Journal of Biopesticides and Environment
ISSN: 2449-1284 (Online); 2449-1292 (Print)
http://www.biopjournal.com

ANTIDERMATOPHYTIC INVESTIGATION OF ETHANOL EXTRACT OF *Mucuna urens* (Linn.) AGAINST *Microsporum canis*

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ABSTRACT

Extract of *Mucuna urens* was evaluated against *Microsporum canis*, a pest found on household pets, domestic animals and on man, and also the causative organism in the dermatophytosis, *Tinea capitis*. Phyto-chemical screening of the test plant extract using standard procedures, showed the presence of cardiac glycosides, flavonoids, Saponnins, reducing sugar and anthraquinones. *In vitro* antifungal activity of the extract using standard cup plate method, gave a zone of inhibition (ZI) of 5.0mm at 1000 $mgkg^{-1}$. Hence, the ethanol extract of *M. urens* exhibited inhibitory activity against the pest *M. canis* and can be employed as a safe biopesticide against this fungal pest, that causes the dermatophyte, *Tinea capitis*.

Keywords: *Microsporum canis*; *Mucuna urens*; Dermatophytosis; *Tinea capitis*.

1. INTRODUCTION

A pesticide is a chemical used to prevent, destroy, or repel pests. Pests can be insects, mice and other animals, weeds, fungi, or microorganisms such as bacteria and viruses (US EPA, 2012, 2014). Some examples of pests are termites causing damage to our homes, dandelions in the lawn, and fleas on our pets. Pesticides also are used to kill organisms that can cause diseases. Most pesticides contain chemicals that can be harmful to people, animals, or the environment. Biopesticides, on the other hand, are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals, hence, are human and environmental-friendly, (US EPA, 2012, 2014). Dermatophytosis or ringworm is a clinical condition caused by fungal infection of the skin in humans, pets such as cats, and domesticated in humans, pets such as cats, and domesticated animals such as sheep and cattle.

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. (Wikipedia, 2013). Dermatophytosis causes infections such as athlete's foot and jock itch. It has also been reported as a zoonotic disease (Kyle and Dahl, 2004). Dermatophytes are the only fungi that have evolved a dependency on human or animal infections for the survival of the specie (Prescott *et al.*, 2005). The group comprises of three genera, but for the purpose of this study, the Microsporum spp. (*M. Canis*) was investigated. *M. Canis* is implicated in several human and animal disease patterns including *Tinea capitis* (Dermatophytosis that affects skin, hair and nails) (Fenner *et al.*, 2005). Transmission of Tinea infection can occur directly through contact with infected lesions or indirectly through contact with contaminated articles (Chuang *et al.*, 2007).

The pesticides used against dermatophytosis exhibit several side effects and have limited efficacy (Sadeghi-Nejad *et al*., 2007). Therefore, there is a pressing need for the discovery of new safer and more effective biofungicides. The use of medicinal herbs in the treatment of skin diseases including mycotic infections is an age-old practice in many parts of the world (Irobi and Daramola, 1995), because herbal remedies used in traditional folk medicine may help to overcome the growing problem of resistance to pesticides and their relative toxicity (Sadeghi-Nejad and Deokule, 2007). Some of these herbs are *Ixora brachiata*(Roxb.) (Sadeghi-Nejad and Deokule, 2007); *Eucalyptus camaldulensis* (Hassani-Mehraban *et al*., 2005); *Moringa oliefera* Lam (Chuang *et al.,* 2007*); Anagallis arvensis, C. spinosa, F. regia, Pistacia lentiscus* and *Ruta chalapensis* (Ali-Shatayeh and Abu-Ghdeib, 1999); Other plants that have shown antidermatophytic activity against *M. canis* include *Eucalyptus camaldulensis* (Hassani-Mehraban *et al*., 2005); *Juglans* sp. and *Solanum* sp. extracts (Heisey and Gorham, 1992) and *Allium sativum* (Venugopal and Venugopal , 1995).

