ANTIFUNGAL ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *Senna alata* L. LEAVES ON FUNGI THAT CAUSE RINGWORM DISEASE

ISSN: 2141 – 3290
www.wojast.com

[1]BASSEY, I. N., [2]ASAMUDO, N. U.,
[3]UDOINYANG E. P AND [1]EDET, U. A.
[1]Department of Botany and Ecological Studies,
University of Uyo, Uyo, Nigeria.
[2]Department of Microbiology, University of Uyo, Uyo.
[3]Department of Zoology,
University of Uyo
basseyinemesit17@yahoo.com

ABSTRACT

Antifungal effect of aqueous and ethanolic extracts of leaves of *Senna alata* L. on *Microsporum canis* (fungus isolated from ringworm infected human) were studied. This study showed that the effect was concentration dependent in both aqueous and ethanolic extracts at 2mls of each concentration. Ethanolic extract had the highest inhibitory effect (56%) on the growth of *Microsporum canis* at 15% concentration on second day of incubation; fourth day recorded the lowest (38%) while the sixth day had 44%. At 10% concentration highest inhibition was recorded on the second day (38%) and the lowest inhibition was on the fourth and sixth day at 26% each. At 5% concentration the lowest inhibition (8%) was recorded on the second day of incubation.Aqueous extract of *Senna alata* had the least effect on fungi when compared to ethanolic axtract. At 15% concentration of aqueous extract there was no significant difference (0.05) between the effect on second and sixth day with 34 and 36% inhibition. At 10% concentration on the second, fourth and sixth day of incubation it recorded 17, 16 and 17% inhibition respectively. It recorded below 10% inhibition in all days of incubation at 5% concentration. Generally, highest inhibition was recorded on the second day of incubation for both extracts. The study has justified the traditional use of this plant as an antifungal agent.

INTRODUCTION

Cassia alata L, is synonymous with *Senna alata* L. (Globinmed, 2010), and so the names can be used interchangeably. The plants are shrubs of the family Fabaceae (formerly Leguminosae) (Evans, 2002). It is a pantropical ornamental shrub (Abubacker *et al.,* 2008), which grows well in the forest areas of West Africa (Owoyale *et al.,* 2005)and are grown as ornamental plants with diverse medicinal uses (Timothy *et al.,* 2012). Evans (2002) reported that *S. alata* is indigenous to tropical Africa.

It is commonly known as "Rai dore" (Hausa) "Asuwon oyinbo" (Yoruba), "Omirima" (Igbo) (Timothy *et al*., 2012), and Nkimeyo (Ibibio). However, there are some common names given to this plant, such as Candlestick, Candlebush, Christmas candle (Stephen, 2011).

The plant is an erect, branched shrub, 1.5 to 3 meters high. Leaves are pinnate and 40 to 60 centimeters long, with orange rachis on stout branches. Each leaf has 16 to 28 leaflets that are 5 to 15 centimeters in length, broad and rounded at the apex, with a small point at the tip. Inflorescences are terminal and at the axils of the leaves are 10 to 50 centimeters long. Flowers are yellow, about 4 centimeters in diameter, at the axils of thin, yellow, oblong, concave bracts which are 2.5 to 3 centimeters long (Reezal *et al.,* 2012*).*

The use of herbal medicine predates the introduction of antibiotics (Owoyale *et al.,* 2005). In recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases. Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Okonko *et al.,* 2010). Plant-produced compounds are of interest as sources of safer or more effective substitutes for synthetically produced antimicrobial agents (Makinde *et al.,* 2007).

