EFFICIENCY OF CHICKWEED (*Stellaria citrifolia*) LEAVES EXTRACT ON DERMATOPHYTES (RINGWORMS) ISOLATED FROM PRIMARY SCHOOL PUPILS IN WUKARI, NORTH EAST, NIGERIA

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Abstract: Ringworm is a global infection that is treated using various antifungal agent including plant extracts. A survey was carried out among one hundred and twenty (120) pupils (eighty (80) males and forty (40) females) in schools in Wukari to determine the traditional treatment of ringworm infection among children using chickweed (*Stellaria citrifolia*) leaves extracts of ethanol and distill water. A total of eighty (80) isolates (twenty (20) from females and sixty (60) from males) were isolated using standard Microbiological techniques. These isolates belong to three species namely: *Trichophyton* species (A dermatophyte), *Microsporum* species and *Epidermophyton* species. The species *Trichophyton* had the highest isolates with 25% and 10% for males and females respectively. This is closely followed by *Epidermophyton* species isolates 20% for males and 5% for females and *Microsporum* species had 15% and 5% isolates in males and females respectively. All the 120 (100%) of the pupils from the schools investigated had 80 pupils (66.7%) of both males and females harboured the pathogenic fungi while only 40 of the pupils representing 33.3% do not harboured the pathogenic organism. These pathogenic fungi when subjected to *Stellaria citrifolia* ethanol extract all the isolated were susceptible to the extract and when these same pathogenic fungi were tested on *Stellaria citrifolia* distill water extract the organisms were all susceptible the extract. This mean that the effectiveness of the *Stellaria citrifolia* is not affected by both the extract medium used and because all the pathogenic fungi were susceptible to the extract it means that *Stellaria citrifolia* is active against isolates *Trichophyton* species (A dermatophyte), *Microsporum* species and *Epidermophyton* species. In conclusion, *Stellaria citrifolia* can be used as an antifungal agent's as these isolated pathogenic fungi has high degree of susceptibility to it. Also, pupils in primary schools in Wukari harboured a number of pathogenic fungi which have effect on their skin (especially head skin) making it public health concern in term of spreading to other uninfected children. Pupils, parents and other individuals should always be advised to dry and clean their clothing after each use, and to maintain good proper hygiene to avoid dampness as this facilitate the growth of fungi. Those that are affected with the pathogenic fungi should try and seek for medical help immediately it is discovered. More studies should be conducted to determine components of *Stellaria citrifolia* which is active against the fungi and the genetic status of the pathogenic strains. Finally, further work should also be carried out to know the fungi that are susceptible to *Stellaria citrifolia*.

Keywords: *Stellaria citrifolia*, ringworm, Wukari, pupils, North-East, Nigeria

Introduction

Dermatophytes are a group of morphologically and physiologically related molds some of which cause well defined infections (dermatophytoses) (tinea or ringworm) (De Vroey, 2000). They possess two important properties: they are keratinophilic and keratinolytic (Mikali *et al*., 2012). This means they have the ability to digest keratin *in vitro* in their saprophytic state and utilize it as a substrate and some may invade tissues *in vivo* and provoke *tineas* (Mikali *et al*., 2012). The infections are universally present in those people who play sports (Satana *et al*., 2011), and the infections are known as dermatophytoses (Nweze, 2010). Dermatophytoses is caused by the genera *Microsporum*, *Trichophyton* and *Epidermophyton* (Sharma *et al*., 2014). These organisms are pathogenic members of the keratinophilic (keratin digesting) soil fungi (Satana *et al*., 2011). *Microsporum* and *Trichophyton* are human and animal pathogens. *Epidermophyton* is also a human pathogen. Anthropophilic species are responsible for the majority of human infections. The dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissue (skin, hair and nails) of humans (Maraki *et al*., 2007). The occurrence of dermatomycoses is superior in population with little socio-economic status and also in close nearness of animals (Farzana, 2007; Mikali *et al*., 2012). However, flooring, clothing, linens, furniture and barber shop instruments, are the vital foundation of dermatophytes. dermatophytoses has a distinction basis in the affected region of the body, but one of their priorities is a universal indicator in humans (Nweze, 2010; Hapcioglu *et al.*, 2006). Primary infection starts through small skin break. These breaks are coming out through secretion of enzymes that digest keratin (Achterman and White, 2012; Mikaili *et al*., 2012). This enzyme is referred to as keratinase (Gupta and Ramnani, 2006). Dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissues (skin, hair and nails) of humans (Maraki *et al*., 2007). For purposes of nomenclature, they can be divided into two states on the basis of stages in their life cycle, the anamorphic and the teleomorphic states (Sharma *et al*., 2014). The anamorphic is the state where asexual or somatic reproduction occurs and has a distinct morphology. The teleomorphic, on the other hand, is the sexually reproductive ("perfect") state, (Hoog, 1996). Sexual reproduction has been demonstrated in a number of species which requires two compatible isolates on a suitable medium (Achterman *et al*., 2011). Dermatophytes are also classified into three habitual species as Anthropophilic, Zoophilic, and Geophilic species in an environment (Lakshmipathy and Kannabiran, 2010). Their habitat depends on the survival host as human, animals and soil (Achterman *et al*., 2011). Dermatophytosis infections occur most only on dead Keratin at the top layer of the skin, hair and nails (Dekate *et al*., 2011). For example, *Tinea barbae* infects to the bearded area of the face, whereas *Tinea pedis* infects the foot (Hapcioglu *et al*., 2006). The dermatophytes are mainly covered with infections to a single part of the body (Achterman *et al*., 2011). *Tinea capitis* presents a significant endemic problem mainly in school children in the world, (Mahmoudabadi *et al.*, 2013).

