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Effect of the Combination of Stem Bark Extract of *Parkia biglobosa* (Jacq) Benth and Certain Antibiotics against Some Organisms of Medical Importance

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Plant-derived compounds are known to exhibit a direct antibacterial activity and or an indirect activity as antibiotic resistance modifying compounds, and when combined with antibiotics, increased effectiveness may be observed. In this study, effort was directed towards combining the aqueous fraction of the methanol extract of the stem bark of *Parkia biglobosa* with some antibiotics

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to observe their combination effects on some organisms of medical importance. The minimum inhibitory concentration (MIC) of the extract and test antibiotics was determined using the checkerboard assay. Combination studies were carried out to ascertain the activities of the combinations against test organisms using the rate of kill assay and checkerboard assay. Results obtained confirmed interaction between the plant extract and the test antibiotics. It specifically confirmed synergistic interaction between Tetracycline, Erythromycin and Nalidixic acid respectively and the extract against *Pseudomonas aeruginosa* and *Escherichia coli*. Results obtained proved that in the search for alternative ways of combating bacterial infections, combination of plant extract with antibiotics could boost effectiveness and the aqueous fraction of the methanol extract of *Parkia biglobosa* is a possible candidate for this purpose against two of the tested organisms.

Keywords: Stem bark extract; antibacterial activity; Parkia biglobosa; checkerboard.

1. INTRODUCTION

Secondary metabolites from plants have been found to potentiate the efficacy of synthetic antibiotics when they are combined [1]. These compounds are believed to play a role in plants defence against infection by working in synergy with intrinsic antimicrobials [2]. It has therefore been suggested that such compounds can potentially be used to improve the efficacy of antibiotics against even multi-drug resistant (MDR) bacterial pathogens. Recently, scientists have invaded the plant kingdom in search of new bioactive molecules for either the formulation of new agents for the control of emerging resistant organisms or its synergistic combination with commercially available antibiotics especially the early generation antibiotics [3,4]. Generally, scientists have recommended the combination of antibiotics with natural antimicrobial plant products as a remedy to infections associated with multiple drug resistant bacterial strains [5,6,7].

The plant *Parkia biglobisa* used in this study is a member of the family Fabaecea and the subfamily Mimocaceae. Popularly called the African Locust Bean tree, it has been reported by several authors to have vast medicinal uses [8-11] possibly due to the presence of certain secondary metabolites like tannins flavonoids and saponins which are known to have antibacterial activities. It has also been reported to be non toxic [12-15]. This makes the plant a potential candidate that could be experimentally examined for its ability to combine with antibiotics for better effects. This work was designed to investigate the possible antimicrobial effects of the aqueous fraction of *Parkia biglobosa* bark extract when combined with some selected antibiotics on some organisms of medical importance.

2. MATERIALS AND METHODS

2.1 Plant Sample Collection, Identification and Authentication

The stem bark of *Pakia biglobosa* was obtained from Samaru- Zaria, Kaduna State, Nigeria. Identification and authentication were done at the Department of Biological Sciences, Ahmadu Bello University, Zaria in Kaduna state, Nigeria where a reference material No 2864 was deposited.

2.2 Extraction of Plant Sample

The stem bark of *P. biglobosa* collected for extraction was carefully cleaned and air dried on a laboratory bench for five days. It was ground into powder using a mortar and pestle. 500g of the ground stem bark was introduced into a flask containing 2.5litres of 70% methanol. It was then kept for maceration at room temperature for 72 hours with intermittent shaking. The filtrate obtained was allowed to evaporate by drying using mild heat.

