
ANTI-FUNGAL ACTIVITIES OF ETHANOLIC EXTRACTS OF *Moringa oleifera* AND *Calotropis procera* LEAVES ON DEMATOPHYTES ISOLATED FROM CHILDREN WITH TINEA CAPITIS

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ABSTRACT

Tinea capitis caused by dermatophytes is the most common scalp infection among children especially in the tropical regions. The treatment options are quite challenging as it is always systemic and topical antifungal agents do not penetrate down to the deepest part of the hair follicle. Most of the available chemotherapeutic agents have the disadvantage of long duration of treatment, potential adverse effects and they are expensive. The biological control / containment of disease causing pathogens or their disease severity inhibition are always cheaper and sustainable for the larger populace. The focus of this study was to investigate the potency of metabolites from *Moringa oleifera* and *Calotropis procera* as antifungal agents for the control of Tinea capitis. *Microsporium gypseum* and *Trichophyton verrucosum* were isolated from children presenting with Tinea capitis on Mycobiotic Agar using spread plate method. The antifungal activities of the ethanolic extracts of *Calotropis procera* and *M. oleifera* leaves against these two dermatophytes were investigated using the food poison method with extract concentrations of 50, 100, 150 and 200mg/ml. The results obtained indicated that ethanolic extract of both *C. procera* and *M. oleifera* leaves were able to inhibit the mycelial growth of *T. verrucosum* and *M. gypseum* *in vitro* to different degrees at varying concentrations. There was total inhibition (100%) of the mycelia growth of *T. verrucosum* at a concentration of 200 mg/ml while *M. gypseum* mycelial growth had 88% inhibition at the same concentration. The results showed that *T. verrucosum* was more susceptible to the extracts than *M. gypseum*. Therefore, the antifungal potentials of these plants should be harnessed for developing more anti- fungal drugs, in order to effectively control the increasing prevalence of mycotic infections, particularly in Nigeria and in the tropics and subtropics at large.

Key words: *Moringa oleifera*, *Calotropis procera*, *Microsporium gypseum* *Trichophyton verrucosum*, antifungal, ethanolic extracts

INTRODUCTION

In the developing countries of the tropics, skin infections are common diseases and the dermatophytes are responsible for a significant number of these diseases which are commonly called dermatophytosis (Wilson, 2006). Dermatophytes are parasitic fungi that invade the skin and cause infection on the skin nails and hair because of their ability to obtain nutrients from keratinized materials. Some of these fungi reside in the soil and are involved in natural decomposition of organic matter. They are known as geophilic species but occasionally act as parasites (Arenas *et al.*, 2006). Some of them are naturally adapted to man and are usually transmitted from one person to another, they are known as anthrophilic species. The zoophilic species are adapted to animals.

When dermatophytes invade a living host, they are restricted to the non-living layer of the epidermis in the immunocompetent host as they lack the ability to penetrate the viable tissues except when the immune system is compromised. These organisms grow best on areas of the skin that are warm, dark and moist. Infections from dermatophytes may be self-limiting and without any overwhelming effects but it may be discomfoting and disfigure the affected areas. Sometimes these dermatophytes invade the subcutaneous tissues and

other sites especially in the immunocompromised hosts. (Kontoyiannis *et al.*, 2003). Despite the significant reduction in the prevalence of dermatophytosis in many developed nations of the world due to improved social economic health-care and hygiene practice, countries like Nigeria a developing nation which is located in the tropical region, having a wet humid climate fall into the category of regions that still have a high prevalence of dermatophytosis, especially in school age children living in rural, suburban and urban areas (Rudy, 1999; Nweze, 2010). Factors such as environmental hygiene, culture, socioeconomic dispositions, overcrowding, health care geographic location, prevailing climatic conditions (such as temperature, humidity, wind) and immigration, are very important in the proliferation of dermatophytosis. (Hay, 2003; Havlickova *et al.*, 2008). Superficial fungal infections in man and animals are of public health importance and it is one of the most common cutaneous infections worldwide (Havlickova *et al.*, 2008; Ameen, 2010)

The development of novel and efficient antifungal agent is lagging far behind despite the advances in the production of antibacterial drugs due to the fact that fungi are also eukaryotic with mechanisms that is almost the same as that of human beings which makes the development of antifungal agents that will target

the fungi specifically without damage to the host difficult. Many of the conventional antifungal drugs are very effective in the treatment of fungal infections the attendant side effects notwithstanding but many patients cannot afford the cost of these conventional antifungal agents. This study will provide information on the antifungal activities of the ethanolic extracts of *M. oleifera* and *c. procera* leaves on dermatophytes responsible for tinea capitis and the results will determine the probability of the plants being considered in the search of new sources of anti-mycotic drugs.

