transported to the department laboratory for further studies.

***In Vitro* Susceptibility Test:** The *in vitro* method proposed by Notional Committee for Clinical Laboratory Standards for testing molds (NCCLS, 1997) was followed for the present study.

Inoculums Preparation: Among the isolated fungal pathogens only the predominant organisms, *Trichopyton rubrum* and *Trichophyton mentagraphytes* were subcultured on to Sabouraud Dextrose Agar (SDA) plates at 29±1ºC. Stock inoculums suspensions of the isolates of both species were prepared from 7-14 day old cultures grown on SDA medium (NCCLS, 1998).

Well Diffusion Assay (Bauer *et al.,* 1996). *In vitro* antifungal activity of the plant extract was assessed by the well diffusion method. The predominant fungal species in the study, *Trichophyton rubrum* and *Trichophyton mentagraphytes* were taken as test organisms. 1ml volume of the inoculums was uniformly seeded on SDA plates consist of 50 µg/ml of chloromphenicol. After 10 minutes, 5 mm sized wells of 3 numbers in each petriplates were made in the help of cork borer. Ethanolic extract of the test drug *Enicostema axillare* at varying concentrations viz., 2000, 4000 and 6000 µg/ml were aseptically loaded in each well in the help of micropipette. Antibiotic for dermatophytes, Griseofulvin at a concentration of 1000 µg/ml as standard control and 1ml ethanol as negative control were loaded in separate control plates. The plates were incubated at 29±1ºc and were examined daily for the appearance of the fungal colony with inhibition zone. The zone to inhibition was calculated by measuring the diameter of the inhibition zone around the well. For each data, averages of three independent determinations were carried out. The zones of diameter were measured in mm and were tabulated.

Clinical Study: Forty one known skin infected patients clinically diagnosed as superficial mycosis were selected for this study from the Out Patient Unit of Siddha Medicine, The Tamil University, Thanjavur. It is a random trial. All the patients were free from other systemic defects and abnormalities. The age groups of patients were ranging from 0-70 years of both sexes. After careful history and clinical examination, the diagnosis was confirmed and the patients were treated with plant drug *Enicostema axillare* powder (chooranam) 1 gram thrice a day with water for three weeks. Among the forty one patients, six were treated with Ganthaga rasayanam, a Siddha drug at a dosage of 1 gm thrice a day with milk as standard control and the remaining were treated with the plant drug *Enicostema axillare* powder. Uniform diet was advised to all the patients. The treatment period was three weeks (21 days). All clinical data viz., name, age, sex, occupation of the patients, sign and symptoms of the disease, isolated organisms , treatment given, review observation and comments were properly recorded (Table 1). The improvement was noted clinically with signs and symptoms. Specific enquires were made to find out the side effects if any. After every examination, the details were properly registered.

RESULTS AND DISCUSSION

The phytochemical analyses revealed the presence of secondary metabolites that the extract was rich in alkaloid. The presence of volatile oils, tannin and phenols, flavonoids and free amino acid were in moderate amount (Table -1). Six infectious fungi were recorded from the skin infected samples of forty one patients. The distribution of isolates among the patients were recorded as *Trichophyton rubrum - 15(36.59%), Trichophyton mentagraphytes - 13(31.71%) Candida albicans - 7(17.07%) Penicillium janthinellum-3(7.32%) Aspergillus flavus - 2(4.88%) and Monilia* Sp - 1(2.44%). Among the six fungal pathogens, *Trichophyton rubrum and Trichophyton mentagraphytes* were the most abundant and considered as predominant dermatophytic species. The *in vitro* antifungal susceptible test revealed that there was a gradual increase in the inhibition zone with increasing concentrations of the test drug. The drug at a concentration of 6000 µg/ml showed a well defined inhibition zone in the test organisms. *T. rubrum* showed a better inhibition zone than *T. mentagraphytes* at all concentration (Table – 2). According to the clinical evaluations among the forty one patients, 11 were male (26.8%), 25 were female (60.98%) and 5 were children (12.2%). The females were mostly affected than male. The different age group of the patients reported in the clinical study. The maximum number of patients were recorded in the age group of 46-60 (34.15%) and the minimum number of patients were observed in the age group above 60 years-5 (12.2%), 1-15 years - 7 (17.1%), 16-30 years - 7 (17.1%) and 31-45 years – 8 (19.5%). The socio-economic status report revealed that 16 were middle class (39.02%), 20 patients were below middle class (48.78%) and 15 were above middle class (36.59%). This revealed that the maximum skin infected patients in the present study were under poor socio – economic status. Among the forty one patients treated, thirty five were given the herbal drug Vellarugu chooranam and the remaining six were given Ganthaga rasayanam. The patients treated with the Vellarugu chooranam, twenty seven patients showed good result (77.1%); five patients (14.3%) recovered moderately and three patients did not attend for review (8.6%). In clinical trial, the herbal drug Vellarugu chooranam (*Enicostema axillare* powder) showed better result than the standard drug Ganthaga Rasayanam and a drastic reduction in symptoms and absence of infectious organisms (Table -3).