2.3 Fractionation

The methanol extract obtained was further partitioned into petroleum ether, chloroform and aqueous fractions according to the method of Udobi et al (2008). 20g of the extract was dissolved in 200ml of water and shaken vigorously in a separating flask and the mixture obtained was filtered using a filter paper to remove debris. 200mL of petroleum ether was then added to the mixture in a separating funnel, shaken vigorously and allowed to settle. The petroleum ether layer (on top) was removed and concentrated while a further 200ml of chloroform was added to the aqueous layer, shaken vigorously and allowed to settle. The aqueous

and the chloroform layers were further separated. While the chloroform and petroleum portions were concentrated to dryness by allowing them to stand on the laboratory bench until all the solvent evaporated, the aqueous layer was concentrated to dryness using mild heat.

2.4 Test Organisms

Standard cultures of *Staphylococcus aureus* NCTC6571, *Escherichia coli* NCTC10418 and *Pseudomonas aeruginosa* ATCC27853 were used for the studies. The organisms were obtained from the laboratory of the Department of Pharmaceutical Microbiology, University of Uyo, Nigeria. They were used after their identity had been confirmed using standard microbiological methods.

2.5 Test Antibiotics

All antibiotics (Tetracycline, Ampicillin, Nalidixic acid and Erythromycin) that were used for the combination assays were purchased from reputable Pharmacies in Uyo metropolis.

2.6 Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of the plant extract as well as the test antibiotics was determined using the 96 well broth microdilution procedure. 30µL of nutrient broth was introduced into all the 96 wells using a micropipette. To the first well/ row of the plate, 30µLof a double strength stock solution of the agent was added. Then serial two fold dilutions were done using a micropipette to dilute the agents appropriately to the required concentrations. Each well was then inoculated with an equal volume of the test organism standardised to 10^5cfu/ml and the plates were incubated at 37°C for 24hours.The MIC was obtained as the lowest concentration without visible turbidity.

2.7 Combination Studies Using Checkerboard

The interaction between the antibiotics and the plant extracts was determined using the checkerboard method involving the 96 wells microdilution procedure. Stock solutions containing the extract and each antibiotic were made respectively. Into each well 30 µL of nutrient broth was introduced and then 30µL of the stock solution with double MIC was added to the first well in order to be diluted in series along the wells of the ordinate of the first line. The antibiotic was serially diluted along the x-axis while the extract was diluted along the Y-axis in such a way that each well contained the combination of the two agents. Subsequently, a drop of the bacterial inoculum of 10^5 cfu/mL was added to each well of the plate. Incubation of the plates was carried out at 37 °C for 24 hours under aerobic conditions.

2.8 Rate of Kill

The rate of kill of the extract, antibiotics and their combinations was determined using the method of Basri *et al.,* 2019. Four set of bottles labelled 1, 2, 3 and 4 were used for each isolate, where bottle set 1 served as the control. To each set of bottle, 9mL of nutrient broth was added. To each of bottles of set 2, 3 and 4, a drop of an overnight culture was added and then, 1mL of either of the extract at its 1/2MIC, MIC and 2MIC concentration respectively was also added. To determine their combination effects, combinations of different concentrations ranging from 0.25xMIC to 2xMIC of each of the antibiotics and the extract at MIC were used. The bottles were then incubated at 37°C and viable counts taken at 30min intervals by withdrawing 1mL of the mixture in the bottle and diluting in nutrient broth. The diluted mixture was plated out on nutrient agar plates and incubated at 37°C for 24 hours. The process was repeated for the different antibiotics used for this study and also with the combined solution of the plant extract with the different antibiotics used respectively. The developed colonies were counted and the colony forming units (cfu/mL) for each were calculated and expressed in Log 10. This was used to generate a time-kill curve for each isolate by plotting Log 10 colony forming unit against time.

3. RESULTS

The interpretation of the results was done by calculating the Fractional Inhibitory Concentration Index (FICI) = FIC A + FIC B.