The disease arises with settling separately on the skin, hairs, and nails in living beings, (Khaksari and Bassiri, 2009). *Tinea* is the allusion of mycosis which presents as a considerable widespread problem generally in school children. Among all *Tinea* implement, *Tinea pedis* is a universal infection, monitored in one in five adults. *Tinea cruris* cause crotch itch, crotch rot, eczema marginatum, gym itch, jock itch, jock rot. *Tinea nigra* disease also recognized as "*Tinea nigra palmaris or plantaris* is present as dark brown to black effortless patches on the palms of the hands and the soles of the feet (Satana *et al*., 2011). Dandruff (*Pityriasis capitis*) caused by *Pityriasis ovale* is another superficial dermatophytosis. This is a common chronic scalp condition marked by itching and flaking of the skin on the scalp, (Achterman *et al*., 2011). The disease is commonly caused in billions immunocompromised persons by dermatophytes, yeast and non-dermatophytes agents, (Abbas *et al*., 2012). *Tinea barbae* is identified as "Barber's itch, ringworm of the beard, and "*Tinea sycosis*" (Satana *et al*., 2011). It is surface disease of the hair, in the beard and mustache region of men (Marcus *et al.*, 2008). *Tinea barbae* starts from the face and neck. It is mostly caused by shaving and abuse of steroids. The main clinical indications are classified into two forms as inflammatory and non-inflammatory. These symptoms depend on kind of fungus and patient's resistant response (Sharma *et al*., 2014). In general ways, the common symptoms of disease are loose and broken off hairs, kerion-like plaques, rash, itching and pimples near a hair follicle in the neck, and genital area (Sharma *et al*., 2014; Achterman *et al*., 2011). Reddening and swelling also occur in the entire area with barbeque. Habitually, the zoophilic dermatophytes such as *T. mentagrophytes, T. verrucosum, T. megninii, T. rubrum* and *T. violaceum* are responsible for infection. *M. canis* and *T. mentagrophytes Varerinacei* also cause Tinea barbae, but these are exceptional (Marcus *et al*., 2008). Dermatophytes are fungi obtaining a mid-transmittable disease which are acquired from infected animals or birds and fomites. Detection of dermatophyte Texas is correlated to epidemiological apprehension. These are important to manage infection and public health issues associated with types of Dermatophytosis (Lin *et al.*, 2004).

Traditionally, the dermatophytosis is normally referred to as "tinea" or "ring-worm" infections (Lakshmipathy and Kannabiran 2010). Damp foot circumstances lead to irritated symptoms due to mixed infection by dermatophytes and bacteria. Tinea of the extremities, T*inea cruris* and onychomycosis caused by zoophiles are exceptional (Sharma *et al*., 2014). In humans, pruritus is a widespread symptom. The skin lesion is usually characterized by inflammation with erythema, scaling and occasionally blister formation. The habitual signs of inflammatory reactions such as redness, swelling, heat and alopecia are distinguishing at the infection position (Laksmipathy *et al*., 2010). The identification of dermatophytes is based on methods that focus on morphological, physiological, ecological and genetic features. Anthropophilic and zoophilic dermatophytes has mostly been recognized via internal transcribed spacer (ITS; sequencing of the rRNA gene) (Sharma and Swati, 2012).

