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ANTIFUNGAL EVALUATION OF *DICHROSTACHY cinerea* (LEAVES AND STEM) EXTRACTS AGAINST SOME DERMATOPHYTES

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ABSTRACT
This study was carried out to evaluate the antifungal efficacy of *Dichrostachys cinerea* extracts against some isolates of dermatophytic fungi. The powdered plant materials were extracted via soxhlet extraction technique using methanol, dichloromethane and petroleum ether as extraction solvents. The extracts were subjected to phytochemical screening for qualitative detection of plant secondary metabolites. The extracts were further tested for antifungal activity against human isolates of *Microsporum canis, Trichophyton terrestre, Trichophyton mentagrophytes and Trichophyton verrucosum*. The results of phytochemical screening showed the presence of some secondary metabolites including; alkaloids, tannins, saponins, glycosides and steroid. Antifungal activity testing indicated that all the extracts were active against the isolates tested with the exception of *T. verrucosum*. Pet ether extracts showed the least activity as reflected in the zone of inhibition (24 mm at 50 mg/ml), against the isolates compared to methanol (30 mm at 25 mg/ml) and dichloromethane (30 mm at 50 mg/ml) extracts. The findings of this study, indicated that the plant is a potential for the drug candidate for the treatment of dermatophytic diseases and support the claim for their traditional use against skin diseases.

Keywords: Antifungal activity, *Dichrostachy cinerea*, *Microsporum canis*, *Trichophyton terrestre, Trichophyton mentagrophytes and Trichophyton verrucosum*

INTRODUCTION

For several years, there has been an increased intrest in the study of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents. The search for new antimicrobial agents is an important line of research because of the resistance acquired by several pathogenic microorganisms (Hossain *et al*., 2012; Riffle *et al*., 2002).

In many developing countries, plant materials have been known to be used as traditional medicine. Thus plants become readily accessible and economical sources available for the treatment of many infections and disease (Savoia, 2012). In recent years, medicinal plants had increased their appeal to pharmaceutical companies and the scientific research community. This was due to evidences that these plant derived compounds have a potential for many biological activities which include antimicrobial activity (Savoia, 2012). Scientist's aim is to extract and characterize active phytocompounds found in plants,since they have provided high activity profile drugs (Vaghasiya *et al.,* 2011).

Dermatophytosis is a superficial infection caused by a group of fungi Called dermatophytes. The infection is common world-wide with higher prevalence in tropical countries (Ramaraj *et al.*, 2016). The dermatophytosis infection referred as ringworm due to the appearance of the lesion.

Dermatophytes comprise of three major genera, Trichophyton, Microsporum and Epidermophyton. They are keratinophilic in nature and have the ability to colonise keratinized non-living tissues such as skin, hair and nail in human and animals (Kaufman *et al*.,2007). Although the infection is not invasive and easy to cure, it's wide spread nature and cost of the treatment is a major public health problem and causes colossal damage to economic status of the tropical countries (Ramaraj *et al.,* 2016).

Dichrostachys cinerea belong to the family fabacea. It is commonly referred to as sickle bush or sekelbos, Marabou-thorn, Christmas tree and Chinese lantern tree. It is commonly called "Dundu" among the Hausa speaking people of Northern Nigeria and "Kore" among the Yoruba speaking people of western Nigeria (Gill, 1992). In Zulu it is called as Ugagene, Umthezane and Umzilazembe (pacific Island Ecosystem at risk (Pier, 2005). It is widely distributed throughout South Africa and has now encroached upon many overgrown bushveld

regions. Despite this undesirable ecological impact, it is a valuable medicinal plant (Vanwyk and Gericke, 2003).

The Haikum Bushmen of Namibia chewed the fresh leaves to treat diarrhea, toothaches and Earache (Vanwyk and Gerick, 2003). It is also applied directly to snake bites. Extracts of the leaves and bark, as well as powdered bark are used for wound healing. Infusions of the roots are used to treat abdominal pain, cough and pneumonia (Vanwyk and Gerick, 2003). Powdered roots are sniffed to curb nose bleeds, while the leaves and roots are smoked to relieve head colds, and to treat tuberculosis and for treatment of epilepsy.

