www.cenresin.org
ISSN 2277-0054

ANTIMICROBIAL PROPERTY OF AQUEOUS AND ETHANOL LEAF EXTRACT OF CELOSIA ISERTII

[1] Emudainohwo J.O.T., [2]Ewharieme, A. D.; [1]Ejebe, D.E., [2]Ewhre, L., [1]Agam, J.I.
[1]Department of Pharmacology and Therapeutics Delta State University, Abraka
[2]Department of Microbiology, Delta State University, Abraka, Delta State, Nigeria
Email: j.o.t.emudainohwo@yahoo.com

ABSTRACT

Studies on the aqueous and ethanolic extracts of medicinal and traditional plants/herbs has become a major focus of recent biochemical researches; in order to propose better models for manufacturing of modern drugs and design of microbes inhibition. This present study investigated the antimicrobial property of the aqueous and ethanolic leaf extract of Celosia isertii against micro-organisms: *E.coli, S. aureus, P. aeruginosa, B. subtilis, S. typhi and K. pneumonia,* using antibiotics; Ampiclox and Erythromycin as control. The disc diffusion techniques was used to test the sensitivity of the micro-organism to the extracts of *C.isertii*and the results obtained show mean zones of inhibition between (7 $\pm$ 0.2mm) to (10 $\pm$ 0.3mm) for coldwater (aqueous) and (10 $\pm$ 0.5mm) to (13 $\pm$ 0.8mm) for ethanol extract. Micro-organisms showed sensitivity in the order: *E.coli;(*10 $\pm$ 0.3mm) and (12 $\pm$ 0.8mm), *S.aureus*; (8 $\pm$ 0.2mm) and (11 $\pm$ 0.6mm) and *P.aeruginosa;* (10 $\pm$ 0.4mm) and (13 $\pm$ 0.8mm), *B.subtilis;* (10 $\pm$ 0.1mm) and (11 $\pm$ 0.7mm), *S.typhi* (8 $\pm$ 0.2mm) and (10 $\pm$ 0.6mm) and *K.pneumoniae*; (7 $\pm$ 0.2mm) and (10 $\pm$ 0.5mm) for aqueous and ethanolic extracts respectively. The minimum inhibition concentration (MIC) ranged from 0.2 to 0.5mg/mL for ethanol extract while aqueous extracts appeared less effective. This result thus suggest the potency of *Celosia isertii* as an antimicrobial agent especially its extraction with ethanol at 0.5mg/ml although, further studies are recommended for its phytochemical screening/analysis.

Keywords: *Antimicrobial Property, Leaf Extracts, Celosia Isertii*

INTRODUCTION

For many generations, several plants were used for their therapeutic virtues, and this was before knowing the exact origin of their benefits. In fact, they were used for treating infections (Bussmann and Sharon, 2006), malaria (Willcox et al., 2005), burns, edema, allergies and prevent several diseases (Bussmann and Sharon, 2006). Since the last decades, researchers have began to explain these virtues by the ability of plants to limit infections (Ulanowska et al., 2006; Kuster et al., 2009), prevent lipid peroxidations (Yamanaka et al., 1996), prevent some cancers (Ames et al., 1995), cure allergies (Park et al., 2008) and many other associated diseases (Rein et al., 2000; Martin and Andriantsitohaina, 2002). Among all these virtues, the anti-infectious activity was considered as one of the most important activities (Park et al., 2008). *Celosia* is a small genus of edible and ornamental plants in the Amaranthaceae family (Rabes, et al., 1997). The generic name is derived from the Greek word "Kelos" meaning "burned" and refers to the flower-heads species. *Celosia* are commonly refer to as wool flowers or cockscombs and is well known