Fungal infections are diseases caused by the growth of fungi in or on the body like the skin, hair, nails, or other superficial sites. They include the familiar ringworm and athlete's foot. The term ringworm or ringworms refers to fungal infections that are on the surface of the skin. The fungi that cause parasitic infection (dermatophytes) feed on keratin, the material found in the outer layer of skin, hair, and nails. These fungi thrive on skin that is warm and moist, but may also survive directly on the outsides of hair shafts or in their interiors (Hees and Naafs, 2001)
The fungi may spread from person to person (anthropophilic), from animal to person (zoophilic), or from the soil to a person (geophilic). Children are most likely to get ringworm. Ringworm of the scalp can spread from child to child when children share hats, combs, or brushes. Ringworm of the body can be spread on towels, clothing, or sports equipment. Dogs and cats can also be infected with ringworm, and they can pass it to people (Hees and Naafs, 2001).

For control of microbial infections and diseases, various synthetic drugs and chemical formulations have been used. But due to their indiscriminate use, microbes have developed wide resistance against these synthetic drugs such as broad-spectrum antibiotics (Okonko *et al.,* 2010). Several reports have shown that *S. alata* contain antimicrobial substance that may be responsible for its reported activity in bacterial and fungal infections. Timothy *et al*. (2012) reported that *S. alata is* recommended for primary health care to treat ringworm and skin diseases in Thailand.

Owoyale *et al.* (2005) and Okonko *et al.* (2010) reported that *Senna alata* is used locally in Nigeria in the treatment of several infections, including ringworm and parasitic skin diseases. Okonko *et al.* (2010) stated that the leaf exudates and the ethanol extract of the leaf of *S. alata* had marked antifungal effects on *Microsporum canis, Trichophyton jirrucosum, T. mentagrophytes.*

On the same note, Palanichyamy (1990) confirms that an extract of *S. alata* completely inhibited the growth of ringworm at a dose of 2.5%$^{w}/_{v}$. Several studies have been conducted to provide scientific basis for the efficacy of plants used in herbal medicine. It has been observed that antimicrobial activity of these plants is associated with the presence of some chemical components such as phenols, tannins, saponins, alkaloids, steroids, flavonoids and these components were also investigated as a scientific assessment of the claim of therapeutic potency (Owoyale *et al.,* 2005; Okonko *et al.,* 2010).

The objective of this work therefore is to investigate the antifungal effect of extracts of *S. alata* on the isolated fungi that causes ringworm in human.

MATERIALS AND METHODS

Collection of Leaf Samples

The leaves of *S. alata* were collected from Anua village in Uyo,Akwa Ibom State. The area falls between latitude $4^0$45"North and longitude 7^0 30" and $8^0$30"(Aksgonline,2012).The leaves were stored in sterile polythene bag and taken to the laboratory for extraction within 24 hours of their collection.

Isolation of Fungal Sample

Using a sterile inoculation loop, scalp of persons infected with ringworm was scrapped and inoculated into prepared sterile PDA media in Petri dishes and incubated at 28°C. They were observed for five days for fungal growth.

Preparation of Extracts

Maceration Method

Fresh leaves of *S. alata* used for the experiment were washed thoroughly, under running tap water and rinsed with sterile distilled water. They were air dried for 8 to 10 days and ground to powder with the aid of a mortar and pestle. The powdered leaves were accurately weighed (20g) and then macerated cold in 70% ethanol for 72 hours (3days) at room temperature following the method of Sofowora (2008). The liquid extracts were recovered by filtration using cotton wool and glass funnel. The filtrate obtained was concentrated in a vacuo at 40^0C to yield semi-solid mass. This same method was carried out with distilled water for aqueous extract. The extract obtained were accurately weighed and then used for phytochemical screening.

Fifteen grams of the extract was reconstituted in 100ml sterile distilled water to obtain 15% concentration of the extract. This method was also used for 10g and 5g to make up for 10% and 5% respectively. These were used for the antifungal tests.

Identification of Fungal Isolates

This was based mainly on the morphological features as seen on the culture plates as well as slides viewed under the microscope. Morphological features like nature of colony, colour, extent of growth, presence or absence of mycelia and spores. Slides of test isolates were prepared and microscopic examination of morphological features were observed and with the help of the standard manual of Barnett and Hunter (1999) the isolates were identified.

