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Original article

Evaluation of antimicrobial activity of *Ricinus communis. L* leaves and seeds extracts

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Abstract

The antimicrobial activity of the leaf and seeds extracts of *Ricinus communis*. L extracted using soxhet and maceration extraction methods were assessed using disc diffusion method. A Gram- positive bacterium (*Bacillus subtilis* (Bs). as well as gram negative bacteria (*Pseudomonas aeruginosa* (Ps), *Salmonella* (Sa), *Escherichia coli* (EC) and a fungus *Candida* (Ca) were used in the bioassay. Comparison of the antimicrobial efficacy of the methanolic extracts of seeds and leaf showed that the latter were more potent against the test organisms with the exceptions of *Escherichia coli* (EC). The maximum zone of inhibition was obtained using seed methanolic extract on *Candida albicans* , followed by Chloroform and Methanolic extracts (maceration) on E. coli, whereas, all other microbes where found resistant to the different extracts or exhibited very weak sensitivity. The maximum antimicrobial activity for the leaf extract was obtained by methanolic extract (Maceration) on C. *albicans* and *Bacillus subtilis* , and the petroleum ether extract on *Psedomonas aeruginosa*. Whereas, the other microbes exhibited moderated sensitivity to the different extracts used. The antimicrobial activity of leaf methanol maceration extract against EC decreases at higher concentrations while increases at higher concentrations against other pathogens.

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Introduction

Traditional medicine based on plant-derived remedies is the primary source of relief from a variety of diseases in Sudanese cultures. It should be promoted, investigated and its potential developed for wider use and benefit to mankind (WHO, 1978).

Primary health care has been adapted by all WHO member states including those in African continent as the appropriate strategy for developing national health system (Akerele 1988). In Sudan about 90% of the population relies on medicinal plants to treat various illnesses.

Castor plant, *Ricinus communis.L*, is a species of flowering plant in the spurge family, *Euphorbiaceae.* Its seed is the castor bean which, despite its name, is not a true bean. Castor plant is indigenous to the South Eastern Mediterranean Basin, Eastern Africa, and India, but is widespread throughout tropical regions (Philips, *et al*. 1999).

The oil extracted from the seed has been used in small doses in clinical setting for numerous medical conditions such as liver and gallbladder disturbances, abscesses, headaches, appendicitis, epilepsy, hemorrhoids, constipation, diarrhea, intestinal obstructions, skin diseases, hyper activity in children and to avert threatened abortion in pregnant women (Christopher *et al.*1996)

Ricin, a toxic protein in the seeds, acts as a blood coagulant. Oil used externally for dermatitis and eye ailments. Seeds which yield 45%-50% of a fixed oil also contain alkaloids ricinine and toxic albumin ricin and they are considered purgative, counter-irritant in scorpion stings and fish poison, leaves applied on head for headache and as poultice for boil (Duke and Wain.1981).

Ricinus communis.L contains toxic organic substance present in the plant. These compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, phenol compounds, flavonoids resins, fatty acids and gums, which are capable of producing definite physiological action on body (Erdogrul. 2002).

The chemical compositions of the leaves ethanol extract of *R communis.L* are investigated using gas chromatography-mass spectroscopy (GC-MS). GC-MS analysis of *R. communis.L* alkaloid leaves ethanol extract revealed the existence of the n- hexadecanoic acid, octadecanoic acid, 1-hexadecanol. 2-Methyl, gibb-3-ene-1. 10-decarboxylic acid, 2,4a 7trihydroxy-1-methyl-8-methylene, 1.4a-lactone.L-valineethyl ester, triethyl citrate, diethyl phthalate, and 3-octadecene. (Ameera, *et al*.2015).

Ricinine is a toxic alkaloid found in the leaves and seeds of *Ricinus communis L*. It can cause vomiting and various other toxic reactions, including liver and kidney damage, convulsions, and hypotension, and can even lead to death (Pinqpinq *et al.* 2015).