among the East Africans as 'Mfungu" and other West-Africa Countries (such as Nigeria), where it is called "Fulfulde" or "bokangida" (Edeoga et al., 2005). Though still in controversy, the *C. isertii*is claimed to have originated from Africa not withstanding its abundance in Indonesia and India (Janarj, et al., 1999). It has been shown to be well-cultivated in Nigeria due to the humid weather found in this west African country, Nigeria and the presence of wet season seem to be an advantage in its cultivation (Diallo, et al., 1999).*Celosia isertii*(Amaranthaceae) grows as a weed during rainy season throughout India and other tropical regions of the world such as Sri Lanka, South Asia, Africa and America (Thangarasu et al., 2002). The leaves are used for the treatment of inflammations, fever and itching. The seeds are bitter, useful in blood diseases and mouth sores (Thangarasu et al., 2002). They are an efficacious remedy in diarrhea (Kirtikar and Basu, 1935).Based on ethno botanical practice, the plant has been investigated for anti-inflammatory (Patil et al.,2003), anti-pyretic, anti-diabetic, antibacterial and diuretic properties (Bhujbal et al.,2006;Park et al., 2008), and with the increased interest shown by researcher in folk medicine for new leads to develop better drugs against microbial infection, (Benkebia, 2004), there is a need therefore, for a study on the determination of possible antimicrobial properties of extract of *Celosia isertii.* Although the antimicrobial activity of some medicinal plants is documented, their antimicrobial activities vary widely, depending on the type of spice or herb, test medium and micro-organism (Synder, 1997). The aim of this study was to investigate the antimicrobial property of aqueous and ethanol leaf extract of *Celosia isertii*on some selected microorganisms such as *Bacillus sp., Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas sp., Staphylococcus aureus*and *Salmonella typhi.*

METHODS

Source and Collection of Plant Parts

The leaves of *Celosiaisertii* plant sample used for the research was collected from Abraka and identified at the Department of Botany, Delta State University, Abraka, Nigeria. Samples were conveyed in black polythene bags to the Microbiology laboratory, where they are then washed with clean water.

Preparation of Plant Extracts

Plant extract was obtained as described by previous research (Doughari et.al., 2007). To a volume of 1500ml of distilled water (for aqueous) and ethanol (for alcohol), was added 300g of the powdered plant extract. The suspension was allowed to stand in position for 3 days in the laboratory. The mixture was agitated at intervals on each day. On the third day, the extract was filtered out into a clean sterile flask with the aid of millipore filter and later concentrated to dryness in a rotatory evaporator in vacuum.

Standardization of Isolates

Test organisms were sub-cultured onto fresh plates of MacConkey agar and incubated aerobically at 37°C for 24 hours. Colonies from these plates were suspended in Mueller-Hinton broth (Oxoid, UK) to a turbidity matching 0.5 McFarland standard (10^8cfu/ml).

Mueller-Hinton agar was then used for antimicrobial assay. All the broth cultures were incubated at 37°C (Aibinu et al., 2007).

Antimicrobial Assay

Suspensions of the bacteria obtained contained approximately 1 x 10^8cfu/ml. Each labelled plate was uniformly seeded with a test organism by means of sterile swab stick rolled in the culture medium. Aliquots were dropped in each well to fullness (Shahidi, 2004). Each plate was kept in the refrigerator for 1 hour to allow the extracts to diffuse into the culture medium while the immediate growth of the organism was stopped from taking place. These plates were then incubated at 37°C for 24hours. The zones of inhibition around the wells were measured in millimeter (mm). Control antibiotics and ethanol solvent were placed in a well on each plate along with the test extracts as control.

Determination of Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms in triplicate in test tubes (Doughari et.al., 2007). To 0.5 ml of varying concentrations of the extracts (0.5, 0.4, 0.3, 0.2 and 0.1mg/ml) in test tubes, Nutrient broth (2ml) was added and then a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standard, was introduced. The procedure was repeated on the test organisms using the standard antibiotics (Ampiclox and Erythromycin). A tube containing Nutrient broth only was seeded with the test organisms, as described above, to serve as controls. The culture tubes were then incubated at 37^{o}C for 24 hours. After incubation the tubes were then examined for microbial growth by observing for turbidity.