Phytochemical Screening

The powdered leaves of the plants were screened for the presence of phytochemicals (alkaloids, saponins, tannins etc) using standard procedures(Sofowora,2008) in the Department of Pharmacognosy, University of Uyo, Nigeria.

Laboratory Screening of Plant Extract

In-vitro Test

Two milliliter of each plant extract at 15, 10 and 5% concentrations were pipetted separately and aseptically into 12 milliliter of cool, molten Potato Dextrose Agar (PDA) medium in each of the Petri-dishes. Each medium was thoroughly homogenized by gentle circular rotations in order to achieve uniform dispersal of the extract. The media was allowed to solidify, and each plate was inoculated with fungal isolates by placing a 5mm disc taken from the advancing edges of 7day old cultures in the centre of each Petri-dish. All dishes were incubated in an incubator at 28^0C and growth of the fungus were measured daily along three axis with a transparent ruler at two days interval for six days.

Zones of inhibition were measured in millimeters on the second, fourth and sixth day of incubation. Control plates containing the test organism without any plant extract were incubated. All the work done was aseptically carried out. Each examination was carried out in triplicates for all isolates.

Statistical Analysis

Analysis of variance (ANOVA) was employed in all numerical data to test for significance test ($P = 0.05$) in the treatment and Least Significant Difference (LSD) test was used to separate the means.

RESULTS

Isolated Fungi

The fungi isolated were identified as *Microsporum canis* and was used for this test.

Plate 1: Culture of *Microsporum canis*

Phytochemical Screening of Test Plant

Senna alata tested positive for alkaloids, saponins, tannins, anthraquinones.

Test for Antifungal Activity

Results shown in Fig. 1 indicate as follows:

Effect of the extracts of *S. alata* on the fungal growth of *M. canis* was concentration dependent. At 5% concentration effect of ethanolic extract on the fungal growth was the least, giving a value as low as 15% inhibition on the fourth day of incubation. The percentage inhibition was highest at 15% concentration, which recorded 56% of inhibition of test fungi on the second day of incubation. However, this was higher than percentage inhibition (44%) recorded on the fourth day of incubation.

Figure 2 indicates less inhibitory activity by aqueous extract when compared with ethanolic extract. Percentage inhibition of test fungi was highest at 15% concentration of extract on the 6th day of incubation (36%). Percentage inhibition at 10% concentration for 2, 4 and 6 days were not significantly different ($p<0.05$). The lowest percentage inhibition (6%) was recorded on the second day of incubation at 5% concentration. At 0% concentration being the control, there was o% of inhibition of test fungi.

The comparison between the different concentrations of the extract of *S. alata* showed that generally, increase in concentration of plant material gave decrease in the fungal growth of the tests fungi.

DISCUSSION

Microsporum canis was isolated from human infected with ringworm (Plate 1). The antifungal properties of some plant extracts in controlling different pathogens that cause fungal diseases in crops have been affirmed by the works of several researchers (Amadioha, 2000; Okigbo and Nmeka, 2005; Bediakao *et al*., 2007).

The ability of extracts of *S. alata* to inhibit the growth of *M. canis in-vitro* could be as a result of the presence of some phytochemicals. This supports findings of Caragay(1992), Okwu (2004), Banso and Adeyemo(2007) that the tannins, flavonoids, saponins, and cardiac glycosides present in medicinal plants possess remarkable toxic activity against fungi and bacteria.According to the study the ethanolic and aqueous extract of *Senna alata* tested on fungal growth of *Microsporum canis* showed different levels of antifungal activity. This agrees with some reports in which similar observations were made (Abubacker *et al*., 2008; Okonko *et al*., 2010; Timothy *et al*., 2012).

The differences observed in the inhibitory ability of the extract used in this research work are due to the susceptibility of the test isolate to different concentrations of the extract. This agrees with the findings of Okigbo *et al.* (2009).

