



## *In vitro* antifungal activity of plants collected from Salem

Harivignesh TS, Gowthaman G, Jothi Nantha G, Udayanithi N, Sampath kumar B, Karthik L\*, Shree devi MS

Department of Gunapadam and Microbiology, Sivaraj Siddha Medical College, Salem, TN, India. \*For correspondence: lkarthik2006@yahoo.co.in

Article Info: Received 12 Sep 2014; Revised: 24 Nov 2015; Accepted 28 Nov 2015.

### ABSTRACT

The objective of the study was focused on the antifungal activity of herbal plants obtained from Salem, Tamil Nadu. The collected fresh leaves of *Cassia alata*, *Cynodon dactylon*, *Ocimum tenuiflorum*, *Curcuma aromatica* and *Hydnocarpus laurifolia* were made into an ointment after standard processing. The ointment showed significant activity against the fungi *Aspergillus niger*. The aqueous extract of *Cassia alata* showed maximum relative percentage inhibition against *Aspergillus niger* (13±1mm). Minimum inhibitory concentration was performed by modified agar well diffusion method on Potato Dextrose Broth and Potato Dextrose Agar. Minimum inhibitory concentration values of aqueous extract of *Cassia alata* varied from 25-100 mg/ml. This result obtained here suggests that the extract bear antifungal metabolites and considered as the potential source for antifungal drug development.

**Keywords:** *Cassia alata*, *Cynodon dactylon*, *Ocimum tenuiflorum*, anti-fungal activity.

### 1. INTRODUCTION

Plants are rich in medicinal properties since ancient times. Hence, these plants and plant derived products are used as a medicine in traditional and folk medicinal system. These medicinal plants are initially prepared in the form of dried powder, gums and aqueous extracts of more than one product. Eventhough they have less side effects, a lot of processing is required to develop a drug from the natural sources [1].

*Cassia alata* is also known as *Senna alata* or Vandukolli which belongs to the family Fabaceae.

The inflorescence looks like a yellow candle. It is also called Ringworm bush because of its effective fungicidal properties, for treating ringworm and other fungal infections of the skin [2]. *Ocimum tenuiflorum* (Thulsi) is an aromatic compound belongs to the family 'Lamiaceae' which is native to the Indian Sub-continent and widespread as cultivated plant throughout the Southeast Asian tropics. It is also known as holy basil. It acts as germicidal, fungicidal, anti-bacterial and resolving fevers [1].

*Cynodon dactylon* (Arugampul) is also known as durva grass, dhoob, Bermuda grass, dubo, dog's tooth

grass, grama, couch grass and devil's grass. It belongs to the family 'Poaceae' [2]. It is used to treat skin infections. *Curcuma aromatica* (Wild turmeric) belongs to the family 'Zingiberaceae'. The species is found in the South Asian regions, predominantly in eastern Himalayas and in the warm forests of the Western Ghats (India). It has rhizomes with a peculiar fragrance and attractive deep yellow color. The rhizomes are often used in cosmetic herbal medicines and as a culinary ingredient in limited quantities as a food flavor. It is used as anti-inflammatory agent, to promote blood circulation and for the treatment of cancer [3].

*Hydnocarpus laurifolia* is also known as Garudaphal. Among Kerala Ayurveda specialists and therapists, *Hydnocarpus laurifolia* is locally called as Marotti. In forest area and reserved forests Kerala, People also call *Hydnocarpus laurifolia* plant as Neerutti [4]. It is used to cure styptic, wound healer, musculo-skeletal disorder, abscess, boils, snake bite, sudation therapy, herpes, fumigation in wounds, wounded patient ward and epilepsy [5].

The *Aspergilli* have always been a factor in the human environment. Micheli was the first to distinguish stalks and spore heads, but it was not until the middle of 19<sup>th</sup> century that these fungi began to be recognized as active in decay processes, as causes of human and animal diseases and as fermenting agents capable of producing valuable metabolic products [6]. The *Aspergilli* is less susceptible to antifungal emerge. This has launched a new phase in handling *Aspergillo*sis. Resistance is currently reported in Belgium, Canada, China, Denmark, France, Norway, Spain, Sweden, The Netherlands, UK and the USA. The reason for drug resistance lies in the fact that the periodical giving of drugs to the patients decreases the power of the drugs but increases the resistance of the fungi [7]. Due to drug resistance in the *Aspergilli*, many researches are going on to find the alternative drug for the disease caused by *Aspergilli*. Hence, the current study focused on screening of antifungal activity of medicinal plants collected from Salem, TN, India.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and media

Potato Dextrose Broth and Potato Dextrose Agar were purchased from Himedia Pvt. Ltd, Mumbai, India.