Where,
FIC A is the MIC of extract in the combination / MIC of extract alone,
FIC B is the MIC of antibiotic in the combination / MIC of antibiotic alone.
and,
FICI ≤ 05= Synergistic effect, 0.5 < FICI ≤ 1= Additive effect, 1< FICI ≤ 4= Indifference effect, FICI > 4= Antagonism

Table 1. Minimum inhibitory concentration of the extract

Organism	MIC value
Staphylococcus aureus	3.125
Pseudomonas aeruginosa	1.563
Escherichia coli	3.125

Table 2. Minimum inhibitory concentration of the antibiotics

Organism	Antibiotic	MIC value
Stapylococcus aureus	TET	0.015
	ERY	0.015
	AMP	0.012
	NAL	0.025
Pseudomonas aeruginosa	TET	0.015
	ERY	0.015
	AMP	0.012
	NAL	0.049
Eschericia coli	TET	0.015
	ERY	0.015
	AMP	0.012
	NAL	0.025

Keys: TET = Tetracycline, ERY = Erythromycin, AMP = Ampicilin, NAL = Nalidixic acid

Table 3. Table showing the outcome of the combination of aqueous fraction of methanol extract of *Parkia biglobosa* and different test antibiotics used to challenge the test organisms

Antibiotic	Organism	MIC of Extract	MIC of Antibiotic	MIC of Extract incombination	MIC of the antibiotics in combination	Result (FIC A + FIC B)	Conclusion
Tetracycline	*S.aureus*	3.125	0.015	1.563	0.008	1.03	INDIFFERENCE
	E.coli	3.125	0.015	1.563	0.004	0.77	ADDITIVE
	P. aeruginosa	1.563	0.015	0.391	0.002	0.38	Synergistic
Erythromycin	*S.aureus*	3.125	0.015	0.782	0.008	0.78	ADDITIVE
	E.coli	3.125	0.015	0.391	0.004	0.39	Synergistic
	P. aeruginosa	1.563	0.015	0.391	0.008	0.78	ADDITIVE
Ampicillin	*S.aureus*	3.125	0.012	1.563	0.003	0.74	ADDITIVE
	E.coli	3.125	0.012	1.563	0.006	1.00	ADDITIVE
	P. aeruginosa	1.563	0.012	0.782	0.003	0.75	ADDITIVE
Nalixidic Acid	*S.aureus*	3.125	0.025	1.563	0.012	0.98	ADDITIVE
	E.coli	3.125	0.025	0.782	0.006	0.49	Synergistic
	P. aeruginosa	1.563	0.049	0.782	0.025	1.00	ADDITIVE

Key: S. aureus = Staphylococcus aureus, E.coli = Escherichia coli, P. aeruginosa = Pseudomonas aeruginosa, FIC A is the MIC of extract in the combination / MIC of extract alone and FIC B is the MIC of antibiotic in the combination / MIC of antibiotic alone

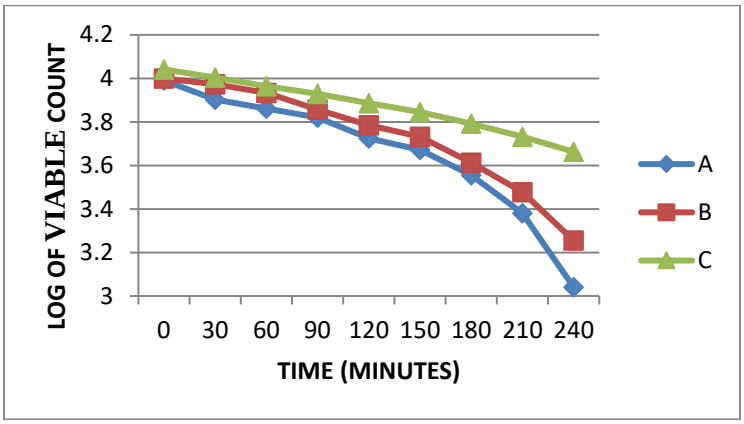

Fig. 1. A chart of the rate of reduction of viable count of *Staphylococcus aureus, Escherichia coli and Pseudomonas aeroginosa* challenged with the mixture of Tetracycline and Aqueous fraction of methanol extract of *Parkia biglobosa* at their minimum inhibitory concentrations