Ricinus communis .L plant is reported to have many medicinal activities, (Almeida, e*t al.,* 2009) study reveals that a lactin isolated from *Ricinus communis* is ricin A, possesses antitumor activity that was more toxic to tumor cell than non-transformed cells, judged from ED_{50} of lectin towards tumor cells and non-transformed cells (Lin and Liu, 1986). The crude extract from root bark of *Ricinus communis* possesses central analgesic activity in tail flick response model to radiant heat at a dose of 250mg\kg f.w. The ethanolic extract of pericarp of fruit of *Ricinus communis* possesses typical CNS stimulant and neuroleptic effects.

(Kumar 2011) reported that the ethanolic extract of *Ricinus communis* roots resulted in antihistaminic activity at the dose 100,125, and 150 mg\kg intraperitoneally by using clonidine induced catalepsy in mice.

The pant *Ricinus communis* seeds extract showed to have cytotoxic effect due to the presence of toxic material called ricin which mainly inhibits the synthesis of protein in cells and cause cell death; therapeutically it can be used to target cancer cells (Lord, *et al.,* 2003)

This study was intended to determine the antimicrobial activity of different extracts of *Ricinus communis .L* leaves and seeds using disc diffusion method.

MATERIALS AND METHODS

Plants Materials

The leaves and seeds of *Ricinus communis.L* were collected from the farms of agriculture college *SUST, Bahri* area. The taxonomy and identification of the plant carried out at the department of botany, college of science, Alneelain University. The leaves were washed with water from dusts and contaminants and dried under the room conditions, the seeds grinded with clean mortar and pestle.

Chemicals

Petroleum ether, chloroform, methanol, chemicals for antibacterial assays (Suphadex), normal saline, Saboraud dextrose agar, Mueller hinton agar.

Test microorganisms

Bacterial microorganisms

Bacillus subtilis NCTC 8236 (A Gram positive bacterium).
Salmonella ATCC 25923 (Gram negative Bacterium).
Escherichia coli ATCC 25922 (Gram negative bacterium).
Pseudomonas aeruginosa ATCC 27853 (Gram negative bacterium).

National Collection of Type Culture (NCTC), Colindale, England.

American Type Culture Collection (ATCC) Rockville, Maryland, USA.

Fungal microorganism

Candida albicans ATCC7596

Preparation of different extracts

Soxhlet extraction

20g of powdered leave, 15g paste of seeds were accurately weighed and extracted in Soxhlet apparatus with 200ml petroleum ether, chloroform and methanol respectively. The temperature of the heater adjusted according to the boiling point of each solvent, the solvents then evaporated under room temperature then dry extracts were obtained and calculation of the yield percentage was carried out.

Maceration extracts

20g of powdered leaved and seeds were macerated in methanol\water (80%) solvent for 3 days, then the solvents evaporated in room temperature, methanol leaves and seeds extracts were obtained and calculation of yield percentage was carried out.

Preparation of the test organism

Preparation of bacterial suspensions

1 ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 108- 109 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing of antibacterial susceptibility

Disc diffusion method

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 108cfu/ ml (turbidity = McFarland standard 0.5). One hundred

microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 ℃ for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

RESULTS AND DISCUSSION

The effect of extraction method and solvents on antioxidant activity

Percentage yields

The extraction of *Ricinus communis,L* leaves and seeds was performed successively using different solvents (petroleum ether, chloroform and methanol respectively) using Soxhlet apparatus and maceration with methanol.

Results in table 1 and table 2 show the extraction percentages of *Ricinus communis.L* seeds and leaves respectivelyTable

Table (1): Percentage Yields of maceration and Soxhlet extraction of *Ricinus communis* leaf and seeds

Plant Sample	**Soxhlet Extraction Method**			**Maceration Extraction Method**
	Petroleum Ether	**Chloroform**	**Methanol**	**Methanol**
Ricinus communis.L leaf	4.89	4.07	11.31	9.99
Ricinus communis.L seed	43.36	12.66	8.90	25.32