RESULTS AND DISCUSSION

The use of plants extract; water (aqueous) and other alcoholic concentrations has become a common practice among traditional medical practitioners (Goncalves, et al., 2008). Although, the active potential phytochemicals present in both aqueous and alcohol extracts of leaves and herbs are the same, there is always a variation in their inhibition potency especially at different concentrations (Androgan, et al., 2002). Therefore, the comparison of these aqueous and alcoholic extracts at their different concentrations serves to propose a model for the pharmacological studies of plants/herbs (Androgan, et al., 2002). In this present study, the levels of inhibition observed ranged between 5mm – 9mm Ampiclox, 6 – 10mm for erythromycin, 7 – 10mm for coldwater (aqueous) extract and 10 – 13mm for ethanolic extracts of *Celosia Isertii*(Table 1). For *E.coli*, the antibiotics (Ampiclox and Erythromycin) showed equal inhibition (8mm) while ethanol extract showed a greater inhibition (12mm) than aqueous (10mm) and the antibiotics. For *S.aureus*, ethanol showed greater inhibition (11mm) than aqueous (8mm) and the antibiotics. Also, ethanol and aqueous extract showed greater inhibition than antibiotics (Table 1) against *P.aeruginosa* while *S.typhi*s resistant to Ampiclox (6mm) but inhibited by ethanol extract (10mm) and Erythromycin (10mm). *K.pneumoniae* also showed a greater resistance to Ampiclox but strongly inhibited by aqueous and ethanol extracts (Table 1). Comparison of aqueous and ethanolic extracts of

C.isertii against microorganisms showed that ethanolic extract have greater inhibitory potency. Comparison of aqueous extract with antibiotics showed that aqueous extract have stronger inhibition with exception to *Staph. aureus*and*Salmonella.typhi* while equal inhibitory effect was observed against *K.pneumoniae*. Ethanolic extract showed a stronger inhibitory effect against all microorganisms when compared with the antibiotics (Ampiclox and Erythromycin). The minimum inhibitory inhibition (MIC) of the aqueous and ethanolic extracts show values indicating a possible adoption of *Celosia isertii* extract as an antibacterial agent. The MIC of ethanolic extracts against *E.coli, S.aureus, P.aeruginosa, B.subtilis, S.typhi* and *K.pneumoniae*were 0.3, 0.4, 0.3, 0.4, 0.3 and 0.2mg/mL respectively (Table 2). On the other hand, *E.coli, S.aureus, P.aeruginosa,* and*S.typhi*appeared to be resistant to the aqueous plant extract. *B.subtilis,* and *K.pneumoniae*which showed susceptibility, were inhibited at a concentration of 0.5 and 0.4 mg/mL.respectively (Table 2). The results obtained from the study may not be unexpected as natural plants/herbs have been shown to possess various medicinal potency as well as their inhibitory effects on various human-disease causing microorganisms. Ethanolic leaf extract of *Aloe vera*burm have been shown to inhibit the growth of *E.coli, K.pneumoniae, P.aeruginosa, B.subtiilis and S.aureus* (Thiruppathi et al., 2010). In 2009, Agatemor showed that *E.coliB.subtiilis, P.aeruginosa, and S.aureus* are strongly inhibited by nine Nigerian spices/herbs of which this result is consistent as all microorganisms were strongly inhibited by both the aqueous and ethanolic extract of *C.isertii* (table 4.1 and 4.3). Although not much work has been published on *C.isertii* especially its phytochemical constituents, it is suspected in this study, that the mechanism of its antimicrobial potency may not be unconnected to the presence of tannins, phlobatannins, saponins, flavanoids, steroids, terpenoids, glycosides and reducing sugars associated with the phytochemical screening/analysis of most Nigerian medicinal and traditional herbs (Egwaikhide, et al., 2007, Edeoga et al., 2005 and Shittu et al., 2007). From the results obtained in the study in which all micro-organism investigated were inhibited and highly sensitive toethanolic leaf extract of *Celosia Isertii.*,*Celosia Isertii*can be employed in the treatment of ailments and diseases caused by tested microbes at 0.5mg/ml of ethanolic extract.Further studies on animal toxicity are recommended.