From the results obtained in this research, it is evident that *Senna alata* has the potential of controlling skin infecting fungi. This can provide alternative way of treatment for reducing and controlling these fungi.

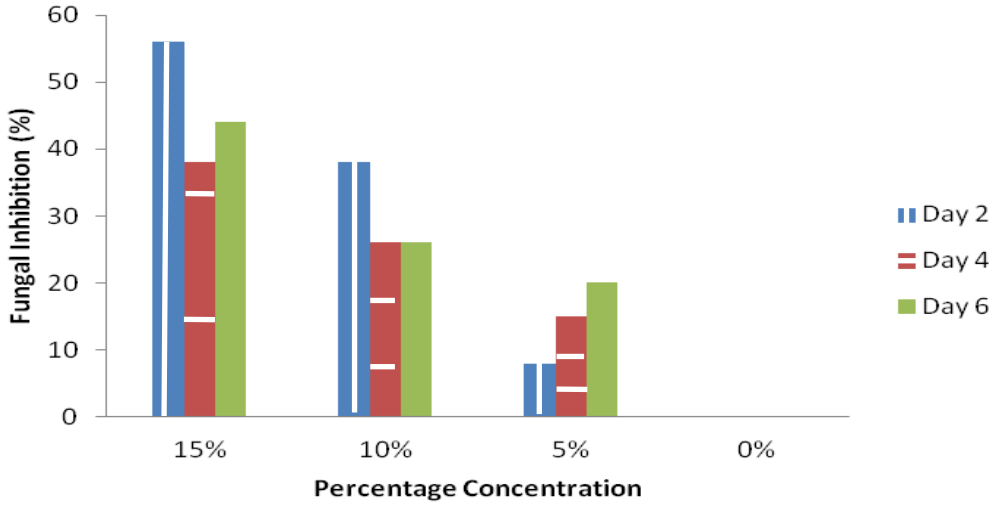

Figure 1: Inhibitory effect of ethanolic extract of *Senna alata* on *Microsporum canis.*

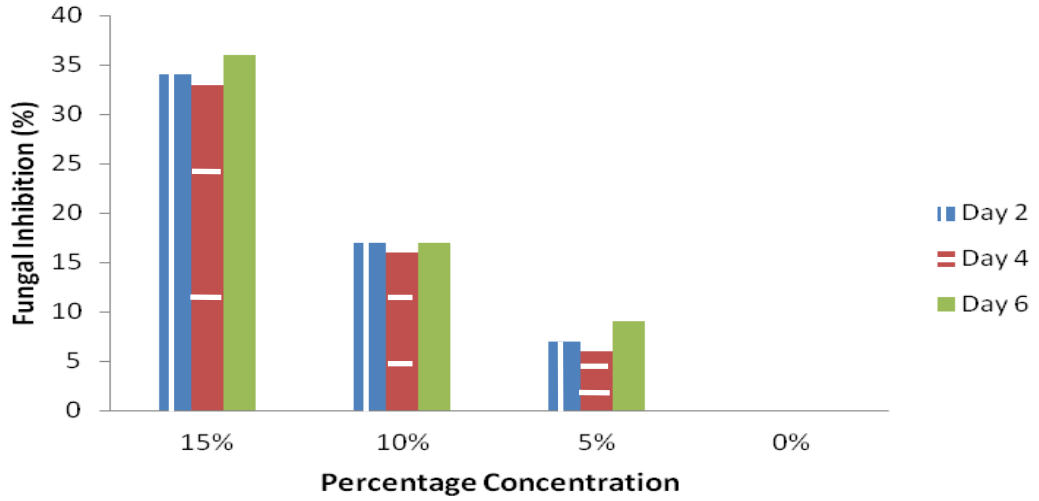

Figure 2: Inhibitory effect of aqueous extract of *Senna alata* on *Microsporum canis*

REFERENCES

Abubacker, M. N., Ramanthan, R. and Kumar, T. S. (2008). *In-vitro* antifungal activity of *Cassia alata* Linn. Flower extract. *Natural Product Radiance,* 7(1): 6-9

Amadioha, A. C. (2000). Fungitoxic effects of some leaf extracts against *Rhizopus oryzae* causing tuber rot of potato. *Arch phytopathol Pflanzo:* 34, 1-9.