KEY: A = Staphylococus aureus, B = Pseudomonas aeroginosa, C = Escherichia coli

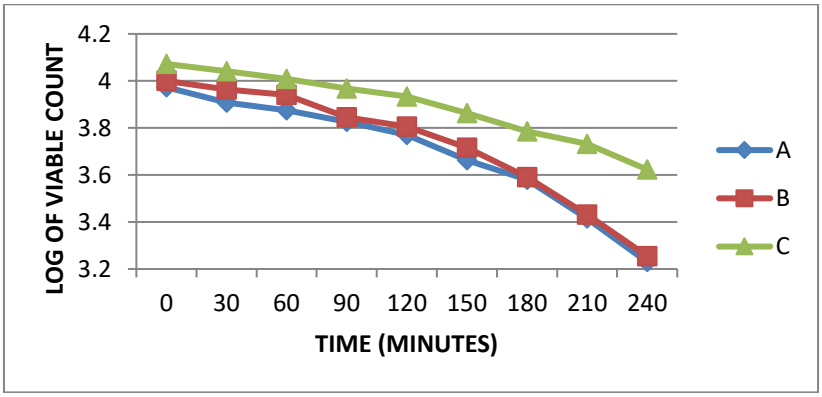

Fig. 2. A chart of the rate of reduction of viable count of *Staphylococcus aureus, Escherichia coli and Pseudomonas aeroginosa* challenged with the mixture of Ampicilin and aqueous fraction of methanol extract of *Parkia biglobosa* at their minimum inhibitory concentrations

KEY: A = Staphylococus aureus, B = Pseudomonas aeroginosa, C = Escherichia coli

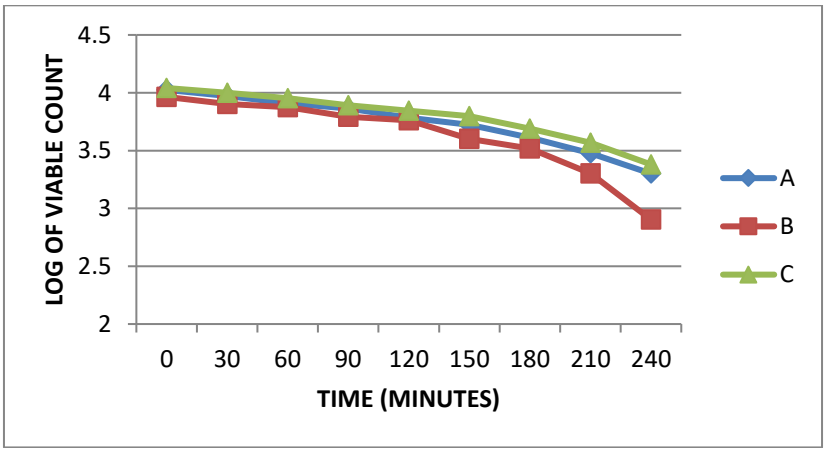

Fig. 3. A chart of the rate of reduction of viable count of *Staphylococcus aureus, Escherichia coli and Pseudomonas aeroginosa* challenged with the mixture of Erythromycin and Aqueous fraction of methanol extract of *Parkia biglobosa* at their minimum inhibitory concentrations

KEY: A = Staphylococus aureus, B = Escherichia coli, C = Pseudomonas aeroginosa

Table 4. Table showing percentage reduction in population of test organisms (cfu/ml) when challenged with the aqueous fraction of the methanol extract of *Parkia biglobosa*

Concentration of Methanol Extract	*Staphylococcus aureus*	*Pseudomonas areuginosa*	*Escherichia coli*
MIC	4.0	3.71	2.3
2MIC	5.05	5.63	3.47
4MIC	7.77	7.82	3.84