Table 1: Mean zones of inhibition (mm) of extract and selected antibiotics on test isolates.

Group	Test Isolates	Antibiotics		Leaf extract of *C. isertii*	
		Ampiclox	**Erythromycin**	**cold water**	**Ethanol**
1	*E. coli*	8 ± 0.2	8 ± 0.2	10 ± 0.3	12 ± 0.8
2	*S. aureus*	9 ± 0.3	10 ± 0.4	8 ± 0.2	11 ± 0.1
3	*P. aeruginosa*	8 ± 0.1	6 ± 0.3	10 ± 0.4	13 ± 0.8
4	*B. subtilis*	7 ± 0.6	7 ± 0.2	10 ± 0.1	11 ± 0.7
5	*S. typhil*	6 ± 0.4	10 ± 0.4	8 ± 0.2	10 ± 0.6
6	*K. pneumoniae*	5 ± 0.1	7 ± 0.2	7 ± 0.2	10 ± 0.5

Table 2: Minimum inhibitory concentration of leaf extracts obtained from aqueous and ethanolic extracts on micro-organisms.

Group	Microorganism	Extracts	Concentrations (mg/ml)				
			0.5	**0.4**	**0.3**	**0.2**	**0.1**
1	*E. coli*	CW	+	+	+	++	++
		E	-	-	-	+	+
2	*S. aureus*	CW	+	+	++	++	++
		E	-	-	+	+	+
3	*P. aeruginosa*	CW	+	+	+	+	++
		E	-	-	_	+	+
4	*B. subtilis*	CW	-	+	++	++	++
		E	-	-	+	+	+
5	*S. typhil*	CW	++	++	++	++	++
		E	_	_	_	+	+
6	*K. pneumonia*	CW	-	-	+	++	++
		E	_	_	_	_	+

Note:
+ = Small growth
++ = major growth
- = No growth
E = Ethanol and CW; cold water

REFERENCES

Agatemor, C.(2009). Anti-microbial activity of aqueous and ethanol extracts of nine Nigerian spiceagainst four food Borne Bacteria: *Internet J. of Food Safety. –IAFSQ***100:** 77 – 80.

Aiyelaagbe, O.O. (2001). Antibacterial activity of *Jatrophamultifida*roots.*Fitoterapia***72**: 544-546

Alonso-Paz E, Cerdeiras, M.P., Fernandez, J., Ferreira, F., Moyna, P, Soubes, M., Vazquez,A, Veros, S, Zunno, L. (1995). Screening of Uruguayan medicinal plants for antimicrobial activity.*J Ethanopharm*.**45**: 67-70.

Ames, B.N, Gold, L.S. and Willett, W.C. (1995).The causes and prevention of cancer.*Proc. Natl. Acad. Sci.*, **92**: 5258-5265.

Anibujuwon, I.I. and Udeze, A.O. (2009).Antimicrobial activity of *Caricapapaya*(Pawpaw) leaf on some pathogenic organisms of clinical origin from south-western Nigeria.*Ethnobotanical Leaflets***12**:850 – 864.

Aridogan, B.C., Baydar, H. and Kaya S. (2002).Antimicrobial activity and chemical composition of some essential oils.*Arch. Pharm. Res.***25:** 860 – 864.

Ayyanar, M. and Ignacimuthu, S. (2005).Traditional knowledge of kani tribal in Kouthalai of Tirunelveli Hills, Tamil Nadu, India.*Journal of Ethnopharmacology***102**:493 – 496.

Benkeblia, N. (2004). Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and Garlic (*Allium sativum*). Lebensmwiss u-Technol. **37**: 263-268.

Bhujbal S, Patil K, Patil M. (2006). Evaluation of anti-pyreticpotentials of *Celosia Argentea*Linn leaf extract.*PlantaIndica*. **2**: 19-20

Bogovazova, G.G., Voroshilova, N.N and Bondarenko, VM (1991)."The efficacy of *KlebsiellaPneumoniae*bacteriophage in the therapy of experimental *Klebsiella*infection.*ZhurnalMikrobiologii, Epidemiologii, I immunobiologii* (4): 5–8.

