Nig J. Biotech. Vol. 25 (2013) 37-45
ISSN: 0189 17131
Available online at www.biotechsocietynigeria.org.

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Antimicrobial Activity of the Methanol and Aqueous Leaf Extracts of *Emilia coccinea* (Sims) G. Don

Erhabor, J.O.[1], Oshomoh, E.O.[1], Timothy, O., Osazuwa, E.S.[2] and Idu, M.[1]

[1]Department of Plant Biology and Biotechnology, University of Benin, P.M.B. 1154, Benin City, Nigeria. [2]Rubber Research Institute of Nigeria, P.M.B. 1049, Iyanomo, Benin City, Edo State, Nigeria.

(Received 01.11.12, Accepted 10.04.13)

Abstract

Assessment of the aqueous and methanol extracts of *Emilia coccinea* leaves was investigated in order to verify its claimed folkloric usage in treatment of microbial infections. Sensitivity tests for antimicrobial properties/activities of the leaf extracts were determined using Agar well diffusion method. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of extracts were also determined using doubling dilution and agar diffusion method respectively. Commercial antibiotics were used as positive reference standards to determine sensitivity of tested organisms. All test organisms (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*, *Aspergillus niger, Penicillium notatum* and *Candida albicans*) were found to be sensitive to both extracts. Methanol extract recorded highest mean inhibition diameter of 20.33±2.73mm against *S. aureus*. Aqueous extract had least mean inhibition diameter of 0.00±0.00mm against *P. notatum*. MIC values showed that methanol extract was more active than aqueous extract. MBC values for methanol extract varied between 5mg/ml – 25mg/ml as against 25mg/ml – 35mg/ml for aqueous extract. It was also observed that aqueous extract had no bactericidal action against *P. aeruginosa.* The results demonstrate that extracts of *E. coccinea* leaf has a broad spectrum of activity and can be a potential source of antimicrobial agents. Thus, the folkloric usage of this plant for the treatment of microbial diseases is justified.

Keywords: *E. coccinea*, Aqueous and Methanol extracts, Antimicrobial activity

Correspondence: erhaborjoseph@yahoo.com

Introduction

Generally, plants serve various purposes and their usefulness can be in the form of food, textile, shelter, as medicines and in religious practices. The use of these plants particularly medicinal plants and traditional medicines in most developing countries as therapeutic agents for the maintenance of good health have been widely observed (UNESCO, 1996). Medicinal plants constitute the base of the health care systems in many societies. Globally, about 85% of the traditional medicines used for primary health care are derived from plants (Farnsworth, 1988). It has also being observed that the rising costs of prescription drugs in the maintenance of personal health and well being, and the bioprospecting of new plant-derived drugs had fuelled the interest in medicinal plants as a re-emerging health aid (Lucy and Edgar, 1999).

However, the clinical efficacy of many existing synthetic antibiotics today is being threatened by the emergence of multidrug-resistant pathogens such as bacteria, fungi and viruses (Bandow *et al.,* 2003). Reports show that Infectious disease accounts for one half of all deaths in tropical countries irrespective of efforts made in controlling the incidence of epidemic

(Iwu, 1993, Okigbo and Ajalie, 2005). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio, 1996). Again, phytomedicines have shown great promise in the treatment of intractable infectious diseases (Idu *et al.,* 2007).