The plant (*Stellaria citrifolia*) with *Stellaria* is a genus of about 90-120 species of flowering plants in the family *Caryophyllaceae*, common names include starwort and chickweed, (Satana *et al*., 2011). This annual plant produces stems about ½–1 long that usually sprawl across the ground. It branches abundantly near the base, but very little toward the tips of the stems. The somewhat succulent stems are green or burgundy; they often have lines of white hairs (Laksmipathy *et al.*, 2010). Pairs of opposite leave occur at intervals along these stems. These leaves become larger toward the tips of the stems, spanning up to ¾" in length and ½" across (Sharma *et al*., 2014). The leaves toward the base of the plant usually have short petioles that are slightly hairy, while the leaves near the tip of each stem are usually sessile. The leaves are oval-ovate, entire (toothless) along their margins, and hairless on the upper surface; the lower surface is occasionally hairy (Laksmipathy *et al.*, 2010). Individual flowers occur from the axils of the outer pairs of leaves, while the stems terminate in small cymes of white flowers. Each flower is about ¼" across, consisting of 5 white bifid petals (appearing to be 10 petals), 5 green sepals, 3 white styles, 2-10 stamens, and a light green ovary in the center. The sepals are lanceolate, hairy on the outer surface, and longer than the petals; each sepal is at least 1/8" (3 mm.) long. The slender pedicels are finely pubescent. The blooming period occurs during the spring for plants that are winter annuals, and during the summer or autumn for plants that are summer annuals. A typical plant will bloom sporadically for 1-2 months. Each flower is replaced by a cylindrical seed capsule that is light brown with 6 small teeth along its upper rim; it contains several seeds. Each mature seed is reddish brown, somewhat flattened, and Orbicular-Reni form; its surface is minutely bumpy (Laksmipathy and Kannabiran 2010). The root system is shallow and fibrous. This plant spreads by reseeding itself; it can also spread vegetative by rooting at the leaf nodes along the stems (Lacaz *et al*., 1998). Typical growing conditions consist of partial or full sun, moist to mesic conditions, and a fairly fertile loam or clay-loam soil. Light shade and temporary flooding are tolerated. Habitats include woodland areas prone to flooding or standing water, thickets, cropland and fallow fields, lawns and gardens, nursery plots, areas adjacent to buildings, and miscellaneous waste areas (Laksmipathy and Kannabiran 2010). While Common Chickweed occurs to a limited extent in natural habitats, where it is sometimes invasive, this plant prefers areas with a history of disturbance (Havlickova *et al.*, 2008). The plant has medicinal purposes and is used in folk medicine. It has been used as a remedy to treat itchy skin conditions and pulmonary diseases. 17th century herbalist John Gerard recommended it as a remedy for mange. Modern herbalists prescribe it for iron-deficiency anemia (for its high iron content), as well as for skin diseases, bronchitis, rheumatic pains, arthritis and period pain (Imarenezor *et al*., 2017). The plant was used by the Ainu for treating bruises and aching bones (Imarenezor *et al.*, 2017). Stems were steeped in hot water before being applied externally to affected areas (Imarenezor *et al.*, 2017). Nature provides preliminary needs of beings for self-care. Plant remedies have a strong efficacy against several assorted diseases such as skin disease caused by fungi and moulds. Their essential oils are best candidature in presence of their cytotoxic aptitude against fungus (Sharma *et al*., 2014). The *Otacanthus azureus* (Linden) *Ronse* essential oil alone or in combination with azoles is a promising antifungal agent in the treatment for human dermatomycoses caused by filamentous fungi (Satana *et al*., 2011). The combination of ketoconazole and *P. graveolens*'s essential oil for treatment of infections caused by *Trichophyton* species reduce the minimum effective dose of ketoconazole, and thus minimize the side-effects of ketoconazole (Shin and Lim 2003).