Bussmann, R.W. and Sharon, D. (2006). Traditional medicinal plant use in northern Peru: tracking two thousand years of healing culture. *J. Ethnobiol. Ethnomed.*,**2**:47.

Cheesbrough M. Medical Laboratory (2000).*Manual for Tropical Countries*.Microbiology, Linacre House, Jordan Hill Oxford, pp. 260, 2000.

Cook, H., Furuya, E, Larson, E, Vasquez, G. and Lowy,F. (2007). "Heterosexual transmission of community-associated methicillin-resistant staphylococcus aureus".*Clin Infect Dis***44** (3): 410–413.

De Lalla ,F. (1999).Antimicrobial chemotherapy in the control of surgical infectious complications. *J Chemotherapy***11**: 440-445, 1999.

Diallo, D., Hveem, B., Mahmoud, M.A., Betge, G., Paulsen, B.S. and Maiga, A. (1999).An ethnobotanical survey of herbal drugs of gourma district, Mali.*Pharmaceutical Biology***39:** 80 – 91

Eckburg PB, Bik EM, Bernstein CN, Purdom E, and Dethlefsen L.(2005).Diversity of the human intestinal microbial flora.*Science***308** (5728): 1635–1638.

Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O.(2005).Phytochemical constituents of some Nigerian medicinal plants.*African Journal of Biotechnology***4**:685 – 688.

Egwaikhide, P.A., Okeniyi, S.O. and Gimba, C.E. (2007).Screening for anti-microbial activity and phytochemical constituents of some Nigerian medicinal plants.*Advances in Biological Research*.**1** (5 – 6): 155 – 158

Euzéby, J.P. (1997). "List of bacterial names with standing in nomenclature: a folder available on the internet". *Int J SystBacteriol***47** (2): 590-2.

Feng, P, Weagant,S. and Grant, M (2007). "*Enumeration of Escherichia Coli and the coliform bacteria".Bacteriological Analytical Manual (8th ed.)*. FDA/Center for Food Safety & Applied Nutrition.

Genus, (2001).*Celosia L. Germplasm Resources Information Network*.United States Department of Agriculture.

Goncalves, F.A., Andrade, N.M., Bezerra, J.N.S., Macrae, A., Sousa, O.V., Fonteles-Filho, A.A. and Vieira, R.H.S.F. (2008).Antibacterial activity of guava.*PsidiumGuajava*(L) leaf extracts on diarrhea causing enteric bacteria isolated from seabob shrimp; *Xiphopenaeuskroyeri*(Heller). *Rev. Inst. Med. Trop. S.paulo*.**50**(1): 11 – 15.

Hase, K., Kadota, S, Basnet, P., Takahashi, T., Namba, T. (2002). Protective effect of celosian, an acidic polysaccharide, on chemically and immunologicaly induced liver injuries. *Pharma bull***19**(4): 567-72.

Hindler, A.J. and Jorgensen, J.H.B.C. (1995).Procedures in antimicrobial susceptibility testing. In: Mahon, C.R. and Manuselis, Jnr. *Testbook of Diagnostic Microbiology*. W.B. Saunders, Philadelphia. p. 63 – 64.

Hudault S, Guignot J and Servin A.L (2001). "*Escherichia coli* Strains colonizing the gastrointestinal tract protect germ-free mice against*Salmonella Typhimurium*infection". *Gut***49** (1): 47–55.

Iwase, T. U., Yoshio, H., Tajima, A.,Seo, H., Takada, K.,Agata, T. and Mizunoe, Y. (2010). "*Staphylococcus epidermidis*Espinhibits *Staphylococcus aureus*biofilm formation and nasal colonization". *Nature***465** (7296): 346–9.

Jaiarj, P., Khoohaswan, P. and Wongkrajang, Y. (1999). Anti-cough and antimicrobial activities of *P. GuajaveLinn* leaf extract. *J. Ethnopharmacol*.**67:**203 – 212.