Emilia coccinea is one medicinal plant that has been widely used traditionally for medicinal purposes to treat a variety of ailments. Ethnomedicinal reports reveal that in Nigeria, the leaf is use to manage or cover sores (Burkill, 1985). The leaf sap is used to treat vertigo (Oliver, 1960). The leaf decoction is also reported to be used as a febrifuge and has a mild laxative effect (Ainslie, 1937). *E. coccinea* have also been effective in treating ulcers, lice, ringworm, gonorrhea, measles, cough and convulsion in children (Edeoga *et al.,* 2005; Odugbemi and Akinsulire, 2006). In Tanzania, the leaves mixed with *Ipomoea eriocarps,* is used as eye drop for eye infections, the crushed green leaves to treat wounds, sores and sinusitis while the dried powered leaves is used to manage sores (Bosch, 2004). The leaf sap can also be used in treating epilepsy in Ivory Coast (Kerharo and Bouquet, 1950) and in Congo for managing gonococcal infection, hernia and for syphilis (Burkill, 1985). Some of the bioactivities of the plant have been confirmed in the laboratory. These include antidiarrhoeal, antimicrobial and antifungal activity (Ogbebor and Adekunle, 2005; Teke *et al*., 2007; Okiei *et al.,* 2009). This present study was carried out to assess the antimicrobial activities of the methanol and aqueous leaf extracts of the plant.

Materials and Methods

Collection and Identification of Plant material: The leaves of the annual herb *Emilia coccinea* was collected between the months of June and July, 2008 from within and around the University of Benin, main campus, Benin City, Edo State, Nigeria. The plant was identified by Professor MacDonald Idu of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.

Preparation and Extraction of Plant material: The fresh leaves of *E. coccinea* were air-dried on the laboratory tables under room temperature and transferred to the oven set at 40^0C for 5-10 minutes. The plant materials were then reduced to fine powder with the aid of a mechanical grinder and stored in a tightly covered glass jars for further studies. Five hundred (500) g of the powdered leaf was macerated in 2.5L of methanol with the resultant solution filtered using Whatman filter paper No 1 after 48 hours under room temperature (25^0C) to obtain the methanol extract. For the aqueous extract, 700g of the powdered material was boiled in 1000L of water and the resultant solution filtered using filter paper. Both extracts were then concentrated via evaporation using an oven set at 40^0C and the extracts stored in a refrigerator until required for use.

Test Organisms: The test organisms used were *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger, Penicillium notatum* and *Candida albicans.* They were obtained from the laboratory stock of the Department of Microbiology, University of Benin Teaching Hospital (UBTH) and Edo Environmental Laboratory, Edo State, Nigeria. The identity of the bacteria isolates were confirmed using standard biochemical and physiological tests as described by (Cheesebrough, 2000; Harley and Prescott, 2002). The macroscopic features of the test fungi isolates were noted. The microscopic characters of the fungi isolates were ascertained with the aid of wet mount technique using lacto phenol cotton blue and distilled water as mountants respectively. The structures observed were recorded and compared with illustrations stated by Barnett and Hunter (1972).

Standardization and Preparation of Microbial inocula for susceptibility testing: All the test organisms (bacteria and fungi isolates) were sub-cultured on freshly prepared nutrient agar plates and malt extract agar plates and incubated for 24 hrs and 48 hrs respectively. The various portions of the already streaked colonies were placed in test tubes containing 5ml of the sterile broth and incubated for 3 hrs at 37^0C (Oloduro *et al*., 2011). The respective growth of the bacteria and fungi suspensions were compared to that of freshly prepared Barium sulphate

solution (0.5ml of 1% Barium in Chloride to 99.5ml of 1% H_2SO_4 (0.36 Normal) (Vandepitte *et al*., 2003). The turbidity was adjusted by adding more sterile nutrient broth to match 0.5 McFarland standards (10^6 cfu/ml). After incubation, 1ml of the standardized cultures of the microbial isolates were inoculated unto the solidified nutrient agar at 45^0C for bacteria and Potato dextrose agar for fungi under room temperature.