Similarly, a number of plant's essential oils have been search out as plant remedies of plant families, that is, *Asteraceae, Liliaceae, Apocynaceae, Solanaceae, Caesalpinaceae, Rutaceae, Piperaceae, Sapotaceae, Caricaceae, Euphorbiaceae, Moraceare, Solaneaceae, Papaveraceae* (Natarajan 2009). For example, antifungal effect of *Hypercom perforatum, Eucalyptus globules* (88%), *Catharanthus roseus* (88%) *Ocimum sanctum* (85.50%), *Azadirachta indica* (84.66%), *Ricinus communis* (75%), *Lawsonia inermis* (74.33%) *Jatropha curcas* (10%) *Eucalyptus intertexta* and *Eucalyptus largiflorens* are determined more active against

Epidermophyton, Microsporum and Trichophyton Genera, (Ghasemi *et al*., 2014). Ringworm is a global infection that is treated using various antifungal agents. This study therefore, will enlighten the public on the traditional treatment of ringworm infection among children using *Stellaria citrifolia* (chickweed) leaves extracts.

Materials and Method

Study population

A total number of one hundred and twenty (120) pupils aged 4 to 12 years from Wukari East primary school (A) and Laden primary school (B) in Wukari, out of which 80 were males and 40 were females. The pupils chosen where all affected with the ringworm of the head.

Media and reagents

The media used in this study were Sabouraud Dextrose Agar (SDA) and Mueller Hinton Agar for sensitivity testing, Ethanol for extraction and lactophenol blue was used for staining. The identification of the fungi was done using method by Dekate *et al.* (2011).

Sample collection

Samples were collected by scraping aseptically from two different primary schools within wukari metropolis, sixty (60) samples from each school and were immediately transported to the laboratory for culturing.

Preparation of Stellaria citrifolia leaves and extraction

Stellaria citrifolia leaves was aseptically dried using hot oven and the leaves were then pounded or crushed into Powder form and stored in a sterile bottle. Twenty gram (20 g) powdered form of the *Stellaria citrifolia* was dissolved in 20 ml distill water (for aqueous solution) and the same grams was dissolved in 20 ml ethanol Both solutions were filtered or sieved using filter paper and collected in separate sterile conical flasks (Dekate *et al.*, 2011).

Sensitivity test

Sensitivity testing was carried to determine whether the *Stellaria citrifolia* leaves extract is susceptible to the fungi (ringworm). The sensitivity test was performed using Mueller Hinton Agar. In this method, 5 ml of the aqueous extract were transferred into ten (10) different Petri dishes, and then newly prepared Mueller Hinton Agar was added into each of the ten (10) different Petri dishes with constant agitation or stirring to ensure a homogeneous mixture, then the medium was allowed to gel. After gelling, the subculture colonies were inoculated (point inoculation) in the medium and incubated at room temperature for 14 days. This same procedure was applied to the ethanol extract (Dekate *et al.*, 2011).

Results and Discussion

The result of the study shows the presence of three (3) types of fungi isolates of *Trichophyton* species, *Microsporum* species and *Epidermophyton* species from collected samples as shown on Table 1. The number of viable and non – viable growth count from both male and female pupils of primary school A as shown in Tables 2 and 3 showed the number of viable and non – viable growth count from both male and female pupils of primary school B. Figures 1 and 2 showed bar chart representation of male and female frequency of the fungi isolates. Skin has been known to harbour microorganisms; hence skin contact is one of the major ways fungi infection can be contacted.

Table 1: Frequency of fungi isolates from both male and female

S/N	Isolates	Frequency	
		Male	Female
1	*Trichophyton* species	25%	10%
2	*Microsporum* species	15%	5%
3	*Epidermophyton* species	20%	5%

Table 2: Fungi growth among pupils in primary school A in Wukari

Sex	Number of Samples	Number of viable growth count	Number of non - viable growth count
Males	40	35	5
Females	20	15	5
Total	**60**	**50**	**10**

Table 3: Fungi growth among pupils in primary school B Wukari

Sex	Number of Samples	Number of viable growth count	Number of non - viable growth count
Males	40	25	15
Females	20	5	15
Total	60	30	30