MATERIALS AND METHODS

Collection and Identification of Plants

The leaves of actively growing *c. procera* and *M. oleifera* plants were randomly collected by hand plucking around Polo area in Maiduguri, Borno state, Nigeria. The plants were first identified on the field using standard keys and descriptions (Keay, 1989). The botanical identification was confirmed at the Biological science department, University of Maiduguri.

Preparation of Plant Extract.

This was carried out in accordance with the method of Kareem *et al.*, (2003). The leaves were thoroughly washed under running water, rinsed with distilled water and air dried. The dried leaves were ground into powdered form by

using mortar and pestle as described by Fatope *et al.*, (1993). The powdered form of the leaves was then stored in air tight containers until required for use.

Extraction protocol

Extraction was carried out using the method of Fatope *et al.*, (1993) at the pharmacognosy laboratory, University of Maiduguri using soxhlet extraction techniques. 100grams of the fine powder of the leaves of each plant was weighed and suspended into 250 ml capacity conical flask. This was percolated with 1000ml of 95% ethanol and allowed to soak for 5 days with constant shaking at regular intervals. The percolates were then filtered with whatman filter paper and the solvent was evaporated using Soxhlet rotary evaporator. It was further left to evaporate to dryness at room temperature for 48 hours.

Phytochemical screening

Phytochemical screening was done in order to detect the presence of bioactive constituents such as alkaloids, tannins, saponins, phenols, glycosides, flavonoids and glycosides using the methods described by Waterman (1993): Talukdar *et al.*, (2010).

Test for saponins

Two milliliter of the aqueous and ethanolic extracts in a test tube was shaken for two minutes. Frothing which per-

sisted on shaking was taken as evidence for the presence of saponins. (Waterman, 1993)

Test for alkaloids

Three milliliter of the ethanolic extracts was stirred with 5 ml of 1% HCL on a steam bath for twenty minutes. The solution obtained was cooled and filtered and few drops of Mayers reagent/picric acid were added to the filtrate. An orange coloration indicated the presence of alkaloid (Talukdar *et al.*, 2010).

Test for phenolics

Two drops of 5% ferric chloride were added to 5 ml of the crude ethanolic extract in a test tube. A greenish precipitate was taken as indication of phenolics.

Test for tannins

A volume of 1 ml of freshly prepared 10% potassium hydroxide was added to a volume of 1 ml of the ethanolic extracts. The presence of a dirty white precipitate was taken as indication of tannins.

Test for steroids

To a volume of 1 ml of the extracts, five drops of concentrated Tetraoxosulphate(VI) acid was added. Red coloration indicated the presence of steroids (Talukdar *et al.*, 2010).

Test for flavonoids

To a volume of three milliliter of the ethanolic extract, a volume of 1 ml of 10% Sodium hydroxide was added. A yellow coloration indicated the presence of flavonoids (Waterman, 1993).

Test for glycosides

To 5ml of extract in a test tube, 2 ml of dilute Sulphuric acid was added and boiled for 15 minutes, cooled and neutralized with 10% NaOH, and then 5ml of Fehling's solution was added. Glycosides are indicated by a brick red precipitate. (Talukdar *et al.*, 2010).

Isolation of test organisms

Sample Collection

Scalp scrapings of children presenting with tinea capitis at Molai General Hospital Maiduguri was collected. Scrapings were collected from the infected portion of the head using a blunt scalpel blade after disinfection of the affected area with methylated spirit. The sample was collected into a sterile sample bottle, properly labeled and transported to the Microbiology laboratory of the Department of Microbiology University of Maiduguri for culturing.

Preparation of Culture Media

The media used in this study were Mycobiotic agar(MA), and Sabourauds dextrose agar and they were prepared according to the manufacturer's directive.

Isolation of test organism

The dermatophytes were isolated using direct culture method. The skin scrapings were placed directly on the surface of prepared plates of Mycobiotic Agar and incubated at 37°C until growth was observed (Karimzadegan-Nia *et al.*, 2007)

The colonies of the isolates were sub cultured by taking inoculums from the growing edge of each colony and transferring into sterile Sabourauds Dextrose Agar plates. The pure cultures obtained were used for identification.