Petroleum ether solvent has extracted castor oil from *Ricinus communis.L* seeds about 43.36% which is the highest yield compared with chloroform and methanol extracts, the yield percentage of the present study was in accordance with (Jibrin *et al*. 2015) who carried out study on optimization percentage of castor oil yield extracted from castor beans using response surface methodology and Box-Behnkin design. The maximum yield of oil extracted was 50.9% (hexane), 49.0% (petroleum ether). In *R.communis.L* leaves, methanol has given highest yield (11.31%) compare to petroleum ether and chloroform. The present results is in accordance with a study carried by (Jennifer, *et al*, 2017), the study showed that for 100 g each in the different solvent the percentage yields were determined and were found to be 5.2, 6.0, 6.8, 7.2 and 8.3% for ethanol, water, petroleum ether, ethyl acetate and methanol respectively, thus methanol extract has the highest yield (8.3%)

Antimicrobial activity

Antibacterial activity of *Ricinus communis L.* seeds and leaves was tested against different pathogens (gram positive and negative bacteria and a fungus *Candida*) using the disc diffusion method.

Table (2) shows the comparison between antibacterial activities of *Ricinus communis.L* seeds and leaves extracts.

Extracts Concentration (100mg/ml)	**Mean Diameter of Inhibition zone (MDIZ)/mm**							
	Leaf Extracts				**Seeds Extracts**			
	PE	**CH**	**ME (sox)**	**ME (mac)**	**PE**	**CH**	**ME (sox)**	**ME (mac)**
Microorganism								
Escherichia coli	10	-	-	-	14	-	-	15
Pseudomonas aeruginosa	15	-	14	14	-	-	-	-
Salmonella	11	14	-	13	10	-	10	14
Bacillus subtilis	-	14	-	15	-	-	-	-
Candida albicans	10	14	14	15	-	15	17	12

Keys: PE: Petroleum ether, CH: Chloroform., ME: Methanol., ME(sox): Methanol soxhlet extract., ME(mac): Methanol Maceration extract., <14 resistant, 14-18 moderate, >18 sensitive

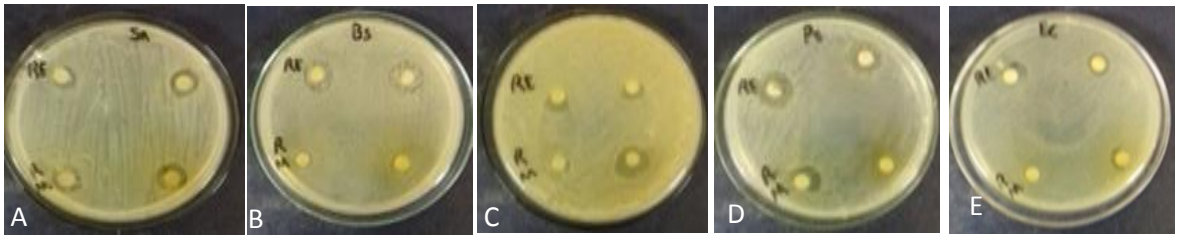

Plate 1: Inhibition zones of Petroleum ether (RE) and methanol extracts (RM) of leaf methanol extracts (RM) of leaf against (A) *Salmonella (Sa), (B) Bacillus subtilis* (Bs) (C) *Candidda albicans (Ca), (D)Pseudomonas aeruginosa (Ps) and (E) Escherichia coli (EC)*

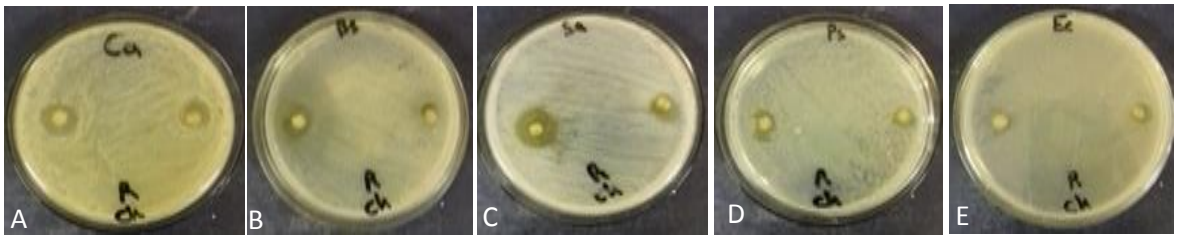

Plate 2: Inhibition zones of chloroform extract of leaf against (A) *Candidda albicans (Ca),* (B) *Bacillus subtilis (Bs),* (C) *Salmonella (Sa), (D) Pseudomonas aeruginosa (Ps), and (E) Escherichia coli (EC)*