Mucuna urens seeds (Fig. 1), are commonly found in home gardens in the South eastern parts of Nigeria, West Africa, where the Efiks, Ibibios and Igbos use it as a major soup condiment for thickening. It is called "Ibaba" by the Efiks/Ibibios, "yerekpe" by the Yorubas and "Ukpor" by the Igbos (Etta, 2008) and is usually sold in the local markets during its harvest season which is in the month of January (Elittä and Carsky, 2003). In other localities where *M. urens* is found, it is known as velvet bean, *pica-pica*, bengal bean, *nescafé*, *ojo de venado*, *pois mascate, kara benguk*, *olhos de burro* (Armstrong, 1998). Horse eye bean, ox-eye bean and devil bean are all common English names for *Mucuna* (Esonu *et al*., 2001). The seeds are also known as "sea beans," because they are commonly carried by rivers into the ocean (Armstrong, 1998). The medicinal and nutritional properties of the *Mucuna urens* plant has been well documented. It is used as a green manure/cover crop by small holder farmers in tropical regions of the world (Tripathi and Upadhyay, 2002). It is also known to be a rich source of protein (Umoren *et al*., 2007). The plant has been used in herbalism to treat or manage a range of health conditions such as urinary tract infection, menstruation disorders, Oedema, constipation and recently it is being investigated for treating Parkinson's disease due to its' significant L-DOPA content (Sridhar and Bhat, 2007).

Despite the detailed studies on the uses and efficacy of extracts of *M. Urens*, there is no record of its' antifungal effect or effects on dermatophytosis. This informed this investigation to ascertain if the seed extract of *M. urens* could present as a cheaper, safer and easily accessible alternative for treatment of *Tinea capitis*, especially, in rural and remote areas where access to conventional antifungal cream or drug may be difficult.

2. MATERIALS AND METHODS

2.1 Collection and preparation of plant materials.

The dry seeds of *Mucuna urens* were bought from a local market (Watt market) and identified by the chief technologist in the Pharmacology Department of the University of Calabar, Calabar. The seeds were further air-dried for 3 days and then pulverized using an electric blender (Laprivia 3000, China). The powdered sample was then extracted in a Soxhlet apparatus using ethanol as the extracting solvent. The ethanol extract was evaporated using a hot air oven (STUARC scientific, England) for 24hrs. at 50°C.

2.2 Phyto-chemical Screening of the ethanol extract of *M. Urens*.

Phyto-chemical Screening of the ethanol extract of *M. urens* seeds were done using standard procedures for the phytochemical screening of medicinal plants according to (Trease and Evans, 1989, Soforowa, 1996 and Evans, 2001). The compounds screened included the following: Polyphenols, Phenobabinins, Cardiac glycosides, Saponins, Tannins, Alkaloids, and Anthraquinone. Others

are Flavonoids, Steroids, Hydroxy methyl anthraquinone, Anthranoid and Reducing Sugars.

2.3 Antidermatophytic Investigation of the ethanol extract of *M. urens*.

Antidermatophytic investigation of the ethanol extract of *M. urens* was also carried out using isolates of the *Microsporum* spp. The dermatophyte isolates were collected from the Microbiology Department of the University of Calabar Teaching Hospital, Calabar. These were cultured using Sabouraud dextrose agar (S.D.A.) as the culture media. Standard procedures for culturing, sub-culturing and fungal inoculation of media, were carried out. The inoculum of conidia suspension was obtained according to Shin and Lim , (2004); Wright *et al*., (1983). Microscopic evaluation showed that the culture was positive for the growth of *Microsporum canis*.

Antifungal activities of ethanol extract of *Mucuna urens* was evaluated by standard cup plate method according to the methods of Pati and Kurade, (2009). 10mgml^{-1} Keteconazole (Janssen pharmaceutical) was used as the standard positive control. Three concentrations of the extract were tested against the fungi after which zones of inhibition (ZI) were recorded. These are 250 mgml^{-1}, 500 mgml^{-1} and 1000mgml^{-1}.