Identification of Fungal Isolates

Macroscopic Examination

The pure cultures of the isolated fungi were identified on the basis of cultural characteristics by observing the growth pattern, color, and texture of the colony. The colour of the reverse side of the colony was also observed (Ellis *et al.*, 2007).

Microscopic Examination

A drop of lacto phenol cotton blue was placed at the center of a clean slide, a small amount of fungal material was transferred to the lacto phenol cotton blue, teased out with two inoculating needles, covered with a cover slip and observed under the microscope using x10 and x40 magnification. Identification of fungal structures (hyphae and spores) was carried out according to the method

Ellis *et al.*, (2007). They were stored on Sabourauds Dextrose Agar slant in the refrigerator at 4°C prior to use.

Antifungal Activity Assay

The crude extracts were made into a suspension using Dimethyl Sulphoxide (DMSO) for susceptibility testing. The concentrations of the extracts were made to 200mg/ml and other concentrations were prepared by serial dilution. The antifungal activity of the ethanolic extract of *C. procera* and *M. oleifera* was evaluated against the dermatophytes by using poisoned food technique (Pundir *et al.*, 2010). The two test dermatophytes were separately inoculated on Sabouraud Dextrose Agar (SDA) plates that contains chloramphenicol supplement to suppress bacterial growth and incubated for 7 days at 37°C, to obtain young, actively growing colonies of dermatophytes.

The pH of the plant extracts were adjusted to 5 and 1 ml of each plant extract at four (50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml) different concentrations was mixed with 15ml of cooled (45°C) molten SDA medium in separate petri dishes and allowed to solidify at room temperature for thirty minutes. A mycelial disc 6 mm diameter, cut out from periphery of 7 day old cultures, were aseptically inoculated onto the agar plates containing each of the plant extract, SDA plates containing

1ml of griseofulvin were used as positive control. Sabouraud dextrose agar plates with 1ml of distilled water were used as negative control (Georgii and Korting, 1991; McCutcheon *et al.*, 1994). The inoculated plates were done in triplicates and incubated at 37°C. Colony diameter was measured and recorded after 7 days and the mean of three readings is recorded (Pundir *et al.*, 2010). Percent mycelia growth inhibition was calculated as given below.

$$\text{Percent mycelia growth inhibition (\%)} = \frac{Da - Db}{Da} \times 100$$

Where Da represents control colony diameter and Db represents treated colony diameter. The colony diameter is in millimetres.

RESULTS

Microsporum gypseum and *T. verrucosum* were isolated from the scalp scrapings of children presenting with tineacapitis at Molai General Hospital Maiduguri.

Phytochemical properties of the ethanolic extract of *C. procera* leaves revealed the presence of, glycosides, flavonoids, saponins, steroids and tannin. Phenolics and alkaloids were not detected (Table 1). Phytochemical properties of the Ethanolic extract of *M. oleifera* leaves revealing the presence of

alkaloids, flavonoids, saponins, steroids, and tannin. Phenolics and glycosides were not detected (Table 2). The antifungal activity of ethanolic extract of *M. oleifera* against *T. verrucosum* and *Microsporum gypseum* is presented in Table 3. The result shows that the extract has inhibitory effect against the two dermatophytes but a higher inhibitory effect against *T. verrucosum* than *Microsporum gypseum* at the various concentrations was observed. At 200 mg/mm concentration, the growth of *T. verrucosum* was completely inhibited. The positive control had maximal growth while there was no growth in the negative control which contained griseofulvin. The percentage of dermatophyte mycelial growth inhibition by different concentrations of ethanolic extract of *M. oleifera* leaf extract is presented on Table 4. The extract shows a higher percentage of mycelial inhibition in *T. verrucosum* than that of *Microsporum gypseum*. At 50mg/ml concentration, there was 42% inhibition of *Microsporum gypseum* mycelia growth while *T. verrucosum* growth had 50% inhibition. Inhibition of mycelia for *Microsporum gypseum* at concentration of 100mg/ml was 50% while that of *T. verrucosum* at the same concentration was 65%. At 150mg/ml concentration *Microsporum gypseum* had 65% mycelia inhibition, while *T. verrucosum* had 77% mycelia inhibition at the same concentration. The

mycelia growth of *Microsporium gypseum* was 86% at a concentration of 200mg/ml. The mycelia of *T. verrucosum* was completely inhibited (100 %) at the same concentration. The antifungal activity of ethanolic extract of *C. procera* against *T. verrucosum* and *Microsporium gypseum* is presented in Table 5. The result shows that the extract has a higher inhibitory effect against *T. verrucosum* than *Microsporium gypseum* at the various concentrations. *Microsporium gypseum*. There was complete inhibition of *T. verrucosum* growth at 200mg/mm concentration.