MATERIALS AND METHODS

Collection, Identification and handling of plant materials

The plant used for this research was *Dichrostachy*s *cinerea* (leaves and stem) which was obtained from International Institute for Tropical agricultural research (IITA) at Tarauni, Kano state .The plant was identified and confirmed with voucher specimens at the Department of Plant Biology, Bayero University Kano, on 4th February 2016, as demonstrated by (Demetrio *et al*., 2015).

Leaves and stem were air dried by spreading on sacks in an aerated room. This was pulverized in to powdered form using a laboratory pestle and mortar, it was then sieved and packed in a clean, sterile container as described by Mukhtar and Tukur (1999).

Extraction procedure

Extraction of plant material was done using Soxhlet extractor. A thimble containing one hundred grams of dried powdered plant materials was attached to the round bottom flask containing one thousand milliliters of solvents (1L). Extraction was done using three different solvents separately i.e. methanol, dichloromethane and pet-ether. The solvents were heated using heating mantle and begin to evaporate, moving through the apparatus to the condenser. The condensate was then dropped in to the reservoir containing the thimble, once the level of the solvent reaches the siphon it pours back in to the flask and the cycle repeats. The process was run exhaustively. The solvents were evaporated using a rotary evaporator leaving the yield of extracted material in the flask. All extracts were kept at 4ºC until use as demonstrated by (Fatope and Hamisu, 1997)

Determination of some physical properties of the plant extract

Texture was assesed manually with the help of glass rod and feeling of the particulate nature of the resultant fraction in between globe fingers as described by Adoum *et al.,* (1997)

Phytochemical screening

Test for saponins

This was carried out as demonstrated by Sofowora (1993), where one milliliter of plant extract was transferred into a test-tube. Distilled water was added to the test-tube and shaken vigorously. Persistent froth that lasted for about 15 minutes was observed indicating the presence of saponins.

Test for tannins

This was done by measuring two milliliters each of the extracts and diluted with distilled water in separate test tube and 2-3 drops of 5% ferric chloride ($FeCl_3$) was added. A green- black or blue coloration was observed indicating the presence of tannin as described by Ciulci, (1994).

Test for flavonoids

To four milliliters (4ml) of each of the fractions a piece of magnesium ribbon was added in to a test tube followed by conc. HCl drop wise. A colour ranging from orange to red magenta indicated the presenceof flavonoids (Sofowora, 1993).

Test for alkaloids

This was carried out according to the method reported by Ciulci (1994). To one milliliter of each extract in separate test tube, 2-3 drop of Meyer's reagent was added. A white precipitate indicates the presence of alkaloids.

Test for steroids

Two milliliters (2ml) of each extract was transferred in to a test tube containing chloroform (2ml), conc. $H_2S0_4$was subsequently added to form a lower layer. A reddish-brown ring at the interface of the two liquids and a violet colour in the supernatant layer indicate the presence of the steroids (Sofowora, 1993).

Test for glycosides

Ten milliliters (10ml) of 50% of H_2S0_4 was added to one milliliter of the extract in a test tube, the mixture was heated for 15 minutes. Ten mililiters of Fehling solution was added and the mixture boiled. Appearance of brick red precipitate indicates the presence of glycosides (Sofowora, 1993).

Test for terpenoids

Two milliliters (2ml) of chloroform was added to 0.5ml of the extract, 3ml of conc. $H_2S\ 0_4$ was added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids (Ciulci, 1994).

Test for anthraquinone

Zero-point five (0.5) ml of the extract was taken in to a dried test-tube and 5ml of chloroform was added and shacked for 5min. The extract was filtered and a drops of ammonia solution was added and shacked vigorously. A pink violet or red color in the ammoniacal layer (lower layer) indicates positive results (Ciulci, 1994).