Susceptibility testing: The agar-well diffusion method, suitably modified was adopted for the susceptibility studies (Irobi *et al.,* 1996; Oboh *et al.,* 2007). Nutrient agar and Potato dextrose agar were the general purpose media of choice used in the susceptibility test. The media were prepared according to the manufacturer's guide and sterilized in an autoclave at 121^0C for 15minutes after which they were poured into Petri dishes and allowed to set. The plates were inoculated with the respective test organism in triplicate culture plates. Using a sterile cork borer of 6mm diameter, four (4) adequately spaced wells per plate were made into the culture Agar plates respectively. Varying concentrations of 35 mg/ml, 25 mg/ml, 15 mg/ml and 5 mg/ml of the plant extracts were poured into the four holes that have been labeled previously to correspond to each of the concentrations of the extract. The plates were left standing on the work bench for 30-40 minutes to allow pre-diffusion time. The bacterial inocula plates were incubated at 37^0C for 24hours while the fungal plates were incubated on the laboratory bench at 28 ± 2^0C for 72 hrs. Zones of inhibition around the wells indicated antimicrobial activity of the extracts against the test organisms. The diameters of these zones were measured diagonally in millimeter with a ruler and the mean value for each organism from the triplicate cultured plates was recorded. Using the agar-well diffusion technique, an already made gram positive and gram negative (Asodisks Atlas Diagnostics, Enugu, Nigeria) standard antibiotic sensitivity disc bought from a laboratory chemical equipment store in Benin city was used as positive control for bacteria while Ketoconanzone was used as positive control for fungi. Distilled water was used as negative control for all the test organisms. All the plates used for control were incubated at 37^0C for 24hours for bacteria and at 28^0C$\pm$ 2^0C for 72 hrs for fungi. The zones of inhibition were then measured after incubation and recorded.

Determination of Minimum Inhibitory Concentrations (MICs): The standard agar dilution protocol with doubling dilution was used to determine the MICs of the extracts (Oboh *et al.,* 2007). A 100 mg/ml concentration of each of the extract was prepared in sterile distilled water, and then diluted to achieve a decreasing concentration of 35, 25, 15 and 5 mg/ml respectively. Each dilution was introduced into nutrient agar plates and potato dextrose agar plates already seeded with the respective test organismAll test plates were incubated at 37^0C for 24 hrs for bacteria and 28^0C$\pm$ 2^0C for 72 hrs for fungi. The minimum inhibitory concentration (MIC) of the extracts for each test organism was regarded as the agar plate with the lowest concentrations without growth.

Minimum Bactericidal Concentration (MBC) Determination: The minimum bactericidal concentration (MBC) of the extracts was determined by the method as described by Igbinosa *et al.* (2009). Samples were taken from the MIC test bacteria plates with no visible growth. These were sub-cultured on freshly prepared nutrient agar plates and incubated at 37^0C for 24 hrs. MBC was taken as the concentration of the extract that did not show any growth on the new set of agar plate.

Statistical analysis: Results are expressed as mean $\pm$ standard error of mean (S.E.M) and the level f significance between means were computed by student's t – test using SPSS 10.0 computer software package. The level of significance was determined at 0.05.

Results

The results of the antimicrobial activity of the aqueous and methanolic extracts of the leaf of *Emilia coccinea* are shown in Table 1 and 2. It was observed that all the test organisms were sensitive to the aqueous extract at all the tested concentrations except *P. notatum* which was not sensitive to the extract at 5 mg/ml (Table 1). The activity of the aqueous extract in all the tested concentrations was significantly different from one another (Table 1). Table 2 revealed that the activity of the methanol (MeOH) extract of the leaf of *E. coccinea* was

significantly different from one concentration to another on each organism. *P. notatum* was the least sensitive organism particularly at the test concentration of 5 mg/ml while *S. aureus* showed the highest susceptibility range of 9.00 mm to 20.33 mm.

Table 1: The effect (zone of inhibition in mm) of various concentrations of the aqueous leaf extract of *E. coccinea* on test organisms