The percentage of dermatophyte mycelial growth inhibition by ethanolic extract of leaves of *C. procera* is presented in Table 6. The extract shows a higher percentage of mycelial inhibition in *T. verrucosum* than in *Microsporium gypseum*. At 50mg/ml concentration of the extract, *Microsporium gypseum* had 46% mycelia growth inhibition while at the same concentration *T. verrucosum* mycelial growth had 56% inhibition. The mycelial growth inhibition of *Microsporium gypseum* at extract concentration of 100mg/ml was 56% while *T. verrucosum* mycelial growth had 73% inhibition. At a concentration of 150 mg/ml the mycelial growth of *Microsporium gypseum* had 74% inhibition while *T. verrucosum* mycelial growth had 95% inhibition. There was total inhibition (100%) of mycellia growth of *T. verru-*

cosum at a concentration of 200mg/ml while *Microsporium gypseum* mycelial growth had 88% inhibition at the same concentration.

DISCUSSION

The detection of alkaloids, flavonoids, saponins, steroids, and tannin in the etanolic extracts of the leaves of *M. oleifera* is in agreement with the work of Patel *et al.* (2014) who also detected the presence of these same phytochemicals in the ethanolic extracts of *M. oleifera* leaves. The ethanolic extract of *C. procera* leaves revealed the presence of glycosides, flavonoids, saponins, sterols and tannin. These same phytochemicals were detected by Sharma (2011) from the ethanolic extracts of the leaves, rots and stem of *C. procera*. Coumarins and triterpenes were other phytochemicals reported in their study which was not detected in this study. This may be due to differences in geographical locations and soil composition. According to Akinyemi (2006) these naturally occurring compounds found in plants have been shown to possess antifungal activities. The Phytochemical components of these extracts detected in this work are responsible for both pharmacological and toxic activities in plants. These medicinally bioactive components exert antimicrobial action through different mechanisms.

The ethanolic extract of both *C. procera* and *M. oleifera* leaves were able to inhibit the mycelial growth of *T. verrucosum* and *M. gypseum* to different degrees at varying concentrations which is in agreement with the work of Chuang *et al.* (2005). He stated that the essential oil of *M. oleifera* seed and leaves had inhibitory effects on *T. rubrum*, *T. mentagrophytes* and *M. canisin-vitro*. Manguro and Lemmen (2007) also in their study showed that ethanolic extracts of seed and leaves of *M. Oleifera* had antifungal activity against *T. rubrum* and *M. canis*. Domenico *et al.* (1999), reported that the ethanolic extract of the bark *C. procera* was active against dermatophytes which is in consensus with the result of this study. Additionally Gupta *et al.* (1998) stated that the ethanolic extract of *C. procera* inhibited all the species of dermatophyte used in their study. According to Zaffer *et al.* (2015) ethyl acetate and methanol extracts of *M. oleifera* leaves had inhibitory effects on *M. gypseum* which is in line with the result of this study. *T. verrucosum* was more susceptible to the ethanolic extracts of both plants used in this study than *M. gypseum* his corroborates the work of Aliyu (2015) which showed that the extracts of *C. procera* screened for antifungal activity against *Trichophyton* spp., *Microsporum* spp., and *Epidermo-*

phyton spp. revealed *Trichophyton* spp. being the most susceptible.

CONCLUSION

T. verrucosum is a zoophilic fungus causing ringworm in cattle. Infections in humans result from direct contact with cattle or infected fomites and are usually highly inflammatory involving the scalp, beard or exposed areas of the body while *M. gypseum* is a geophilic fungus with a world-wide distribution which may cause infections in animals and humans, particularly children. These dermatophytes can hardly be avoided by children and adults who have occupational exposure. The susceptibility of these two dermatophytes to ethanolic extracts of both *C. procera* and *M. oleifera* leaves which are plants that are readily available and easily accessible, present a huge biological potential as safe and effective agents in the search for new therapeutic agents that could be used as an alternate source for the treatment of skin diseases caused by these dermatophytes since the pharmaceutical arsenal currently available against them is limited and has many attendant side effects. Most antifungal drugs are expensive and have various degrees of toxicity. It is therefore necessary to carry out more research with a view to developing more anti-fungal drugs, in order to effectively control the increasing prevalence of mycotic infections, particularly in the tropics and

subtropics. Natural materials such as those from plant products which have been found to possess one or more medicinal properties should be harnessed by pharmaceutical research centers and industries in this search. The potentials of these plants should be harnessed.