Collection and Identification of test organisms

Sample collection

The test microorganism used for the antimicrobial activity screening are fungi. The test micro-organisms used for the antimicrobial activity screening are fungi. The samples were collected from infected individuals at "Tsangayar Almajirai' at Daurawa Tarauni LGA Kano State. Children of ages 5-15 years

old were randomly screened during the months of February to July, 2016 for fungal infections consistent with dermatophytosis on the skin (hands, face), scalp and feet from five selected schools. A Total of 100 students that showed visible clinical signs (erythema, alopecia, scaling etc.) of dermatophytic infection constitute the study population using Cochran's formula, $n=z^2pq/d^2$. Scraping from the edges of the skin or dull broken hairs from the margin of lesions consistent with dermatophytosis which were previously swapped with 70% ethanol were collected using scalpel blade and epilator forceps respectively in to folded aseptic envelope. Each of these envelopes was level with the age, date and site of collection and subsequently taken within 24 hours to the microbiology department at Bayero University Kano for identification. Information about the state of the classroom /school compound (environmental hygiene) and the children play pattern were also carefully gathered using both direct observations as described by (David, 1989).

Isolation of organisms

Isolation of the organisms was carried out in microbiology laboratory, Bayero University Kano. Collected samples (scales, hairs and lesion) were cultured on sabouraud's dextrose agar medium supplemented with 0.5mg/ml each of chloramphenicol and cyclohexamide and incubated at 37^0C for a period of 3-6 weeks. Pure isolates were generated by sub-culturing on sabouraud's dextrose agar and potato dextrose agar. Cultures were incubated for one month before discarding them as negative (Ellis *et al.*, 2007).

Identification of test organisms

Cultures were examined thrice weekly for the appearance of growth. Pure isolates were sub cultured on dermatophytes test medium and Trichophyton media (1, 2, 3, and 6) for both visual and macroscopic examination of cultural and morphological characteristics (reversed pigmentation, colour and growth pattern) as well as microscopic examination by tease mount technique in lacto phenol cotton blue (Polari *et al.*, 2015).

Lactophenols stain

Sterilized inoculation needle was used to remove small portion of the suspected colony which was then mounted in a drop of lactophenol stain on a clean, greese free microscope slide. The specimen was covered with a cover slip, the preparation was squashed with the butt of the needle and then otted off the excess fluid. The mount was examined under microscope at 40 magnifications for the presence and nature of macro and micro conidia (David, 1989)

Preparation of the different concentration of the extract

Different concentrations of the extracts were prepared by dissolving 50mg of each of the extracts in 5milliters of dimethyl sulfoxide (DMSO). Therefore, each stock solution had a concentration of 10,000mg/ml. from these stock solution four different concentrations of each of the plants were prepared. These are 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml respectively as reported by Yusha'u *et al*. (2008).

Inoculum Standardization

The inocula were prepared as described by Yusha'u *et al.,* (2009) Strains were sub cultured on Sabouraud's dextrose agar at 37^0C for 3-5 days. A loopful of the sub cultured isolate was picked using sterile wire loop and needle and emulsified in 5milliliter of 2% tween 80. The turbidity of the suspension was matched with that of 0.5 McFarland standard.

Antifungal Assay

A sterile Sabouraud's Dextrose Agar media incorporated with chloramphenicol and cyclohexamide was poured in to Petri plates. As soon as the agar solidified, five wells were made in to the plates with the help of a cork borer sterilized with alcohol and flame. A sterile swab was used to evenly distribute the standardized inoculums over the surface of the sterile sabouraud's dextrose agar; the plates were allowed to stand for 15minutes before use in the test. The extracts concentrations (0.1ml) were introduced in to the wells. The antibiotic (Ketoconazole, 25mg /ml) was introduced in to the central well to serve as control. The plates were labeled and left to stand for about 30 minutes before 72hrs incubation at 25^oC. The activity was determined by measuring the diameter of zone of inhibition (zones of clearance produced around the or well). The diameter of inhibition zones was measured in mm and the results were recorded. The assay was done in triplicate (Jagesser, 2008).