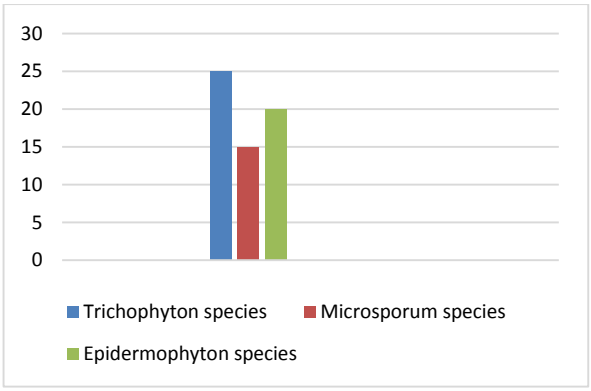

Fig. 1: Bar chart showing frequency of fungi isolates in males

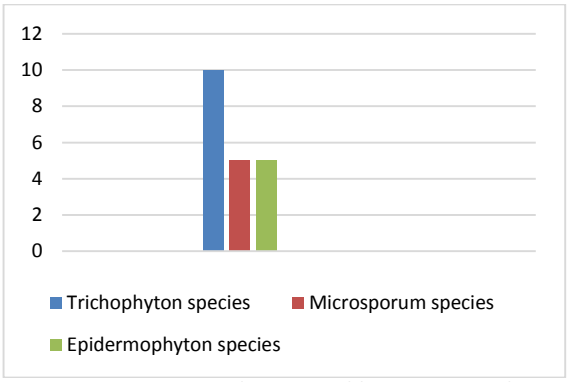

Fig. 2: Bar chart showing frequency of fungi isolates in females

These fungi isolated except *Microsporum* species have earlier been reported by other workers (Sharma *et al*., 2014). The *Microsporum* species isolated in this study may be due to the fact that yeast was observed to be a normal flora of the animal skin (Lin *et al*., 2004). From the overall result, the males had the highest number of isolate (60.0%) compared to that of the females which was 20.0%. In males, it was observed that the incidence of *Trichophyton* species (25%) is greater than (>) *Epidermophyto species* (20%) > *Microsporum* species (15%). In females, *Trichophyton* species (10.0%) > *Epidermophyto species* (5%) and *Microsporum* species (5%). The fungi isolated in this study are those that thrive on skins (Satana *et al*., 2011). The implication of their occurrence shows the ability to survive the effect of different types of creams and other skin treatment (Laksmipathy and Kannabiran 2010). The variation is the number of fungi isolated from skin suggested that fungi are able to tolerate the conditions and treatments in

skin, and possible higher ability to carry out metabolic activities in human (Ghasemi *et al*., 2014). In addition, increased acidity and salinity resulting from sweat in skin may support growth of fungi (Gupta and Ramnani 2006). The fungal species *Trichophyton* species, *Microsporum* species and *Epidermophyton* species isolated in this study are opportunistic pathogen commonly colonizing human skin surfaces as a component of the normal microflora.

However, when host defenses are weakened or when there is a disruption in the host environment, these opportunistic organisms can proliferate, causing an array of infections ranging from mucosal to systemic that are often life threatening. Of all the organisms that were isolated, it was only *Trichophyton* species that was reported pathogens by Ghahfarokhi *et al.* (2004). These pathogenic fungi when subjected to *Stellaria citrifolia* ethanol extract all the isolated were susceptible to the extract and when these same pathogenic fungi were tested on *Stellaria citrifolia* aqueous extract the organisms were all susceptible the extract. This mean that the effectiveness of the *Stellaria citrifolia* is not affected by both the aqueous and ethanol extract medium, this is in agreement with the work of Sharma and Swati (2012); Achterman *et al*. (2011). Because all the pathogenic fungi were susceptible to the extract it means that *Stellaria citrifolia* is active against isolates of *Trichophyton* species (A dermatophyte), *Microsporum* species and *Epidermophyton* species.

Conclusion

These fungi have effect on the wearers and the shoe itself. This corresponds with the result obtained from previous researched work. Students and other individuals should always try to dry and clean their shoes after each use, to avoid dampness as this facilitate the growth of these pathogenic fungi reported in this study. Those affected with the pathogenic fungi should seek for medical help immediately it is discovered. Proper methods of preserving the leather shoes including dehydration and use of antifungal agent should be developed. More studies should be conducted to determine the genetic status of the pathogenic strains which is hitherto nonpathogenic. Finally, work should also be carried out to know the various pathogenic organisms associated with shoes worn at different times of the day to determine any relationship between organisms and the weather condition, that is, hot or cold weather.

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