Table 5. Table showing percentage reduction in population of the test organisms (cfu/ml) when challenged with the test antibiotics

	Percentage reduction											
Concentration	**Ampicillin**			**Tetracycline**			**Nalidixic acid**			**Erythromycin**		
	S. A	**E. C**	**P. A**	**S. A**	**E. C**	**P. A**	**S. A**	**E.C**	**P.A**	**S. A**	**E.C**	**P.A**
HALF MIC	10.20	14.00	8.60	14.70	19.34	10.22	7.48	7.37	6.45	9.96	11.60	9.49
MIC	13.99	16.80	13.70	19.17	11.56	10.59	13.09	15.10	14.61	17.22	18.53	13.95

KEY: S.A = Staphylococcus aureus , P.E = Pseudomonas areuginosa , E.C = Escherichia coli

Table 6. Table showing percentage reduction in population of test organisms (cfu/ml) when challenged with the combination of a test antibiotic at different concentrations and the (MIC) of the Aqueous fraction of the Methanol extract of *Parkia biglobosa*

Concentration	**Ampicillin + Extract**			**Tetracycline + Extract**			**Nalidixic acid + Extract**			**Erythromycin + Extract**		
	S. A	**E. C**	**P. A**	**S. A**	**E. C**	**P. A**	**S. A**	**E.C**	**P.A**	**S. A**	**E.C**	**P.A**
MIC	18.70	18.63	11.03	23.80	9.35	18.63	13.65	41.33	9.40	17.87	26.77	16.34
2MIC	27.48	23.62	13.86	-	10.24	25.39	15.71	41.74	10.12	21.5	41.33	19.85
4MIC	-	31.65	18.29	33.42	17.66	41.05	23.81	-	12.17	26.95	-	26.25

KEY: S.A = Staphylococcus aureus, P.A = Pseudomonas aeruginosa , E.C = Escherichia coli

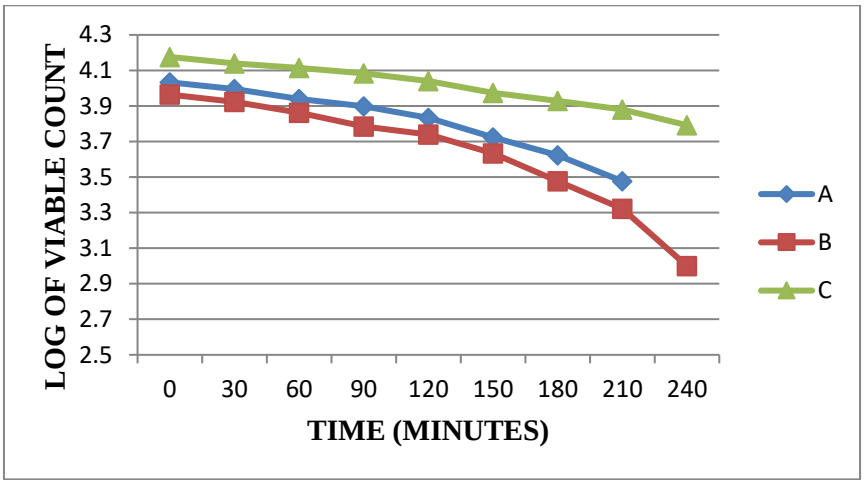

Fig. 4. A chart of the rate of reduction of viable count of *Staphylococcus aureus, Escherichia coli and Pseudomonas aeroginosa* challenged with the mixture of Nalidixic acid and aqueous fraction of methanol extract of *Parkia biglobosa* at their minimum inhibitory concentrations

KEY: A = Staphylococus aureus, B = Escherichia coli, C = Pseudomonas aeroginosa

4. DISCUSSION AND CONCLUTION

Plants generally are known to posses numerous phytochemicals that inhibit the growth of bacteria using different means. They have therefore, continued to be the hope for new compounds which can be used against resistant organisms. Some plants have also been reported to produce drug resistance inhibitors that can improve the activities of antibiotics against drug resistant bacteria pathogens (Isra *et al.,* 2020).