STATISTCAL ANALYSIS

The results obtained after extraction were subjected to correlation analysis to compare between the quantity of the extract yield and the boiling point of the solvents using Microsoft word excel.

RESULTS

Physical characteristics of the plant extracts

Physical characteristics of the plant extracted are presented in Table 1. From the data presented, the initial weight of all the plant powder was 200g. The percentage yield of *D. cinerea* methanol leaves extract (DLM) was (40%), *D. cinerea* leaves Dichloromethane extract (DLD) was (30%), *D. cinerea* leaves petroleum ether extract (DLP) was (15%), *D. cinerea* stem methanolic extract (DSM) was (30%), *D. cinerea* stem Dichloromethane extract (DSD) was (25.1%), *D. cinerea* stem petroleum ether extract (DSP) was (5%),

The colour of DLM, DLD, DLP, DSM, DSD and DSP were dark brown, dark brown, dark green, dark green, dark brown, dark green respectively. The texture of all the extracts was gummy and they were all (extracts) soluble in Dimethylsulfoxide (DMSO).

Some phytochemical component including alkaloids, saponins and flavonoids were present in all the extracts, while Tannin was absent in *D. cinerea* leaves petroleum ether extract, Steroids and Glycosides were absent at (Dichloromethane and

petroleum ether) leaves and petroleum ether stem extracts of *D. cinerea*. Terpenoids is only present in the methanol leaf extract of *D. cinerea*, while Anthraquinone is only present in methanol and dichloromethane leaf and stem extract of *D. cinerea (*Table 2).

The leaves extract contained a higher quantity of phytochemicals than the stems in *(*Table 1*)*.

Identified organism

After seven to thirty days of incubation, the isolated organisms were identified based on their morphological features (colony colour, surface pigment, reverse pigmentation and ability to grow on cyclohexamide containing agar (Table 3), as well as microscopic appearance. They include *M. canis, T. terrestre, T. mentagrophytes and T. verrucosum*

Inhibitory activity of the plant extracts on the test organisms

The inhibitory effect of *D. cinerea* leaves and stem extracts showed that all extracts were effective against all the dermatophytes isolates tested with the exception of *Trichophyton verrucosum* which showed resistance to all the extracts. The methanol extract has the highest activity followed by dichloromethane extract, while petroleum ether extract produces lowest zone of inhibition. The zone of inhibition ranges from 6mm to 30mm at concentration of 6.25 to 50mg/ml, for methanol ranges from 8mm to 30mm, 10mm to 30mm for dichloromethane and 6 to 20mm for petroleum ether. The *M. canis* exhibited the highest zone of inhibition, while *T. verrucosum* was resistant at all the concentrations used. In *M. canis* and *T. terrestre*, the extracts produce higher zone of inhibition than the control (Ketoconazole), while *T. verrucosum* was only sensitive to Ketoconazole at 25mg/ml with 40mm zone of inhibition (Tables 4 and 5).

Table 1: Physical characteristics of the plant extracts

Extract	% yield	Color	Boiling point ^{0}C	Texture
DLM	40	Dark brown	65	Sticky
DLD	30	Dark brown	40	Sticky
DLP	15	Dark green	40	Gummy
DSM	30	Dark green	65	Gummy
DSD	25.4	Dark Brown	40	Gummy
DSP	5	Dark green	40	Sticky

Key: DLM=*Dichrostachys cinerea* leaves methanol extract, DLD=*Dichrostachys cinerea* leaves dichloromethane extract, DLP=*Dichrostachys cinerea* leaves petroleum ether extract, DSM=*Dichrostachys cinerea* stem methanol extract, DSD=*Dichrostachys cinerea* stem dichloromethane extract, DSP=*Dicrostachys cinerea* stem petroleum ether extract.