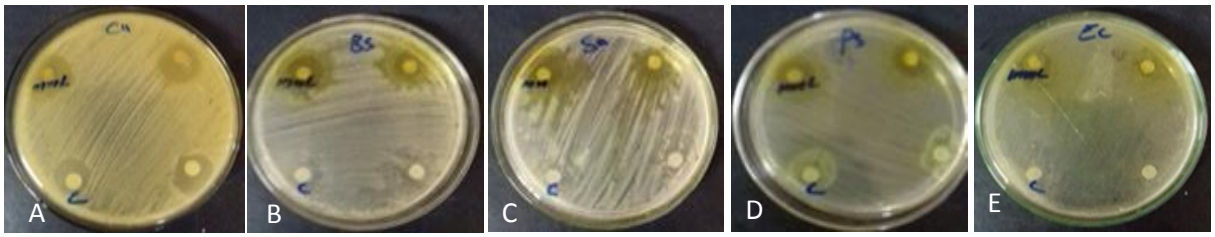

Plate 3: Inhibition zones of methanol maceration (mmL) of *R.communis.L* leaf and chloroform (C) seed extracts against *(A) Candidda albicans* (*Ca), (B) Bacillus subtilis (Bs), (C) Salmonella (Sa), (D) Pseudomonas aeruginosa (Ps)* and (E) *Escherichia coli (EC)*

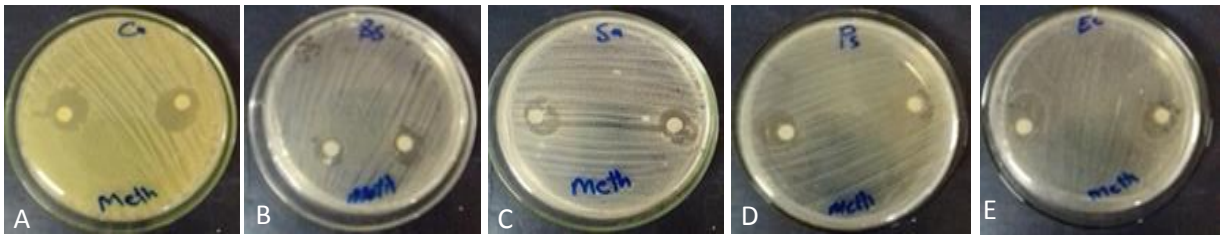

Plate 4: Inhibition zones of methanol extract of *R.communis* seed againist (A) *Candidda albicans* (*Ca), (B) Bacillus subtilis (Bs), (C) Salmonella (Sa, (D) Pseudomonas aeruginosa (Ps)* and (E) *Escherichia coli (EC)*

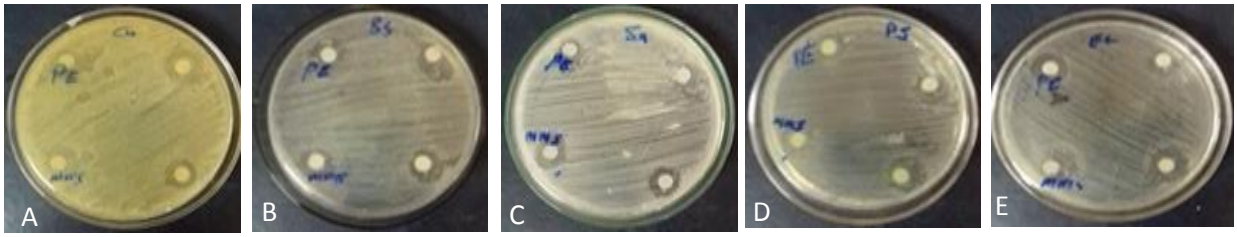

Plate 5: Inhibition zones of petroleum ether and methanol maceration extracts of *R.communis.L* seed against (A) *Candidda albicans* (*Ca), (B) Bacillus subtilis (Bs), (C) Salmonella (Sa), (D) Pseudomonas aeruginosa (Ps) and (E) Escherichia coli (EC)*