Table 1: Phytochemical properties of the Ethanolic extract of *Calotropis procera* leaves

PHYTOCONSTITUENTS	PRESENCE/ABSENCE
Glycosides	+
Alkaloids.	-
Flavonoids.	+
Saponins	+
Tannin	+
Phenolics	-
Steroids	+

Key: + = present, - = absent

Table 2: Phytochemical properties of the Ethanolic extract of *Moringa olifera* leaves

PHYTOCONSTITUENTS	PRESENCE/ABSENCE
Alkaloids.	+
Flavonoids.	+
Saponins	+
Tannin	+
Steroids	+
Phenolics	-
Glycosides	-

Key: + = present, - = absent

Table 3: Antifungal activity of ethanolic extract of *Moringa oleifera* leave (diameter of the zone of mycelial extension, mm) against two dermatophytes

Fungi	Concentration of <i>Moringa olifera</i> leave extract(mg/ml)				Negative control	Positive control
	50	100	150	200		
<i>Trichophyton concentricum</i>	20.3 ±0.80	14.3 ±0.90	9.37 ±0.72	0.00	40.67	0.00
<i>Microsporium gypseum</i>	22.5 ±0.7	19.40 ±0.5	13.43 ±0.4	5.53 ±0.6	39.10	0.00

Table 4: The percentage (%) mycelial growth inhibition of ethanoilc extract of *Moringa oleifera* leaves against two dermatophytes by poisoned food technique

Fungi	Concentration of <i>Moringa oleifera</i> leave extract(mg/ml)				Negative control	Positive control
	50	100	150	200		
<i>Trichophyton verrucosum</i>	50.10%	64.84%	77.00%	100%	0.00%	100%
<i>Microsporum gypseum</i>	42.46%	50.40%	65.65%	85.86%	0.00%	100%

Table 5: Antifungal activity of ethanolic extract of *Calotropis procera* leaves (diameter of the zone of mycelial extension, mm) against two dermatophytes

Fungi	Concentration of ethanolic extract of <i>Calotropis procera</i> leaves (mg/ml)				Negative control	Positive control
	50	100	150	200		
<i>Trichophytonc on centricum</i>	18.13±0.47	11.03±0.56	02.1±0.26	0.00	40.67	0.00
<i>Microsporum gypseum</i>	21.23±0.54	17.4±0.26	9.8±0.52	4.67±0.54	39.10	0.00

Table 6: The percentage (%) mycelial growth inhibition of ethanoilc extract of *Calotropis procera* leave against two dermatophytes by poisoned food technique

Fungi	Concentration of ethanolic extract of <i>Calotropis procera</i> leave (mg/ml)				Negative control	Positive control
	50	100	150	200		
<i>Trichophyton verrucosum</i>	55.52%	72.88%	94.84%	100%	0.00%	100%
<i>Microsporum gypseum</i>	45.70%	55.50%	74.94%	88.10%	0.00%	100%

REFERENCES

- Akinyemi, K.O., Oluwa, O.K. and Omomigbehin, E.O. (2006). Antimicrobial Activities of Three Medicinal Plants Used In South- West Nigerian Folk Medicine on Some Foodborne Bacteria Pathogens Africa Journal of Traditional Complementary and Alternative Medicine 3(4):13-22
- Aliyu, R.M, Abubakar, M.B, Kasarawa A.B., Dabai, Y. U., Lawal, N. and Bello, M., B. (2015). Efficacy and phytochemical analysis of latex of *Calotropis procera* against selected dermatophytes. *Journal of Intercultural Ethnopharmacol*; 4:314-7.
- Ameen, M. (2010). Epidemiology of superficial fungal infections. *Clinical Dermatology*. 28:197-201.
- Arenas, R, Toussaint, S. and Isa-Isa, R. (2006). Kerion and dermatophytic granuloma .Mycological and histopathological findings in 19 children with inflammatory tineacapitis of the scalp. *International Journal of Dermatology*. 45:215-9.
- Chuang, P., Lee, C, Chou, J., Murugan, M., Shieh, B. and Chen, H. (2005). Anti-Fungal Activity of Crude Extracts and Essential Oil of *Moringa Oleifera* Lam. *Bioresource Technology* 98: 232–236.
- Domenico.B. (1999). Novel antifungal drugs. *Current Opinion in Microbial Research*. 2:509-15.
- Ellis, D., Davis, S., Alexiou, H., Handke, R, and Bartley, R. (2007). Descriptions of Medical Fungi 2nd Edition Nexus Print Solutions 153 Holbrooks Road Underdale, South Australia 2032 ISBN 9780959851267