Table 2 Phytochemical component of *D cinerea* leaves and stem

	Extracts											
Phytochemical component	DLM	DLD	DLP	DSM	DSD	DSP	DLM	DLD	DLP	DSM	DSD	**DSP**
Alkaloid	+	+	+	+	+	+	+	+	+	+	+	+
Saponin	+	+	+	+	+	+	+	+	+	+	+	+
Tannin	+	+	-	+	+	+	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+	+	+	+	+	+	+
Steroid	+	-	-	+	+	-	+	+	+	+	+	+
Glycoside	+	-	-	+	+	-	+	+	+	+	+	+
Terpenoids	+	-	-	-	-	-	-	-	-	-	-	-
Anthraquinone	+	+	-	+	-	-	-	-	-	-	-	-

Key DLM=*Dichrostachys cinerea* leaves methanolic extract,
DLD=*Dichrostachys cinerea* leaves dichloromethane extract,
DLP=*Dichrostachys cinerea* petroleum ether extract,
DSM=*Dichrostachys cinerea* stem methanolic extract,
DSD=*Dichrostachys cinerea* stem dichloromethane extract,
DSP=*Dicrostachys cinerea* stem petroleum ether extract,
(+) =Present,
(-) = Absent.

Table 3: Macroscopic characteristics of the isolated organism

DTM	TM 1	TM 2	TM 3	TM 6	ORGANISM
White cottony appearance with closely space radial groove and change the media to red colour	Poor growth +	Fair/Partial growth ++	Fair growth +++	Massive whitish cottony growth with raised center	*Microsporum canis*
Flat yellowish raised center with pheripheral white cottony thallus and red colour around the colony	Growth was enhanced indicating no special nutritious requirements	Growth was enhanced with black reversed pigmentation	Growth was enhanced with no reversed pigmentation	Growth was inhibited	*Trichophyton mentagrophyte*
White cottony with pink center and change the media entirely to red colour	Creamy flat entire colony with pheripheral pringes	Creamy flat entire colony with pheripheral pringes	Creamy flat entire colony with pheripheral pringes	White cottony growth with irregular edges	*Trichophyton terrestre*
	Poor growth	Fair growth	Good growth		*Trichophyton verrucosum*

Table 4: Activity of *D cinerea* leave extracts on the tested organism

	Zone of Inhibition (mm)												
Organisms	**DLM**				**DLD**				**DLP**				**Control**
	50	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**25**
Microsporum canis	30	30	26	20	30	26	0	0	1	0	10	0	30
Trichophyton terrestre	20	8	0	8	12	12	0	0	20	11	0	0	20
Trichophyton mentagrophyte	18	17	15	0	20	15	10	10	20	13	13	8	27
Trichophyton verrucosum	0	0	0	0	0	0	0	0	0	0	0	0	40

Key: DLM= *D cinerea* leaves methanol extract, DLD=*D cinerea* leaves dichloromethane extract, DLP=*D cinerea* leaves petroleum ether extract

Table 5: Activity of *D cinerea* stem extracts on the tested organism

Organisms	Zone of Inhibition (mm)												
	DSM				DSD				DSP				Control
	50	25	12.5	6.25	50	25	12.5	6.25	50	25	12.5	6.25	25
Microsporum canis	30	20	18	10	28	24	20	16	0	0	0	0	30
Trichophyton terrestre	25	20	18	8	28	24	15	8	0	0	0	0	20
Trichophyton mentagrophyte	20	20	16	0	16	14	14	0	18	18	8	0	27
Trichophyton verrucosum	0	0	0	0	0	0	0	0	0	0	0	0	40

Key: DSM= *D cinerea* stem methanol extract, DSD=*D cinerea* stem dichloromethane extract, DSP=*D cinerea* stem petroleum ether

Table 6: Correlation between quantity of yield extract and boiling point of the solvent