### 2.2. Plant materials

*Cassia alata* (Seemaiagathi), *Cynodon dactylon*  
*In vitro* antifungal activity of aqueous extract of *Cassia alata*

(Arugampul), *Ocimum tenuiflorum* (Thulsi), *Curcuma aromatica* (Wild turmeric) and *Hydnocarpus laurifolia* (Neeradimuthu) were collected from the foot hills of Kanjamalai (latitude: 11°37'0"N and longitude: 78°4'0"E) (Fig 1), Salem, Tamil Nadu, India, during November 2014.



Figure 1. Kanjamalai (source:<http://www.google.co.in/imgres>)

### 2.3. Processing of plants

The leaves of freshly collected above stated plant materials (5gm each) were washed thoroughly in tap water and followed by distilled water. The leaves were dried in the shadow at room temperature for three days and powdered.

### 2.4. Preparation of ointment

The powdered leaves were boiled with 100ml of distilled water. Then the boiled herbal extracts were mixed with equal amount of coconut oil and heated until all the water gets vaporized. Finally it was mixed with *Themmelugu* (Bee-wax) to get solidified.

### 2.5. Antifungal assay

Antifungal activity of the crude extracts was determined by agar well diffusion method. All test organisms were inoculated in PDB for 12hours. Isolates were seeded on PDA plates by using sterilizing cotton swabs. Agar surface was bored by using sterilized gel borer to make wells. 100µl of test extract and 100µl of distilled water (negative control) were poured into separate wells. The standard antibiotics disk was placed on the agar surface as positive control. Plates were incubated at 28°C for 72 hours. All experiments was performed in triplicates.

### 2.6. Determination of minimum inhibitory concentration (MIC)

MIC of the plant extract was performed by modified agar well diffusion method. Two fold serial dilution of the stock solution was prepared in sterilized

distilled water to make a concentration range from 20-100 mg/ml.

Test cultures were inoculated in PDA and PDB for fungi '*Aspergillus niger*'. Microbial suspensions were seeded on PDA for fungi using a sterilized cotton swab. In each of these plates, two wells were cut out using a standard cork borer (7 mm). Using a micropipette, 100  $\mu$ l of each dilution was added into the wells. All plates were incubated at 28°C for 48 hours. The minimum concentration of each extract showing a clear zone of inhibition was considered to be MIC [8].

### 2.7. Statistical analysis

The anti fungal activity was performed triplicates. Data were analyzed and values between groups were analyzed using one way ANOVA using Graph Pad Prism (version 6.0.1, USA). All values are shown as mean  $\pm$  standard deviation. Results were concluded significant at  $p < 0.05$ .

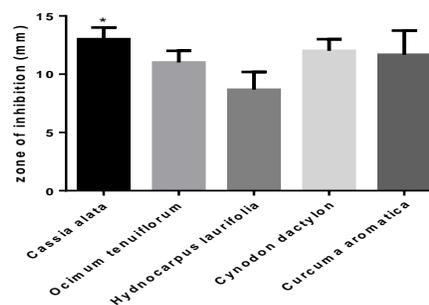
## 3. RESULTS AND DISCUSSION

Fungal infections are one of the major threats to human population. These fungal infections may cause rashes, allergies and some skin diseases. Limitations of the existing drugs lead scientists to develop new drugs from alternative sources such as algae, plants, etc. Hence in this study, we screened *Cassia alata* (Semaiagathi) and *Cynodon dactylon* (Arugampul) for the anti-fungal activity. In this study, aqueous extract of the leaves of *Cassia alata*, *Cynodon dactylon*, *Ocimum tenuiflorum*, *Curcuma aromatica* and *Hydnocarpus laurifolia* were screened for anti-fungal activity and treated against *Aspergillus niger*. Based on the result, the anti-fungal ointment is prepared. The extract exhibits excellent anti-fungal activity. Among these, *Cassia alata* showed a maximum range of zone of inhibition ( $13 \pm 1$ mm) and *Hydnocarpus laurifolia* showed a minimum range of zone of inhibition ( $8.667 \pm 1.5$ mm) (Fig 2).