- Fatope, M.O, Ibrahim H. and Takeda, Y. (1993): Screening of higher plants reputed as pesticides using the brine shrimp lethality assay. *International Journal of pharmacognosy***31**:250-254.
- Georgii, A. and Korting, H.C. (1991). Antifungal susceptibility testing with dermatophytes. *Mycoses*, **1**: 193-199.
- Gupta, A.K., Konnikov N, Mac Donald, P., Rich, P., Rodger, N, V. V. and Edmond, M.W. (1998). Prevalence and epidemiology of toenail onychomycosis in diabetic subjects: A multicenter survey. *British Journal of Dermatology*.**139**:665-671.
- Havlickova, B, Czaika, V.A. and Friedrich, M. (2008). Epidemiological trends in skin mycoses worldwide. *Mycoses***51**:2-15.
- Hay, R.J. (2003). Fungal infections (mycoses). In: Warrell DA, Cox TM, Firth JD and Benz EJ Jr (Eds.). Oxford Textbook of Medicine 4th Ed. Oxford. Oxford University Press Section 7.12.1
- Kareem, S.O., Akpan, I. and Oshon, M.B. (2003). *Calotropis procera* [seldom apple]. A potential plant for enzyme purification. *Journal of Bioscience technology*. **87**:133-135.
- Karimzadegan-Nia, M., Mir-Amin-Mohammadi A., Bouzari N. and Firooz.(2007). Comparison of direct smear, culture and histology for the diagnosis of Onychomycosis. *Australian Journal of Dermatology* **48**(1): 18-21
- Kontoyiannis, D.P, Mantadakis, E. and Samonis, G. (2003). Systemic mycoses in the immunocompromised host: an update in antifungal therapy. *Journal of Hospital Infection*.**53** (4) 243-258.
- Manguro, L.O. and Lemmen, P. (2007). Phenolics of *Moringa Oleifera* leaves. *National Product Research*.**21**: 56
- McCutcheon, A.R., Ellis, S.M., Hancock, R.E.W. and Tower, H.G.W. (1994). Antifungal screening of medicinal plant of British Columbian native people. *Journal of Ethnopharmacol*. **44**: 157-169.
- Nweze, E.I. (2010). Dermatophytosis in Western Africa: a review. *Pakistan Journal of Biological Science*. **13**(13): 649-656.
- Patel, P., Patel, N., Patel, D., Desai, S. and Meshram, D. (2014). Phytochemical Analysis and Antifungal Activity of *Moringa Oleifera* *International Journal of Pharmacy and Pharmaceutical Sciences* **6**(5): 201.
- Pundir, R.K., Pranay, J. and Sharma, C. (2010). Antimicrobial activity of ethanol extracts of *Syzygium aromaticum* and *Allium sativum* against food associated bacteria and fungi. *Ethnobotanical Leaflets*, **14**:344-60

- Rudy, S.J. (1999). Superficial fungal Infection in children and adolescents. *Nurse Practical Forum***10**: 56-66.
- Sharma, A.K., Kharb, R. and Kaur, R. (2011) Pharmacognostical Aspects of *Calotropis Procera* International *Journal of Pharmacognosy and Biological Sciences*. 2(3):480-488
- Talukdar, A.D., Choudhary, M.D., Chakraborty, M. and Duutta, B.K. (2010). Phytochemical screening and TLC profiling of plant extracts cyatheaGigantea (wall. Ex. Hook.) Haltt and Cyatheabrunoniana.Wall ex. Hook (cl. & Bark).*Assam University Journal of Science and Technology: Biological and Environment Science* 2010; 5(1): 70-74.
- Waterman, P.G (1993).Methods in plant biochemistry vol. 8 Academic Press.Pp 222-243.
- Wilson, J. (2006). Infection Control in Clinical Practice. 3rd edition, BallièreTindall Elsevier,London.
- Zaffer, M., Ahmad, S., Sharma, R., Mahajan, S., Gupta, A. andAgnihotri, R. (2012). Antifungal activity and preliminary phytochemical analysis of bark extracts of *Moringa oleifera* Lam. *International Journal of Biosciences* **2**: 26-30.