Kapoor, A. (2001). Neem: the wonder plant. *Pesticides Inform*. **27**: 33-34

Karthikeyan, A., Shanthi, V. and Nagasathaya, A (2009). Preliminary Phytochemical and Antibacterial Screening of Crude Extract of the Leaf of *AdhatodaVasica*L. *Int J Green Pharm*, **3**:78-80

Khandelwal, K.R.(2007). Practical Pharmacognosy techniques and experiments: (9th ed). Pune (India): Niraliprakashan. Pp149-156.

Kilani, A.M. (2006). Antibacterial assessment of whole stem bark of *VitexDoniana*againstsome Enterobactriaceae.*Afr. J. Biotechnol*. **5**: 947-959.

Kirtikar K. and Basu, L (1935).*Indian Medicinal Plants, Vol. III.* (2nd ed)., Allahabad:. pp. 2053-2054.

Kuster, R.M., Arnold, N. andWessjohann, L .(2009). Anti-fungal flavonoids from *TibouchinaGrandifolia*.*Biochem. Syst. Ecol*., **37**(1): 63-65

Levy, S.B. (1994). Drug Resistance: The new apocalypse (special issue) *Trends Microbiol***2**: 341–425

Lozoya, M., Lozoya, X.P.(1989).Harmacologicalproperties *in Vitro* of various extracts of *Mimosa PudicaLinn*.Tepescohuite*Arch Invest Mex*, 87-93

Mahajan, A, Das, S (2003).Plants and microbes- potential source of pesticide for future use.*Pesticides Inform*. **28**(4): 33-38.

Martin S. and Andriantsitohaina R (2002).Cellular mechanism of vasculo-protection induced by polyphenols on the endothelium.*Ann. Cardiol. Angeiol*.,**51**(6): 304-315

Menichetti, F. (2005).Current and emerging serious gram-positive infections.*Clin Microbial Infect***11**Suppl**3**: 22–28.

Neely, A.N, Maley, M.P (2000).Survival of enterococci and staphylococci on hospital fabrics and plastic.*J. Clin. Microbial.***38** (2): 724–726.

Nina Chanishvili, (2009).Aliterature review of the practical application of bacteriophage research. *ClinRheumatol***26** (6): 858–64.

Olowoyo, O.G., Adesina, O.A., Adigun, A.O. and Azike, C.K. (2005). Pro- and antioxidant effects and cytoprotective potentials of nine edible vegetables in south-west Nigeria.*J. of Med. Food***8**: 539-44.

Palleroni, N.J. (2010). The pseudomonas story.*Environmental Microbiology***12** (6): 1377–1383.

Park, H.H., Lee, S, Son, H.Y., Park, S.B., Kim, M.S, Choi,E.J., Singh, T.S, Ha, J.H., Lee, M.G., Kim, J.E, Hyun, M.C., Kwon, T.K, Kim, Y.H. and Kim, S.H (2008). Flavonoids inhibit histamine release and expression of proinflammatory cytokines in mast cells. *Arch. Pharm. Res.*, **31** (10): 1303-1311.

Patil, K., Bhujbal, S. andChaturvedi, S. (2003). Anti-inflammatoryactivity of various extracts *CelosiaArgentea*Linn*. Ind.J.Pharm. Sci*. 645- 647

Postgate, J (1998).*Nitrogen Fixation, 3rd Ed.*.Cambridge University Press.Podschun, R and Ullman, U. (1998). *KlebsiellaSpp*. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clinical Microbiology Reviews***11** (4): 589–603.

Rabe, T. and Van-Staden, J. (1997). Antibacterial activity of south african plants used for medicinal purposes. *Journal of ethnopharmacol*.**56:** 81 – 87.

Rashid, T, Ebringer, A.(2006). "Ankylosingspondylitis is linked to *Klebsiella*-the evidence. *ClinRheumatol***26**(6): 858–64.