Test organisms	Concentration of extract (mg/ml)				Sterile distilled water
	5	15	25	35	
Bacteria					-
Bacillus subtilis	7.67±0.67^a	13.00±1.00^b	16.33±1.86^c	19.33±1.76^d	
Staphylococcus aureus	5.33±1.33^a	7.67±2.33^b	14.00±0.00^c	17.00±0.58^d	-
Pseudomonas aeruginosa	5.33±0.88^a	9.33±1.45^b	13.33±0.67^c	17.33±2.03^d	-
Escherichia coli	3.33±1.76^a	6.33±1.20^b	13.33±0.67^c	17.33±0.67^d	-
Fungi					
Aspergillus niger	1.33±1.33^a	3.33±1.76^b	7.00±0.58^c	8.67±0.88^d	-
Penicillum notatum	0.00±0.00^a	1.33±1.33^b	5.33±0.88^c	6.67±0.88^d	-
Candida albicans	1.33±1.33^a	3.00±1.53^b	7.33±0.33^c	8.00±0.58^d	-

NB: The values are the means ± S.E.M of three measurements (n=3).
Means ± S.E.M with different superscripts within a row are significantly different, $P < 0.05$.
- = No inhibition.

Table 2: The effect (zone of inhibition in mm) of various concentrations of the methanolic leaf extract of *E coccinea* on test organisms

Test organisms	Concentration of extract (mg/ml)				Sterile distilled water
	5	15	25	35	
Bacteria					-
Bacillus subtilis	5.33±0.88^a	10.00±1.53^b	13.33±0.33^c	17.00±0.58^d	
Staphylococcus aureus	9.00±2.89^a	12.67±1.33^b	16.67±1.86^c	20.33±2.73^d	-
Pseudomonas aeruginosa	7.33±0.33^a	11.33±1.33^b	14.00±1.15^c	17.00±1.53^d	-
Escherichia coli	6.33±0.67^a	9.00±0.58^b	13.67±0.33^c	17.00±1.15^d	-
Fungi					
Aspergillus niger	2.33±2.33^a	4.00±2.31^b	9.00±1.53^c	12.33±1.45^d	-
Penicillium notatum	1.33±1.33^a	4.00±1.15^b	6.67±1.45^c	9.00±1.15^d	-
Candida albicans	2.67±1.33^a	7.00±1.53^b	10.00±1.53^c	13.00±0.1.53^d	-

NB: The values are the means + S.E.M of three measurements (n=3). Means + S.E.M with different superscripts within a row are significantly different, $P< 0.05$. - = No inhibition. The activity of the commercial antibiotics (standard sensitivity disc and ketoconanzone) on the test bacteria and fungi respectfully are represented in Table 3 and 4.

Table 3: Sensitivity zone of inhibition of commercial antibiotics (standard sensitivity disc) on the test bacteria

Antibiotics	**Test bacteria** ***Staphylococcus aureus***	**E. *coli***	***Bacillus subtilis***	***Pseudomonas aeruginosa***
Gentamycin (10μg)	+ (18mm)	+ (20mm)	+ (15mm)	+ (17mm)
Ampiclox (30μg)	+ (10mm)	-	-	-
Zinnacef (20μg)	-	-	-	-
Amoxacillin (30μg)	-	+(10mm)	-	-
Rocephin (25μg)	-	-	-	-
Ciprofloxacin (10μg)	+ (13mm)	+(19mm)	+(11mm)	+(18mm)
Septrin (30μg)	+ (10mm)	-	-	-
Erythromycin (10μg)	+ (13mm)	-	-	-
Pefloxacin (30μg)	-	-	-	-
Augumentin (30μg)	-	+(8mm)	-	-
Tarivid (10μg)	-	-	-	+(14mm)

+ = sensitive - = No inhibition

Table 4: Sensitivity zone of inhibition of commercial fungi antibiotics (ketoconanzone) on the test fungi

Test fungi	Ketoconanzone (200mg/ml)
Aspergillus niger	14mm
Penicillium notatum	20mm
Candida albicans	22mm