3. RESULTS

Results showing the qualitative phyto-chemical components of the ethanol seed extract of *M. urens* is presented on Table 1 while results of the Zones of Inhibition(ZI) of *M. canis* by ethanol seed extract of *M. urens* is presented on Table 2. The ethanol seed extract was positive for Cardiac glycosides, Anthraquinone, Saponins, Steroids, Tannins, Flavonoids, Reducing Sugars and Hydroxy methyl Anthraquinone. At 250 mgml^{-1} and 500 mgml^{-1}, the fungi showed resistance against the herb extract, but at 1000 mgml^{-1}, sensitivity of 5.0mm was observed (Fig. 3). It was also observed that 48hrs. later, the fungi again, developed resistance against the extract. The standard positive control showed a ZI of 25mm against the fungi.

4. DISCUSSION AND CONCLUSION

Qualitative Phyto-chemical screening of *Mucuna urens* ethanol seed extract yielded eight phyto-components as earlier stated. Despite being nutritionally and medicinally promising, *Mucuna urens* has been reported to contain some endogenous toxic factors Ukachukwu *et al*., 2003). Relatively high concentrations of tannins, phytic acid, cyanogenic glycoside, oxalate and gossypol have been reported in *Mucuna* (Liener and Kakada ,1980; Laurena *et al*., 1994). Unlike report by Nwokonkwo,(2013), tannins were present in ethanol extracts *of M. urens* from our findings. Other researchers have also recorded tannins in extracts of *M. urens*. (Mohan and Janardhanan, 1995; Vadivel and Janardhanan, 2001b).

Tannins are known to be anti-nutritional and have been recorded to be very high (468.37 mg/100g) in raw *Mucuna urens* seeds but lower in the processed (cooked and roasted) seeds (36.20 and 45.87 respectively) (Umoren *et al*., 2007). Though tannins are known to be anti-nutritional, current information has it that their beneficial or anti-nutrition properties depend upon their dosage and the kind of tannin present (Chuang *et al*., 2007). They are also recorded as being a major component of most antifungal herbs (candida-cure-recipes.com.). Tannins are highly astringent and leave a dry feeling in the mouth if consumed unprocessed (Yisa, 2009). This accounts for the dry feeling in the mouth when *Mucuna urens* seed is chewed raw. The medical potentials of tannins have been well documented (Effiong and Umoren, 2011; Fenner *et al*., 2005; Hammer *et al*., 2003). Recent findings indicate that tannins neither inhibit food consumption nor digestion but rather possess the potential to decrease the efficiency of converting the absorbed nutrients to new body substances (Chung *et al*., 1998), due probably to the great efficiency with which tannins precipitate proteins through interaction that occurs by hydrophobic forces and hydrogen bonding (Yoo *et al*., 2011).

The presence of flavonoids indicates the presence of polyphenolic secondary metabolites in the extract. Flavonoids are simple phenolic compounds which have been reported to possess

a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic, as well as ability to modify the gene expression (Marinova *et al.*, 2005; Beta *et al.*, 2005). Wound healing potentials of *Mucuna urens* has been attributed to the presence of several phytochemicals including flavonoids (Manjunatha *et al.*, 2006). The seeds have also showed antibacterial activity against *B. cereus, E. coli, P. vulgaris and* Staph (Ujowundu *et al.*, 2010). This antibacterial property of *M. urens* presents it as a novel biopesticide deserving further investigations. This could be attributed to its rich store of flavonoids. The antioxidant and scavenging of free radicals ability of flavonoids may also be responsible for the anti-carcinogenic property of *Mucuna urens* reported by Delmulle and Demeyer (2010).

The laxative effects of anthraquinone (2-hydroxylmethyl anthraquinones) have been documented (Ukoha, 2011). Anthraquinones are implicated in most antifungal herbs as a major component, thus, the ethanol extract of *M. urens* may have also derived its' antifungal property due to the rich store of anthraquinones in it. They have also been documented as not being friendly chemicals, as they cause kidney damage and gastro intestinal bleeding (USAEPA, 2014). The presence of anthraquinone in *Mucuna urens* may also be implicated for the highly toxic anti-nutritive property of unprocessed *Mucuna urens* (Umoren *et al.*, 2007). They have also been used as chemotherapeutic agents (Greidanus, 1988; Patterson, 2002) .