Quantity of the yield extract %	Boiling point of the solvent ^{0}C
40	65
30	40
15	40
30	65
25.4	40
5	40
Coefficient value	0.671331

DISCUSSION,

In this study methanol solvent produced a higher extracts yield, followed by dichloromethane, while the petroleum ether yielded the least. Similarly, higher yield of extract was observed in the leaf and seed as compared with the stem, which tallies with the work of Vashka (2009) that higher yield was obtained from leaves. The variability in the percentage yield of the plant parts may be because the stems of most plants act as translocation organs not as storage organs and as expected will have fewer compounds in them.

The phytochemical analysis conducted on *D. cinerea* extracts revealed the presence of tannins, flavonoids, steroids and saponins, which corresponds with the report of Banso and Adeyemo (2007) that *D. cinerea* have tannins, alkaloids and glycosides. The methanolic extracts of the leaves and stems contained tannins, Saponin, alkaloid, flavonoid and steroids, although terpenoids and anthraquinone were only found in the leaf. This corresponds with the report of Vennapoose *et al., (*2013). The dichloromethane extracts contained only alkaloids, Saponins, tannins, flavonoids ad anthraquinone while the stem extracts contained alkaloids, Saponins, tannins, flavonoids, steroids, glycosides and anthraquinone, the petroleum ether extract contain alkaloids, Saponins, tannins and flavonoids, which report agrees with the work of Zumbes *et al*. (2005) where only Anthraquinone is absent.

The variation in phytochemical constituents resulted from the extraction ability of a particular component which appeared to depend on extraction medium polarity (solvent) and the ratio of solute to solvent as well as increase in temperature (Simon, 2015).

The various extracts of *D. cinerea* plant exhibited activities against all the tested isolates with the exception of *Trichophyton verrucosum*. Of the three extraction solvents, methanol and dichloromethane extracts gave wider zones of inhibition of (30mm) as compared to petroleum ether extracts (20mm), this is because the methanol and dichloromethane has high polarity which makes them to extract more substances.

The inhibitory activities exhibited by the test extract tends to agree with the report that antimicrobial properties of these plants are due to the presence of tannins alkaloids, Flavonoid terpenoids or essential oils (Bassole *et al.,* 2003; Viljoe *et al.,* 2003; Erasto *et al.,* 2004). The zone of inhibition produced by

the extracts against the test organism indicated the potency of the active principles which have measurable antimicrobial activities against microorganisms.

The methanol leaves and stem extract of *D. cinerea* inhibited the growth of all the tested organisms with exception of *Trichophyton verrucosum*, which is resistant at all the concentrations used. It produces a high zone of inhibition against *M. canis* with 30mm zone of inhibition, which is similar to that produce by the ketaconazole (standard antifungal), this indicate that, either the concentrations of the extract used is not enough to have effect on *T verrucosum* or the organism has developed resistive material. This result does not agree with the work of Vashka (2009), who reported the inhibition of *Trichoderma spp* which possess innate resistance to most agricultural chemicals including fungicide. The dichloromethane extracts (leaves and stem) were active against the tested organism with the exception *of T. verrucosum*. It produces a high zone of inhibition on *M canis* with 30mm diameter zone of inhibition. The petroleum ether leaf extract exhibited it activity against all the tested isolates with the exception of *T. verrucosum* while the stem extract is only active against the *T. mentagrophytes*, where it produces the highest zone of inhibition of 20mm diameter, this variation occur due to the difference of bioactive compound present in the leaves and stem respectively . This agrees with the work of Vijaylakshimi (2004) and Zumbes *et al.,* (2005), in which they reported that pet ether extracts produce moderate activity at 25mg/ml to above and 250-500mg/ml respectively. the coefficient value indicate that the percentage yield of the extract and the boiling point of the solvents have high positive correlation, since the coefficient value was found to be one (1). That is the higher the boiling points the more the quantity of the extracts to obtained. There is positive relationship between the quantity of the extract yield and boiling point solvent, since the correlation coefficient value was found to be 0.67 (table 6).