Banso, A and Adeyemo, S. O. (2007). Evaluation of Antibacterial properties of Tannis isolated from *Dicrostachys cinerae. Afr. J. Biotechnol.*, 6(15): 1785- 1787.

Barnett, H. L. and Hunter, B. B. (1999). Illustrated general of imperfect fungi. 4th ed. *American Phytopathological Society press*. USA. 218pp.

Bediakao, A., Showemimo, F.A., Asiama, Y. O. and Amewowor, D. H. A. K. (2007). *In vitro* analysis of growth media and the control of yam minisett-rot. *Biotechnology*, 6:1-4.

Caragay, A. E. (1992). Cancer preventive foods and ingredients. *Food Tech.,* 46: 65-69.

Evans, W. C. (2002). "Trease and Evans Pharmacognosy" 15th Edition. W. B. *Saunder, London*. Pp. 16, 231 – 235.

Hees, C.V. and Naafs,B.(2001).Common skin diseases in Africa. An illustrated guide, Studio oss, Zimbabwe, 89 p

Makinde, A. A., Igoli, J. O., TA'Ama, L., Shaibu, S. J., Garba, A. (2007). "Antimicrobial activity of *Cassia alata*" *African Journal of Biotechnology,* 6(13): 1509-1510.

Okigbo, R. N. and Nmeka, I. A. (2005) control of yam tuber rot with leaf extracts of *Xylopia aethiopica* and *Zingiber officinale. African Journal of Biotechnology,* 4(8): 804-807.

Okigbo, R. N., Okorie, R. E. and Ramesh, P. (2009). *In vitro* effect of garlic (*Allium sativum* L.) and African basil (*Occimum gratiasimum* L.) on pathogens isolated from rotted cassava tubers. *Interciencia Journal Venezuela*, 34(10): 7442 – 747.

Okwu, D. E. (2004). Phytochemical and Vitamin content of indigenous spices of South Eastern Nigeria. *Journal Sustainable Agriculture and Environment,* 6(*1):* 30-37.

Okonko, I.O, Sule, W. F, Joseph, T. A., Ojezele, M. O., Nwanze, J. C., Alli, J. A., Adewale, .O. G. and Ojezele, O. J. (2010). "*In-vitro* antifungal activity of *Senna alata* Linn. Crude leaf extract" *Advances in Applied Science Research,* 1(2): 14-26.

Owoyale, J. A., Olatunji, G. A. and Oguntoye, S. O. (2005). "Antifungal and Antibacterial Activities of an Alcoholic Extract of *Senna alata* Leaves" *Journal of Applied Science in Environment Management*, 9(3): 105 – 107.

Palanichamy, S.; Nagarajan, S. (1990): "Antifungal activity of *Cassia alata* leaf extract". *Journal of Ethnopharmacology*, 29(3): 337-340

Stephen, S. B. (2011). "*Senna alata*" University of Florida. IFAS Extension Lee County. (239) 533-7513.

Sofowora, A. (2008). Medicine Plants and Traditional Medicine in African, 3rd. Ed., *Spectrum Book Limited,* Ibadan, Nigeria. p. 19.

Timothy, S. Y., Wazis, C. H., Adati, R. G. and Maspalma, I. D. (2012). "Antifungal Activity of Aqueous and Ethanolic Leaf Extracts of *Cassia alata* Linn" *Journal of Applied Pharmaceutical Science* 02(07): 182-185.