The *R. communis. L* methanolic maceration leaf extract showed the maximum (15mm) antibacterial activity as compared to petroleum ether and chloroform extracts against *Bacillus subtilis* and *Candida albicans* and the lowest activity against *Escherichia coli* (no activity).
The *R. communis.L* seed methanolic extract showed maximum zone of inhibition (17mm) against *Candida albicans* and exhibited (15 mm) zone of inhibition against *Escherichia coli* and the minimum zone of inhibition was recorded against *Pseudomonas aeruginosa and Bacillus subtilis* (no activity).
The chloroform extract of *R.communis.L* seeds showed the lowest antibacterial activity against the gram positive bacterium *Bacillus subtilis* as well as the gram negative bacteria (*Pseudomonas aeruginosa*, *Salmonella, Escherichia coli*) compared to methanol and chloroform of leaf extracts.
Methanolic (80% v\v) leaf extract of R. *communis.L* inhibited the fungal growth of *Candida albicans* by inhibition zone of (15 mm). While the methanol soxhlet extract of leaf exhibited inhibition zone of (14mm).
Methanol leaf extract was found to be more effective in inhibiting the fungal growth as compared to the seeds and other leaf extracts.
Antibacterial and antifungal activities of the (methanolic (sox) and maceration) and chloroform leaf extracts of *R. communis.L* have been evaluated in the present research work. The *in vitro* antimicrobial activity of different plant extracts is a first step towards the development of new potential drugs.
These results are in agreement with the previous work which showed that in plants most of the compounds having antimicrobial potential are soluble in methanol (Chandrasekaran, *et al.* 2004) and methanol leaf extract is more effective against pathogenic bacterial strains than ethanol or water extracts (Naz, *et al*. 2011).
Fungi are major skin disease causing organisms. Many species of fungi are also responsible for several plant pathogens. Antifungal activity of *R. communis.L* was studied and a little bit considerable activity was found. Methanol leaf extract exhibited about (15mm) against *Candida albicans.*
The present study indicated that the antibacterial and antifungal activity vary with the different solvents of plant leaf material used.
R.communis.L is effective even at low concentrations against bacterial and fungal pathogens (Zarai, 2012).
Generally, at present with the spread of resistance against antibiotics almost at regular scale (Olayinka, *et al.* 2004) and noticeable challenges confronted with medical physicians in the treatment of many infectious diseases (Taiwo, *et al*. 2002), such plants should be considered to reap all the possible antimicrobial benefits intrinsic in them. In this way, the actual ingredients of having antimicrobial potential must be extracted and then identified. The tolerable level and toxic effects of such compounds on human as well as on animals should be properly investigated. This work provides a scientific validation to medicinal plant in having potential to be a good drug. This study further requires the isolation and identification of the phytochemicals and active compounds in the plant material used

Antimicrobial activity of different concentrations of methanolic extract
The experiment was carried out for methanol maceration extract of *Ricinus communis.L* leaves using concentration ranges from 12.5, 25, 50, and 100 mg\L
Table (3) shows the inhibition zones of different concentrations of methanol maceration extract of *Ricinus communis.L* leaf.

Microorganisms	**Mean Diameter of Inhibition Zone (MDIZ)/mm**			
	100	50	25	12.5
Escherichia coli	14	15	17	14
Pseudomonas aeruginosa	-	-	-	-
Salmonella	16	15	15	9
Bacillus subtilis	12	-	-	-
Candida albicans	22	19	19	17

Key: *MDIZ* <14 resistant, 14-18 moderate, >18 sensitive.

ifferent concentrations were tested using methanol maceration extracts of *Ricinus communis.L* leaf because it has given the best antimicrobial potential against pathogen among all, as the concentration decreases the inhibition zones decreases except for *E.coli* at concentration of 50 and 25mg\ml