CONCLUSION

The results of this study showed that the *D. cinerea (*leaves and stem) have exhibited varied antifungal activity against the *T. terrestre, T mentagrophytes, T. verrucosum and M. canis* .

RECOMMENDATIONS

Based on the findings from this study, it is recommended that further research should be carried out to identify and isolate the bioactive compounds and what made *Trichophyton verrucosum* to be resistant to almost all the extracts tested. The extracts should also be tested against a wide range of pathogenic microorganisms (viruses, parasitic protozoans, bacteria and fungi). There is need also to find the toxicity of the plant.

REFERENCES

Adoum, O.A, Dabo N.T. and Fatope MO (1997*) Bio-activities of some savannah plants in brine Shrimp lethality test an invitro antimicrobial assay. International journal of pharmacogno s*y **35 (5):334**.

Banso A, and Adeyemo, SO (2007). *Evaluation of antimicrobial properties of tannins isolated from Dichrostachys Cinerea*. *African Journal of Biotechnology*., **6(15)**: 1785-1787.

Bassole, I. N. H, Ouattara, A.S, Nebie, R, Ouattara C. A. T, Kabore, Z. I, and Traore, A.S (2003). *Chemical Composition and antimicrobial activities of the essential oils of Lippia and chevalieri Lippia multiflora* from Burkina Faso. *Phytochemical*, 62:209-212.

Ciulci, I. (1994). *Methodology for the analysis of vegetable drugs chemical industries branch, Division of industrial operations. Unido, Romania*: **24, 26 and 67**.

David, G.W. (1989*). Antimicrobial chemotherapy* oxford mechanical publication pp.91-99.

Demetrio, L; Valle, J. R; Jeamie, I. A; and Windell, L. R, (2015*). Asian Journal of Tropical Bio- Medicine. Antibacterial activities of ethanol extracts of Philippine medicinal plants again- St MDR-bacteria.*

Ellis, D., Stephen, D., Helen, A., Rosemary, H. and Robyn, B. (2007). *Description of Medical Fungi 2nd edition.Published by Authors, Australia, North Adelaide***, P84-170.**

Erasto, P., Bojase-Moleta, G. and Majinda, R. R. T. (2004). *Antimicrobial and antioxidant flavonoids from the roots wood of Bolusanthus spesiosus. Journalof Phytochem*ical; **65:875-880.**

Fatope, M.O. and Hamisu, I. (1997*). Screening of higher plants reported as pesticides using brine Shrimp lethality assay. International journal of pharmacognosy* **31:250-260.**

Gill, L.S. (1992). *Ethno medical uses of plants in Nigeria*. University of Benin press. Benin City, Nigeria**, p. 100.**

Hossain, K.M.Hassan, N., Parvin, M., Hasan, S. and Haque, A. (2012) *Antimicrobial Cyto-toxic And Thrombocytic activity of Cassia Senna leaves Journal of Applied pharmacognosy. Sciences.*

Jagesser R.C., Mars, A. and Gones, G. (2008). *Selective anti-microbial properties of leaf extract against various micro-*

organisms using Disc diffusion and Agar well diffusion method Journal of nature and science ***Vol 6 (2):24-38,***

Kaufman G, Horwitz BA, Duek L, Berdicevsky I. (2007). *Infection stages of the dermatophyte pathogen Trichophyton: microscopic characterization and proteolytic enzymes. Medical Mycology*, **45:149-155**.