Reid,G., Howard, J.andGan,B.S (2001). Ca
n bacterial interference prevent infection?.*Trends Microbiol.***9** (9): 424–428

Rein, D, Paglieroni, T.G, Wun, T, Pearson, D.A., Schmitz, H.H, Gosselin R,.andKeen,C.L. (2000). Cocoa inhibits platelets activation and function. *Am. J. Clin. Nutr.*,**72**: 30-35.

Ryan, K.J Ray,C.G (2004). *Sherris Medical Microbiology* (4th ed.). McGraw Hill.

Sani, C.A., Dzenda, T. and Suleiman, M.M.(2006) Antidiarrhealactivity of the methanol stem-bark extract of *AnnonaSenegalensis Pers*. (Annonaceae).*J of Ethnopharmacology***116**: 125-130.

Santos, P.R.V, Oliveira, A.C.X, Tomassini,T.C.B. (1995). Control microbiogicodeproductos. fitoterapicos. *Rev. Farm. Bioquim*.**31**: 35-38

Saxena, G., McCutcheon, A.R., Farmer, S., Towers, G.H.N. and Hancock, R.E.W. (1994). Anti-microbial constituents of *Rhusglabra*.*J. Ethanol. Pharm*.**42**: 95-99.

Shittu, I.A.J. Bankole, M.A., Ahmed, T,;Bankole,. M.N., Shittu, R.K. Saalu, C.L. and Ashine, O.A. (2007).Antibacterial and antifungal activities of essential oils of crude extracts of *SesamumRadiatum*against some common pathogenic microorganism.*Iranian Journal of Pharmacology and Therapeutics*. (IJPT) **6:** 165 – 170

Thangarasu, V., Manuiappan, J, Bangaru, A.(2002).Anti-diabetic activity of alcoholic extract of *CelosiaArgentea*Linn seeds in rats. *Biol. Pharm. Bull*. **25**:526-528.

Thiruppathi, S., Ramasubramanian, V., Sivakumar, T. and Thirumalai, A.V. (2010).Anti-microbial activity of *Aloe Vera*(L) Burm.F. against pathogenic microorganism.*J.Biosci.Res.***1**(4): 251 – 258.

Ulanowska K, Traczyk A, Konopa G. and Wegrzym G (2006).Differential antibacterial activity of genistein arising from global inhibition of DNA, RNA and protein synthesis in some bacterial strains. Arch. *Microbiol*., **184**(5): 271-278.

Van Eldere, J. (2003). Multi-centre surveillance of *Pseudomonas aeruginosa*susceptibility patterns in nosocomial infections. *J. Antimicrob. Chemother.***51** (2): 347–352.

Voroshilova, G.G. and Bondarenko, V.M. (1991). "The efficacy of *KlebsiellaPneumoniae*bacteriophage in the therapy of experimental *Klebsiella*infection".*ZhurnalMikrobiologii, Epidemiologii, I Immunobiologii* (4): 5–8

Willcox, M., Bodeker, G., Philippe.And Rasoanaivo, P. (2005). Traditional medicinal plants and malaria.*J. Altern*. COMPLEM. Med., **11**(2): 381-382.

Yamanaka,N., Samu, O. and Nagao, S .(1996). Green tea catechins such as (-) epicatechin and (-) epigallocatechin accelerate cu+2 induced low density lipoprotein oxidation in propagation phase.*FEBS Lett.*,**401**: 230-234.

Yarza, P.,Ludwig, W., Euzéby, J., Amann, R., Schleifer, K., Glöckner, Frank O. and Rosselló-Móra, R.(2010). Date of the all-species living tree project based on 16S and 23S rRNAsequence analyses. *Systematic and Applied Microbiology***33** (6): 291–299

Yarza, P.,Richter, M., Peplies, J. R., Euzeby, J., Amann, R.,Schleifer, K. H, Ludwig, W and Glöckner, F. O. (2008). The all-species living tree project: a 16S RNA-based phylogenetic tree of all sequenced type strains. *Systematic and Applied Microbiology***31** (4): 241–250.