CONCLUSION

Soxhlet extracts of *Ricinus communis L*. leaves using petroleum ether, chloroform and methanol have given antimicrobial activity fluctuate between resistant and moderate but maceration extract of the leaves gave a result of moderate against all the pathogens except *EC.*
For the Soxhlet extraction of seeds, extracts have given a result of resistant to most pathogens while the maceration extract of the seeds has showed moderate result against *EC* and *Sa.*
The antimicrobial activity of leaf methanol maceration extract against *EC* decreases at higher concentrations while increases at higher concentrations against other pathogens

RECOMMENDATION

Fractionation of methanol maceration extract of *Ricinus communis.L* leaves using column chromatography and

PTLC and isolation of pure compounds to identify the high antimicrobial compounds and synthesize them in laboratory.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

REFERNCES

Ameera O H, Imad H H, Huda J, Muhanned A K (2015). Determination of alkaloid compounds of *Ricinus communis* by using gas chromatography- mass spectroscopy (GC-MS).*Journal of medicinal plants research*. 10: pp. 349-359.

Akerele O (1988). Medicinal plants and primary health care. An agenda for action.*Fitoterapia*, *LIX*.p.355-63.

Almeida R N, Navarro D S, Barbosa Filho J M (2009). Plants with central analgesic activity. *phytomedicine*, 8(4): 310-322.

Christopher B (1996). *The Royal Horticultural Society AZ Encyclopedia of Garden Plants*. London: *Dorling Kindersley*.2nd edition .pp.884-885.

Chandrasekaran M, Venkatesalu V, (2004).

Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. J Ethnopharmacol. 91: 105-108.

Duke A J and Wain (1981). *Medicinal Plants of the World*.V :3

Erdogrul O T (2002). Antibacterial activities of some plant extracts used In folk medicines. *Pharma.Biol*, 40:pp 269 – 273.

Jennifer S, Richard M, Addai M, Donkor (2017). Antibacterial and antifungal activities and phytochemical profile of leaves extract from different extractants of *Ricinus communis* against selected pathogens.*BMC Research Notes.*volume 10, Article number: 660.

Jibrin M D, Agus B A, Muhammad A Z, (2015). Solvent extraction of castor beans oil; experiemental optimization via response surface methodology.

Kumar A, (2011). I n vitro immunomodulatory activity of *Ricinus communis.*201-201.

Lin J Y, Liu S Y (1986). Studies on the antitumor lectins isolated from seeds of *Ricinus communis* (castor bean). *Toxicon*, 24(8): 757-765.

Lord M J, Jollife N A, Marsden C J, Patemen C S, Smith D C, Sponer R A, Aatson P D, Robertd L M (2003). Ricin mechanism of cytotoxicity. *Toxicol Rev*. 2253-64.

Miles A A, Misra S S (1938). The estimation of the bactericidal power of the blood. *Journal of hygiene*. 38: 732-749.

Naz R, Bano A, Yasmin H, Ullah S, (2011). Antimicrobial potential of the selected plant species against some infectious microbes used. *J Med Plants Res;* 5(21): 5247-5253**.**

Olayinka AT, Onile BA, Olayinka BO, (2004). Prevalence of multidrug-resistance (MDR) *Pseudomonas aeruginosa* isolates in surgical units of *Ahmadu Bello University Teaching Hospital*, An indication for effective control measures. *Zaria, Nigeria, Ann Afri Med*; 3(1): 13-16.

Phillips R, Martyn R (1999). *Annuals and Biennials.* London: Macmillan. V: 2 p.106.

Pinqpinq G, Dandang W, Gunsonq W, Ge D (2015*). State key laboratory of natural medicine*.

National Committee for Clinical Laboratory Standards (NCCLS) (1999).Performance standards for antimicrobial susceptibility testing; ninth informational supplement. *Wayne*, Pensilvania document M100-S9.Vol.19.

Taiwo SS, Okesina AB, Onile BA, (2002). In vitro antimicrobial susceptibilitypattern of bacterial isolates from wound infections in University of Ilorin Teaching Hospital. *Afr J Clin Exp Microbiol*. 3(1): 6-10

WHO (1978).The promotion and development of traditional medicine. Geneva.