The choice of this plant for the present study was based on its medicinal properties and use in traditional medicinal system. The aqueous extract of the leaves of *Cassia alata* is used for the treatment of several fungal diseases like itches, rashes, skin diseases, etc. Timothy et al reported *Cassia alata* is an important medicinal plant with several medicinal uses in traditional and folk medicinal system. But our aqueous extract of the combinations of *Cassia alata*, *Ocimum tenuiflorum*, *Hydnocarpus laurifolia*, *Cynodon dactylon* and *Curcuma aromatica* which tested against *Aspergillus niger* was found to be more

*In vitro* antifungal activity of aqueous extract of *Cassia alata*

effective than the aqueous extract of *Cassia alata*.



**Figure 2.** Antifungal activity of aqueous extract of *Cassia alata*, *Cynodon dactylon*, *Ocimum tenuiflorum*, *Curcuma aromatica* and *Hydnocarpus laurifolia*.

The choice of this plant for the present study was based on its medicinal properties and use in traditional medicinal system. The aqueous extract of the leaves of *Cassia alata* is used for the treatment of several fungal diseases like itches, rashes, skin diseases, etc. Timothy et al reported *Cassia alata* is an important medicinal plant with several medicinal uses in traditional and folk medicinal system. But our aqueous extract of the combinations of *Cassia alata*, *Ocimum tenuiflorum*, *Hydnocarpus laurifolia*, *Cynodon dactylon* and *Curcuma aromatica* which tested against *Aspergillus niger* was found to be more effective than the aqueous extract of *Cassia alata*. The combination of herbal plants, Mixture of *Cassia alata* (Semaiagathi), *Cynodon dactylon* (Arugampul), *Ocimum tenuiflorum* (Thulsi), *Curcuma aromatica* (Wild turmeric) and *Hydnocarpus laurifolia* (Neeradimuthu) 5gm each correspond to activity of  $26 \pm 1$  mm.

The above discussed plants are collected from the foothills of Kanjamalai which is located near Salem, Tamil Nadu, India. Kanjamalai is enriched with iron content. So these plants in the mountains have greater medicinal power than the plants found in other parts of the country [9].

The word 'Kanjam' refers to Gold, Copper and Iron and malai means hill. It is said that Paranthaka Chozha who built Sri Nataraja temple with golden roof, took the gold from this mountain. Also the water falls seen in this hill is called "Ponni Nadhi" [10].

#### 4. CONCLUSION

It is concluded that the fungi *Aspergillus niger* was inhibited by the aqueous extract of the leaves of *Cassia alata*, *Cynodon dactylon*, *Ocimum tenuiflorum*, *Curcuma aromatica* and *Hydnocarpus laurifolia* and this is found to be more effective and could be used as effective agent to inhibit these microbes.

#### Acknowledgement

We thank our God and special thanks for our parents who supported to make this project and our sincere thanks to the management of Sivaraj Siddha Medical College for giving this golden opportunity.

#### Conflict of Interest

The authors declare that they have no conflicts of interest.

#### References

1. Staples, George, Michael S. Kristiansen (1999). Ethnic Culinary herbs, University of Hawaii Press. P. 73. ISBN 978-0-8248-2094-7
2. Farsani TM, Etemadi N, Sayed-tabatabaei BE, Talebi M. Assessment of Genetic Diversity of Bermudagrass (*Cynodon dactylon*) Using ISSR Markers. *Int J Molecular Science* (2012). 13(1):383-92.
3. Shi JH, Li CZ, Liu DL. Experimental research on the pharmacology of *Curcuma aromatica* volatile oil. *Zhong Yao Tong Bao*. 1981; 6:36-8.
4. Medicinal plants in India. URL:<http://indianmediplants.blogspot.in/2009/08/hydnocarpus-laurifolia.html>
5. [http://parisaramahiti.kar.nic.in/Medicinal\\_plants\\_new/med%20plants/p116.html/](http://parisaramahiti.kar.nic.in/Medicinal_plants_new/med%20plants/p116.html/)
6. Tekaiia F and Latge JP. Current Opinion in Microbiology. Host microbe interactions-fungi, Edited by Howard Bussey; pp: 385-392.
7. Desselberger V, (2000). Emerging and re-emerging infectious diseases. *J. Infection and Disinfection*,40: 3-15.
8. Okunji CO et al. An antifungal saponin from fruit pulp of *Dracaena manni*. *International Journal of Crude Drug Research*. 28 (3); 1990: 193-199.
9. Allow Salem steel plants to access iron ore deposits in Kanjamalai, "THE HINDU", Dec.12, 2008

10. Gavanagar Muzhakkam magazine. Issued on Sunday, June 19, 2011. Available from the URL: <http://www.kalanganathar.blogspot.in>