Table 1. Qualitative Phytochemical screening of ethanol extract of whole plant of *Enicostema axillare*

Sl.No.	Name of Compounds	Name of the test	Status of the Substance Ethanol Extract
01.	Carbohydrates	i.Fehling's	+
		ii.Benedict's	+
02.	Alkaloids	i.Mayer's	++
		ii.Hager's	-
		iii.Wagner's	+
		iv.Dragendorff's	+++
03.	Steroids	Chloroform + acetic acid + conc. H_2SO_4	+
04.	Tannin & Phenols	i.10% Lead acetate	++
		ii.5% Ferric chloride	++
		iii.1% gelatin	+
		iv.10% NaCl	+
05.	Saponins	Foam test	-
06.	Fixed oils & Fats	Spot test	+
07	Gums & Mucilage	Alcoholic precipitation	++
08	Proteins and Free amino acids	Biuret test	++
09.	Flavonoids	Na OH/HCI	++
10.	Volatile oils	Hydro distillation method	++

Note : +++ Rich amount: ++ Moderate amount : + Minimum : - Absent

Table: 2. *In Vitro* antifungal susceptibility test on ethanol extract of *Enicostema axillare* against two Predomiant dermatophytic fungal isolates *Trichophyton rubrum* and *Trichophyton mentagraphytes*

Sl.No.	Name of the Dermatophytes	Inhibition Zone Diameter (in mm)				
		Standard Control Griseofulvin 1000 µg/ml	Negative Control Ethanol	Test drug 2000 µg/ml	Test drug 4000 µg/ml	Test drug 6000 µg/ml
1.	*Trichophyton rubrum*	33	0	10	13	17
2.	*Tricophyton mentagraphytes*	30	0	9	11	15

Table 3. Clinical data about skin infected patients and their treatment at Out Patients Unit, Dept. of Siddha Medicine, Tamil University, Thanjavur during September 2008- March 2009

Sl. No	Name of the patient	Age / Sex	Occupation	Infected Area	Identified Organism	Signs and symptoms	Drug	Result
1	Sahaya Mary	45/F	Teacher	Both hands, Rt. Wrist	*Trichophyton rubrum*	Itching and scaly lesion	Vellarugu Chooranam 1 gram thrice a day with water	Good
2	Kalyani	55/F	House Wife	Forearms, Rt. arm	*Trichophyton. Mentagrophytes*	Inflammatory region,itching	"	Good
3	Selvi	48/F	Clerk	Both axilla	*Candida albicans*	Small white patches	"	Good
4	Kesavan	59/M	Cooly	Lt. Limb	*Trichophyton Mentagrophytes*	Vesicles,itching	"	Fair
5	Kavitha	32/F	Cooly	Around the hip	*Trichophyton rubrum*	Itching, scaly lesion.	Ganthaga Rasayanam 1 gram thrice a day with milk	Good
6	Nidyanandham	37/M	Business	Both Groin	*Trichophyton rubrum*	Vesicular patches	Ganthaga Rasayanam 1 gram thrice a day with milk	Good
7	Pragadeeswari	19/F	Student	Chest	*Trichophyton rubrum*	Inflammatory region,itching	Ganthaga Rasayanam 1 gram thrice a day with milk	Good
8	Sureshkumar	33/M	Electrician	Nails	*Trichophyton Mentagrophytes*	Itching with pustules	Ganthaga Rasayanam 1 gram thrice a day with milk	Good
9	Nivedha	6/F/c	-	Rt. Hand	*Trichophyton rubrum*	Irritation, inflammation	Ganthaga Rasayanam 1 gram thrice a day with milk	Did not turn up
10	Jyothi	50/F	Cooly	Lt. axilla, anal region	*Monilia spp.*	White scaly patches	Ganthaga Rasayanam 1 gram thrice a day with milk	Fair
11	Chinnappan	47/M	Mosaic Worker	Forehand	*Trichophyton rubrum*	Slight inflammation, itching	Vellarugu Chooranam 1 gram thrice a day with water	Did not Attend for review
12	Subramanian	67/M	Rtd. Teacher	Both Thighs	*Penicillium anthinellum*	Itching	"	Fair
13	Pappathi	50/F	House Wife	Both hands	*Trichophyton rubrum*	White patches,itching	"	Good
14	Kokila	14/F	Student	Hand, both arms	*Trichophyton Mentagrophytes*	Papules,scaling and irritation	Vellarugu Chooranam 1 gram thrice a day with water	Good
15	Subramanian	35/M	Cooly	Chest, Lower abdomen	*Trichophyton Mentagrophytes*	Circular scaly margin with itching	"	Did not Attend for review
16	Meerabai	64/F	House Wife	Around the chest	*Trichophyton. Mentagrophytes*	Erethematous papular region,	"	Good