Mukhtar, M.D. and Tukur, A. (1999). *Invitro screening for activity of Pistiastratiotes extract NISEB journal* **1 (1):51-60.**

Pacific Island Ecosystem at Risk (Pier), (2005). *Dichrostachys cinerea, Australian/New Zealand Weed Risk Assessment adapted for Hawaii, Research directed by C. Dachler with finding from the Kaulunani Urban Forestry Program and US Forest Service.*

Polari L V, Tyothi, pi and Sai L K (2015). *Clinicomycological Study of Dermatophytosis in South India. Journal of laboratory physicians* **7(2):84-89.**

Riffle, A. L.F., Medina, V., Steffens, R. C., Santos, D.B and Bizani, A.B, (2002). *Invitro antimicrobial activity of a new series of 1, 4-naphthaquinones. Brazilian Journal of Medicine and Biology. Res.***35:811-818.**

Savoia, D. (2012): *Plant-derived antimicrobial compounds; Alternatives to antibiotics*. **7:979-990**

Simon, B., Lidianys M., and Lewis-Lujan C. (2012). *Solvent effects on phytochemical constituents' profiles and antioxidant activities, using four different extraction Formulation for analysis of Bucidabuceras. BioMedCentral Researchnotes*.

Sofowora, E.A. (1993). *Medical plant and Traditional Medicine in Africa* (2nd edition), spectrum Books limited **Pp44-58,210-212.**

Vaghasiya, Y., Dave, R. and Chanda, S. (2011). *Phytochemical analysis of some medicinal plants from western region of India.Resident.Journal of Medicinal Plant* ***5:569-576***

Van Wyk, B; and Gericke, N, (2003) Peoples plants. *Aguide to useful plants of South Africa.* Pretoria: Briza publications. ISBN-1-875093095.

Vashka Hurinanthan. (2009*). Immune modulatory effect of Dichrostachys cinerea, Carpobrotus dimidiatus, Capparistomentosa and Leonotisleonurus. Durban Uni of Tech South Africa.*

Vennaposa, S., Devareddy, S. K., Sumathi, N., and Senthil, K. (2013). *Phytochemical and Antimicrobial Evaluations of Dichrostachys cinerea. International Research Journal of Pharmaceutical Sciences.***4 (1).**

Viljoe, A., Van Vuuren, S., Ernst, E., Klepser, M., Demirci, B., Basser, H., and Van Wyk, B.E. (2003) *Osmitopsisasteriscoides (Asteraceae*)-The antimicrobial and essential oil composition of a Cape Dutch remedy. J*ournal of Ethnopharmacology*, 88:137-143

Vijayalakshmi, K., Periyanayagam (2004) Quantitative microscopy and *Invitro* anti-dandruff activity *of Dichrostachys cinerea. Journal of Pharmaceutical and Scientific Innovation*, (5) 448-452.

Ramaraj Vijayakumar, Rajyogananda S Vijayraman, Sudha Rangaea Jan, Anumpma Jyoti Kindo (2016). *Incidence and prevalence of Dermatophytosis in and around Chennai, India. International Journal of Residence Medical Sciences* Mar; **4(3):695-700.**

Wijesundara, D.S.A. (2003). *Status of medicinal plants research in Srilanka and plans for inventory and documentation of medicinal plants in Sri Lanka*. Paper presented at the Asia Pacific. *Medicinal plant research meeting Kuala Lumpur.* Malaysia.

Yusha'u, M., Garba, L., and Shamsuddeen, U. (2008*). In-vitro Inhibitory activity of garlic and ginger extracts on some respiratory tract isolates of gram-negative organism. International Journal of Biomedical and Health Sciences.* **Vol 4, No 2,** Printed in Nigeria.

Yusha'u, m., Onuorah, F.C., and Murtala, Y. (2009*). In-vitro Sensitivity pattern of some Urinary Tract Isolates to Carica papaya extracts. BAJOPAS* **Volume 2.**

Zumbes, H. J., Mavak,J..D., Babalola, O.B., Chinyedu, C., Ekpiwre, D.V, Gokir J. D. and Dabo, A, D. (2015). Research paper*: Antibacterial and antidiarrhoeal Activities of Dichrostachys cinerea against some enteric pathogens. International Journal of Science and Research*, **4 (II)**, 2277-8179.