Previous studies have shown that plant extracts and certain phytochemical composites combine effectively with antibiotics for use in therapy with resultant greater effect (Danielle *et al.,* 2019). This discovery can therefore be useful in the prevention or blockage of resistance processes hence helping in the containment of multidrug resistant pathogens [16]. Combination between known antibiotics and bioactive plant extracts could be beneficial (synergistic or additive) or deleterious (antagonistic or toxic outcome) [17].

Evaluated in this study is the interaction of the aqueous fraction of the methanol extract of the stem bark of *Parkia biglobosa* with each of four commonly used antibiotics majorly of the first generation which were selected from different classes of antibiotics. Results obtained confirmed the great potentials in *Parkia biglobosa* as has been reported by many researchers and goes further to reveal the nature of interaction between the aqueous fraction of the methanol extract of the stem bark of *Parkia biglobosa* and the test antibiotics (Ampicillin, Erythromycin, Tetracycline and Nalidixic acid) when exposed to certain organisms of medical importance.

Results obtained showed the presence of bioactive compounds like Tannins, flavonoids, anthraquinones, and cardiac glycosides. All of which are known to have antibacterial properties. These bioactive compounds are notably responsible for the antibacterial activities exhibited by medicinal plants. Flavonoids are responsible for varieties of pharmacological activities [18](Pandey, 2007), Tannins are noted to be useful in the tanning process and used as healing agents in inflammation, burn, piles etc [19]. Udobi and Onaolapo [12]; Bukar and Oyeyi, [20] reported non toxicity and good antibacterial activities of the stem bark of *Parkia biglobosa* and its widely reported use in folk medicine in West Africa was the attraction for its selection for this work.

Rate of kill and the checkerboard assays were used to evaluate the interaction of the combinations. Time-kill assay is used to evaluate the antimicrobial efficacy of agents or their combinations. It shows the pattern of reduction of the organisms over time and confirms if the action is bacteriostatic or bacteriocidal. On exposure of the test organisms to the antibiotics and their combinations, it was observed that there was a reduction in the viable cells population over time. Both the plant extract and the antibiotics had reduction effect on the test organisms. At MIC, the extract caused a percentage reduction of 4%, 3.71% and 2.3% on *Staphylococcus aureus,*

Pseudomonas areuginosa and *Escherichia coli population* respectively (Table 4). This reduction increased as concentration of the agents increased confirming the activity of the extract to be concentration dependent.

When the plant extract and the antibiotics were variously combined in different proportions, a higher percentage of reduction of viable cell population was observed (Table 6). The combinations with plant extract showing better antibacterial reduction effect on the test organisms points to the possibility of an interaction where a combination is taking place or one is helping the other to act. The results also confirm that the activity of the combinations are concentration dependent. For time kill assay endpoint determinations, bacteriostatic activity is a reduction between 0 and 3 Log10 Cfu/ml while bacteriocidal activity is a reduction between 3 Log10 and above at different interval from the original population [21]. Our results therefore confirm the action of the agent as bacteriostatic since no 3log cycle reduction was observed.