The results obtained for the minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) for the aqueous and methanolic extracts of *E. coccinea* are presented in Table 5 and 6. The MIC values showed that the methanolic extract was more active than the aqueous extract(Table 5) against the microorganisms used. The aqueous extract showed MIC at 5 mg/ml against *P. aeruginosa* , 25 mg/ml against *E. coli, S.aureus* and *A. niger* while at 35 mg/ml against *B. subtilis, C. albicans* and *P. notatum.* MIC values for the methanolic extract at 5 mg/ml was against *P. aeruginosa, S.aureus* and *A. niger* while at 15 mg/ml it was against *E. coli* and *P. notatum.* At 35 mg/ml the MIC exhibited by the methanolic extract was against *B. subtilis,* and *C. albicans.* The MBC values (Table 6) revealed that the aqueous extract had no bactericidal action against *P. aeruginosa.* All the test bacteria were found to be more sensitive to the methanol extract than the aqueous extract. The MBC value for the methanolic extract varied between 5 – 25 mg/ml as against 25 and 35 mg/ml for the aqueous extract.

Table 5: Minimum inhibitory concentrations (MICs) of the aqueous and methanolic extracts of the leaf of *E. coccinea* against the test organisms

Organisms	MIC (mg/ml)	
	Aqueous extract	Methanol extract
Escherichia coli	25	15
Pseudomonas aeruginosa	5.0	5.0
Bacillus subtilis	35	35
Staphylococcus aureus	25	5.0
Aspergillus niger	25	5.0
Candida albicans	35	35
Penicillum notatum	35	15

Table 6: Minimum bactericidal concentration (MBC) of the aqueous and methanolic leaf extracts of the leaf of *E. coccinea* on the test bacteria

Test bacteria	MBC (mg/ml)	
	Aqueous extract	Methanolic extract
Escherichia coli	25	15
Pseudomonas aeruginosa	-	5
Bacillus subtilis	35	25
Staphylococcus aureus	25	5

- = Not bactericidal

Discussion

The aqueous and methanolic extracts of *E. coccinea* showed inhibitory activities against all the test organisms (Table 1 and 2). It was observed that susceptibility increased with increased concentration of the extracts. *Penicillium notatum* of all the tested organisms recorded the highest resistance ranging from 0.00±0.00 mm to 9.00±1.15 mm for both extracts. The sensitivities of *C. albicans, B. subtilis, S. aureus* and *E.coli* is in agreement with earlier work carried out by Okiei *et al.* (2009) on the leaf of *E. coccinea.*

Generally, the methanolic extracts exhibited more potency and consistency in activity than the aqueous extract. The methanolic extract recorded the highest inhibitory effect of 20.33 ± 2.73 mm than the aqueous extract (Table 2). These results support earlier studies which observed that plant extracts in organic solvent provided more consistent antimicrobial activity compared with those extracted in water (Parekh *et al.,* 2005; Ahmad *et al.,* 1998). *S. aureus* was the most sensitive test bacteria and generally the most sensitive test organism at the highest concentration of 35 mg/ml of the aqueous extract. Following *S. aureus* is *B. subtilis* with an inhibition diameter of 19. 33 ± 1.76 mm at the concentration of 35 mg/ml of the aqueous extract (Table 1).

Methanol extract recorded the highest antifungal activity against *C. albicans* at the highest concentration of 35 mg/ml with an inhibition diameter of 13.00 ± 1.53 mm. *P. notatum* was the least sensitive fungus and test organism(Table 3). The aqueous extract recorded no activity against *P. notatum* at a concentration of 5 mg/ml. However the inhibitory effects were observed across the other concentrations of 15, 25 and 35 mg/ml.

The activity/inhibitory effects of the aqueous extract at 5 mg/ml contradict earlier report by Teke *et al.* (2007) that the aqueous extracts did not have any antimicrobial activity but corroborate same report that the methanolic extract was active on *E.coli.* It was also observed that the two extracts were active when compared with the negative control (sterile distilled water) against all the test organisms (Table 1 and 2). The control recorded no visible activity. The positive control (standard sensitivity disc) used on the test bacteria revealed that only gentamycin and ciprofloxacin had inhibitory effects on all the test bacteria (Table 3). Ampiclox and Septrin had activity on S*. aureus* with the same inhibition diameter of 10 mm while 13 mm was recorded for Erythromycin as against 17 mm and 20 mm inhibition diameter for the aqueous and methanolic extracts. Augmentin and Amoxaciilin were sensitive to *E. coli* with inhibition diameter of 8 mm and 10mm as against 17 mm for both extracts. Another antibiotic-Tarivid only had activity on *P. aeruginosa* with a sensitivity zone of inhibition of 14 mm as against 17 mm for both extracts. Comparatively, the extracts of the leaf of *E. coccinea,* can be said to possess better activity than the standard antibiotics which were used as positive control.