The presence of steroids in the ethanol extract of *Mucuna urens* attests to the possible efficacy of the therapeutic use of *Mucuna urens*. Nwokonkwo (2013), also reported steroids in the ethanol extract of *M. urens*. Steroidal compounds are of importance and interest in the body since they are related to sex hormones and could serve as potential starting materials in the synthesis of sex hormones and ensure such hormonal balance (Delmulle and Demeyer, 2010).

Investigations of the antifungal properties of the ethanol extract of *M. urens* showed no activity against *Microsporum canis* at 250 $mgml^{-1}$ and 500 $mgml^{-1}$. There was however, sensitivity observed at 1000 $mgml^{-1}$ concentration. This could be attributed to the presence of saponins and tannins implicated as possessing antifungal properties too (Cowan, 1999)(Table 2). 48 hours after the culture was inoculated at 1000$mgml^{-1}$ the fungi developed resistance against the *M. urens* extract. Ali-Shatayeh and Abu-Ghdeib (1999), also documented several herbs that showed no activity against *M. canis*. These herbs include *Asphodelus microcarpus, Clematis cirrhosa, Solanum nigrum*. Also in their report, *Juglans regia* showed an inhibitory value of 3 mm, a value lower than that of *M. urens* (from our investigations). *Moringa oleifera* has also been shown to exhibit antifungal activity *in vitro* against *M. canis* (Chuang *et al.*, 2007). The presence of a rich array of phytochemicals in the ethanol extract of *M. urens* may be implicated in its antifungal activity against *M. canis*, albeit at a high concentration of 1000$mgml^{-1}$. Hence, the ethanol extract of *M. urens* exhibited inhibitory activity against the pest *M. canis* and can be employed, as a safe and active biopesticidal agent in pharmaceutical formulations against this fungal pest, that causes the dermatophyte, *Tinea capitis*. It may therefore be suggested that further studies on the synergistic effect of *M. urens* with other antifungal herbs as an herbal remedy against *M. canis* be investigated.

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Fig. 1 *Mucuna urens* seeds
(Source: Wikipaedia, 2013)

Fig.2 *Tinea capitis* on the skin of a dog.
(Source: Field work, 2013)

Table 1: Qualitative Phyto chemical constituents of *M. urens* seed extract

Phyto chemical constituent	**Availability**	**Phyto chemical constituent**	**Availability**
Polyphenols	-ve	Flavonoids	+ve
Phenobabinins	-ve	Hydroxy methyl Anthraquinone	+ve
Cardiac glycosides	+ve	Anthranoids	-ve
Anthraquinone	+ve	Saponins	+ve
Alkaloids	-ve	Reducing Sugars	+ve
Tannins	+ve	Steroids	+ve

+ve – Present; -ve – Not present.

Table 2: Zones of Inhibition (mm) of *M. canis* by ethanol extract of *M. urens* seeds.

Extract Concentration(mgml^{-1})	**Fungal Sensitivity(ZI)**	**At 48hrs.**
250	R	R
500	R	R
1000	5	R
Keteconazole (+ve Control)	25	25

R – resistance

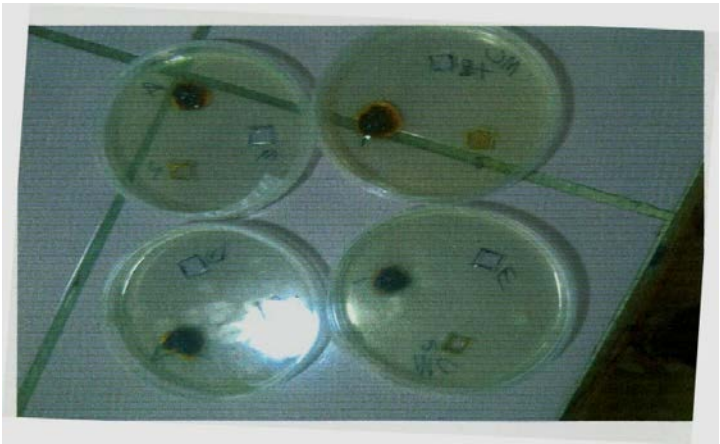

Fig. 3 Antifungal Sensitivity Test

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