Result of the interaction studies using the checkerboard assay method are shown in Table 3 , They show that Erythromycin, Tetracycline and Nalidixic acid combined with the extract to produce a desired synergistic effect with a resultant higher percentage reduction in the viable cell population. Results obtained are in agreement with various other previously reported works which show interactions between plant extracts and a wide range of antibiotics.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ghaleb A, Mohammad M: Synergistic effects of plant extracts and antibiotics on Staphylococcus aureus strains isolated from clinical specimens. Middle-East Jour. Scie Res. 2008;3:134–139
2. Tegos G, Stermitz FR, Lomovskaya O. Lewis K: Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. Antimicrob. Agent. Chemother. 2002;46 (10):3133–3141.
3. Adwan GM, Abu-Shanab BA, Adwan KM. *In vitro* activity of certain drugs in Combination with plantplant extract against *staphylococcus aureus* infection Pakistan Jour Med. Sci. 2008;24:541-544.
4. Ali A, Al-Saady NA, Waly MI, Bhatt N, Al-Subhi AM, Khan AJ. Screening of indigenous Omani legumes for their nutritional quality, phytochemical composition and antioxidant properties. Int. J. Postharvest Tech. 2013;3(4):333-336
5. Ali NH, Kazim SU, Faizi S. Activity of synergistic combination amoxy-cassia against salmonella. Pak. Jour. Med. Sci. 2007;20:140–145
6. Udobi CE. Onaolapo JA: Bioactive Compounds of the stem bark of Parkia biglobosa. Journ. Appl. Pharm. Sci: 2012; 2(7):133-137.
7. Wijsman JA, Rieder MJ, Dekaban GA. Differential toxicity of reactive metabolites of clindamycin andsulphonmide in HIV-Infected cells. Journ. Clin. Pharmacol. 2005;45:346–351.
8. Asase A, Otng-Yeboah AA, Odamtten GT, Simmonds MSJ. Ethnobotanical study of same Ghanaian anti-malarial plants, J. Ethnopharmacol., 2005;99: 273-279.
9. Abo KA, Fred-Jaiyesimi AA, Jaiyesimi AEA. Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in south western Nigeria. J Ethnonopharmacol. 2008;115: 67-71.
10. Grønhaug TE, Glaeserud S, Skogsrud M, Ballo N, Bah S, Diallo D: Ethnopharmacological survey of six medicinal plants from Mali, West-Africa. Jour. Ethnobiol. Ethnomed. 2008;4:26.
11. Kayode J, Oyedeji AA, Olowoyo O. “Evaluation of the effect of pollution with spent lubricant oil on the physical and chemical properties of soil”. Pacific J. Sci. Tech. 2009;10(1):387-391.
12. Udobi CE, Onaolapo JA.: Cell kill pattern and acute toxicity studies of the aqueous fraction of the methanolic extract of parts of Parkia biglobosa. Afri. Journ. Biotech. 2010;8(31):4993-4998.
13. Aiyegoro OA, Okoh AI. Use of bioactive plant products in combination with standard antibiotics Implications in antimicrobial chemotherapy. Jour. Med. Pl. Res. 2009;3:1147-1152.
14. Cunha BA. Antibiotic side effects. Medical Clinics of North America. 2001;85:149–185.

15. Tegos G, Stermitz FR, Lomovskaya O, Lewis K. Multidrug Pump Inhibitors Uncover Remarkable Activity of Plant Antimicrobials, Antimicrob. Agents Chemother. 2002;46(10):3133-3141.
16. Hafid H, dan A Syam. Pengaruh aging dan lokasi otot terhadap kualitas organoleptik daging sapi Buletin Peternakan. 2007; 31(4):209-216.
17. Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine. 2008;15:639–652
18. Mahomoodally MF. Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. Evidence-Based Complementary and Alternative Medicin; 2013.
19. Boroushaki MT, Mollazadeh H, Afshari AR. Pomegranate seed oil: a comprehensive review on its therapeutic effects. Int J Pharm Sci Res 2016;7(2):430
20. Bukar A, Uba A, Oyeyi TI. Phytochemical analysis and antimicrobial activity of Parkia biglobosa (Jacq.) Benth. Extracts Againt Some Food Borne Microrganisms. Advances in Environmental Biology. 2010; 4(1):74-79
21. Drago Li. De, Vecchi E, Lombardi A, Nicola I, Valli M and Gismomdo MR. Bactericidal activity of levofloxacin, gatifloxacin. penicillin, meropenem and rokitamycin against Bacillus anthracis clinical isolates. Journal of Antimicrobial Chemotherapy. 2002;50(6): 1059-1063.

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