This is supported by the fact that the extracts contain both pharmacological and non pharmacological active substances as against pure active substances contained in the antibiotics. The effect of the commercial antifungal drug (Ketoconanzone) tested at the concentration of 200mg/ml against the test fungi (Table 4) can be considered not better in

activity when compared with the extracts, particularly at the highest tested concentration of 35 mg/ml which was five times lower in concentration than that of the fungal antibiotics. This probably implies that if the concentrations of the extracts are increased, it can lead to increased activity. It can be observed from the MIC values that the methanolic extract was better in activity than the aqueous extract; though both extracts have the same MIC values of 5 mg/ml against *P. aeruginosa* and at 35 mg/ml against *C. albicans.*

Antimicrobial substances can be considered as bactericidal agents when the ratio MBC/MIC $\leq$ 4 and bacteriostatic agents when the ratio MBC/MIC > 4 (Gatsing *et al.,* 2010). For the extracts tested, the ratio MBC/MIC was $\leq$ 4 against all the tested bacteria. It suggests that the extracts can be considered as bactericidal agents, although, no bactericidal activity was observed against. P*. aeruginosa* when the aqueous extract was used.

Conclusion

The results from these studies provide evidence for the ethnomedicinal uses of the tested plant. The presence of secondary metabolites in the leaf of *E. coccinea* is thought to be probably responsible for its medicinal properties. The methanol and aqueous leaf extracts of *E. coccinea* showed activity against all the test bacteria (gram positive and gram negative bacteria) and fungi. It can therefore probably serve as a potential source of antimicrobial agent.

However further studies are recommended on the chemical characterization and biological availability of the extracts.

References

Ahmad, I., Mehamood, Z. and Mohammad, F. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *J. of Ethnopharm*. 62**:** 183-193.

Ainslie, J.R. (1937). A list of plants used in native medicine in Nigeria. Imperial Forestry Institute, Oxford University Institute Paper 7.

Bandow, J.E., Brotz, H., Leichert, L.I.O., Labischinski, H. and Hecker, M. (2003). Proteomic approach to understanding antibiotic action. *An. Agents and Chem.* 47**:** 948 -955.

Barnett, H. L. and Hunter, B. B. (1972). Illustrated Genera of Imperfect Fungi. 3rd Edn. Burgess, New York. p.225.

Bosch, C.H. (2004). *Emilia coccinea* (Sims) G.Don. In: Grubben, G.J.H. and Denton, O.A.(Eds). PROTA2: Vegetables/Legumes. (CDROM). PROTA, Wageningen, Netherlands.

Burkill, H.M. (1985). The Useful Plants of West Tropical Africa. The Whitefriars Press Limited,Great Britain. p.960.

Cheesebrough, M. (2000). District Laboratory Practice in Tropical Countries. Cambridge University Press. Cambridge, UK. p.428.

Colombo, M.L. and Bosisio, E. (1996). Pharmacological activities of *Chelidonium majus* L. (Papaveraceae). *Pharm. Res.* 33: 127-134.

Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. of Biotech.* 4(7): 685 – 688.

Farnsworth, N.R. (1988). Screening Plants for New Medicines.*In*: Biodversity. Wilson, E.O. (Ed). National Academy Press, Washington DC, pp.83-87.

Gatsing, D., Nkeugouapi, C.F.N., Nkah, B.F.N., Kuiate, J.R. and Tchouanguep, F.M. (2010). Antibacterial activity, bioavailability and acute toxicity evaluation of the leaf extract of *Alchornea cordifolia* (Euphorbiaceae). *Int. J. of Pharm.* 6: 173-182.