Continue…

17	Vijayalakshmi	27/F	Sales Girl	Both hands	*Trichophyton Mentagrophytes*	Itching, pustules	"	Good
18	Jaya	35/F	Cooly	Rt. Limb, thigh	*Candida albicans*	White inflammatory patches	"	Good
19	Vairam	50/F	House Wife	Chest	*Trichophyton rubrum*	Vesicular rings, itching	"	Good
20	Senthil	28/M	Painter	Both lower limbs	*Candida albicans*	Crusted patches with itching	"	Fair
21	Meenatchi	40/F	House wife	Both hands	*Aspergillus flavus*	Inflammatory patches, irritation	"	Good
22	Sagunthala	36/F	Business	Rt. Side Wrist	*Trichophyton rubrum*	Browny scaly, itching	"	Good
23	Vembu	60/F	Cooly	Both Lower limbs	*Penicillium janthinellum*	Crusted patches with itching	"	Fair
24	Thangam	60/F	House wife	Chest	*Candida albicans*	Inflammed and erethematous lesion	"	Did not Attend for review
25	Saroja	19/F	Cooly	Rt. Lower limbs	*Trichophyton rubrum*	Itching, macules	"	Good
26	Divya	11/F/c	Student	Both axilla	*Candida albicans*	Crusted papules with itching	"	Good
27	Santhosh	4/M/c	-	Shoulder, hand	*Trichophyton rubrum*	Circular margin with itching	"	Good
28	Govindharaj	48/M	Painter	Neck, hand	*Trichophyton rubrum*	Pustular region and burning sensation	"	Good
29	Suganthi	40/F	Sweeper	Fore arm, nails	*Trichophyton Mentagrophytes*	Thickened, friable nail	"	Fair
30	Jeeva	3/M/c	-	Rt. thigh	*Penicillium janthinellum*	Scaly eczematous region,itching	"	Good
31	Malathi	26/F	Teacher	Both Groins	*Candida albicans*	Red, scaly, eruption	Vellarugu Chooranam 1 gram thrice a day with water	Good
32	Ganesh	5/M/c	-	Forehead	*Aspergillus flavus*	Fine scales, small patches	"	Good
33	Femina	16/F	Student	Both shoulder	*Trichophyton rubrum*	Macules, erethematous border	"	Good
34	Paramasivam	58/M	Cooly	Chest, face	*Trichophyton Mentagrophytes*	Itching and scaly lesion	"	Good
35	Jansi	21/F	Sales girl	Hip region	*Trichophyton Mentagrophytes*	Macules,irritation,	"	Good
36	Vengatesh	10/M	Student	Chest	*Candida albicans*	Itching well defined lesion, finely scaling	"	Good
37	Rajan	54/M	L.I.C. Agent	Both Lower Limbs	*Trichophyton Mentagrophytes*	Vesicles and eruption, itching	"	Good
38	Vijayalakshmi	21/F	House wife	Finger, nails	*Trichophyton Mentagrophytes*	Inflammatory region, furrowed nails	"	Good
39	Jamuna	39/F	Constable	Neck, Chest	*Trichophyton rubrum*	Red macules with itching	"	Good
40	Selvaraj	49/M	Mechanic	Both Groin	*Candida albicans*	Brownish scaly patches,	"	Fair
41	Rukmani	55/F	Cooly	Lt. Forearm	*Trichophyton Mentagrophytes*	Itching and eczematous lesion	"	Good

Note: M - Male : F - Female : c - Child

Due to the presence of alkaloid, it strengthens the skin, increase the concentration of antioxidants in wounds and restore inflamed tissues by increasing blood supply. Griensen and Afolyan (1999) and Geda and Bokoidia (1978) stated that the presence of volatile oils and flavonoids were associated in the antimicrobial property. The growth of many fungi, yeast, bacteria and viruses were inhibited by tannin (Chung *et al.,* 1998). Enemur and Amedu (2009) on their survey on the superficial mycoses in 2184 school children in Nigeria revealed that among eight dermatoplytic fungi recovered, *Trichophyton rubrum* was the most abundant species and occupied 30.3%. The same type of result was observed in the present investigation that *Trichophyton rubrum* was the most abundant 36.59%. From the results obtained it was apparent that the ethanolic extract of *Enicostema axillare* at 6000 µg/ml concentration was the most effective as widest inhibitory zone were observed. According to Prescott *et al.,* (2002) the activity of antimicrobial agent is concentration dependent. The antimicrobial activity of the study plant depends upon the phytochemical potentiality and which lead to control, reduce or prevent the microbial population. This result supports the use of this plant in traditional system of medicine for the treatment of infectious skin diseases and the potential against dermatophytes increasing the knowledge on finding new drugs.

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