Harley, J.P. and Prescott, L.M. (2002). Laboratory Exercises in Microbiology. 5th Edn. Mac Graw Hill, New York. p.449.

Idu, M., Omonigho, S.E. and Igeleke, C.L. (2007). Preliminary investigation on the phytochemistry and antimicrobial activity of *Senna alata* L. flower. *Parkistan J. of Biol. Sc.* 10(5): 806-809.

Igbinosa, O.O., Igbinosa, E.O. and Aiyegoro, O.A. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *Afr. J. of Pharm. and Pharmacol.* 3(2): 58-62

Irobi, O., Moo-Yang, M. and Anderson, W.A. (1996). Antimicrobial activity of Annatto (*Bixa orellana* extract). *Int. J. of Pharmacog. 34*: 87-90.

Iwu, M.M. (1993). Hand book of African Medicinal Plants. 1st Edn. CRC Press Inc, Florida.p.435.

Kerharo, J. and Bouquet, A. (1950). Plants medicinales et toxiques de la Cote-d' ivoire-Haute-Volta. Vigot Freres, Paris. p.295.

Lucy, H. and Edgar, J.D. (1999). Medicinal pants: a re-emerging health aid. *Electr. J. of Biotech. 2*(2): 1-15.

Oboh, I.E., Akerele, J.O. and Obasuyi, O. (2007). Antimicrobial activity of the ethanol extract of the aerial parts of *Sida acuta* burm .f. (Malvaceae). *Tr. J. of Pharm. Res.* 6: 809-813.

Ogbebor, N. and Adekunle, A.T. (2005). Ingibition of conidial germination and mycelia growth of *Corynespora caiicola* (Berk and Curt) of rubber (*Hevea brasiliensis* muell. Arg.) using extracts of some plants. *Afr. J. of Biotech.* 4:996-1000.

Odugbemi, T. and Akinsulire, O. (2006). Medicinal plants by species names. *In*: Odugbemi, T (Ed). Outlines and Pictures of Medicinal Plants from Nigeria, University of Lagos Press, Nigeria.pp.73-116.

Okigbo, R.N. and Ajalie, A.N. (2005). Inhibition of some human pathogens with tropical plant extracts. *Int. J. of Mol. Med. and Adv. Sc.* (Pakistan). 1(1): 34-41.

Okiei, W., Ogunlesi, M. and Ademoye, M.A. (2009). An assessment of the antimicrobial properties of extracts of various polarities from *Chasmanthera dependens, Emilia coccinea* and *Cuscuta australis,* herbal medications, for eye diseases. *J. of Appl. Sc.* 9: 4076 – 4080.

Oliver, B. (1960). Medicinal Plants in Nigeria. Nigeria College of Arts, Science and Technology. p.138.

Oluduro, O., Bakare, M.K.O., Omoboye, .O.O., Dada, C.A. and Olatunji, C.I. (2011). Antibacterial effect of extracts of *Acalypha wilkesiana* on gastrointestinal tract pathogens and bacteria causing skin infections in neonates. *Ife J. of Sc.* 13(2):371-380.

Parekh, J., Jadeja, D. and Chanda, S. (2005). Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Tur. J. of Biol.* 29: 203-210.

Teke, G.N., Kulate, J.R., Ngouateu, O.B. and Gatsing, D. (2007). Antidarrhoeal and antimicrobial activities of *Emilia coccinea* (Sims) G. Don extracts. *J. of Ethnopharm.* 112(2): 278-283.

UNESCO (1996). Culture and Health, Orientation texts – World Decade for Cultural Development Documents CLT/DEC.PRO-1996. Paris, France. pp.29.

Vandepitte, J. Verhaegen, J., Engbaek, K. Rohner, P. Piot, P. and Heuck, C.C. (2003). Basic Laboratory procedures in clinical bacteriology. Geneva, WHO, p.175.