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# Evaluation of combined effect of *Azadirachta indica* and *Khaya senegalensis* oils on common fastidious microorganisms

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**Abstract**
The profound use of *Khaya senegalensis* oil (K. S) and *Azadirachta indica* oil (A. I) in Northern Nigeria herbal medicine practice and the promising result outcome have marked these plants oils as those whose curative potentials may be harnessed in the manufacturing of useful drugs that can be viable alternatives to some of the present day antibiotics. The aim of this study was to evaluate the antimicrobial properties of *Khaya senegalensis* oil and *Azadirachta indica* oil. Both oils were obtained from Uba central market of Borno State and tested for antimicrobial activities using serial dilution and agar well dilution using Levofloxacin as the standard. The activity index of the oil was also determined. Result showed that *A. indica* oil demonstrated a lower activity than *K. senegalensis* oil on *Klebsella pneumoniae*, *Staph aureus* and *E. coli*. However, synergistic study showed that the extracts when combined give a higher antimicrobial activity than the crude extracts alone.

**Keywords:** *Azadirachta indica* oils, *Khaya senegalensis* oils, synergistic and antagonistic antimicrobial activities

## Introduction
Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used with considerable importance in international trade. In certain African countries, however, up to 90% of the population still relies exclusively on plants as a source of medicines [1]. The development of resistance to current antibiotics by disease causing microbes has reinforced research for discovery of new ones. It is estimated that approximately one quarter of prescribed drugs contain plants extracts or active ingredients obtained from plant substances e.g. Aspirin, Atropine, Artemisinin, Colchicines, Digoxin, Ephedrine, Physostigmine, Pilocarpine, Taxol, Vincristine and Vinblastine [2].
Essential oils among various communities of Nigeria are extracted from several types of seeds or plants products. These oils are often treasured among its custodians (elderly and herbal medical practitioners especially women) and guarded secrets because of their commercial advantages, medicinal and spiritual purposes; depending on the people's belief [3]. Examples of such oil from plants seeds which are native to Northern Nigeria include *Khaya senegalensis* oil (usually referred to as "Mai or Man Madachi", which means bitter oil in Hausa language), *Ricinus communis* or Castor seed oil (Man gelo), *Butyospermum parkii* or Shea butter oil (Man Kadanya), *Azadirachta indica* or neem seed oil (Man Dogonyaro), *Balanites eagyptica* oil *(*Man Aduwa) and *Sesamum indicum* oil (Sesame oil) [3]. In folk medicine, various parts of *A. indica* have successfully been used as anthelmintic, antiseptic, diuretic and purgative actions, and are also used to treat boils, pimples, eye diseases, hepatitis, leprosy, rheumatism, ringworm and ulcers [4]. Its leaves are used in the treatment of malaria while the twigs are used as toothbrushes, and dentists find its twigs effective in preventing periodontal disease. The very bitter bark has a considerable reputation in its natural range as a fever remedy. The bark is also used as a vermifuge and for treating syphilis. Bark extract is used for treating jaundice, dermatoses, scorpion bite, allergies, infection of the gums, hookworm, bleeding wounds (disinfectant), and as a laxative [4]. Seeds and leaves are used for treating fever and headache; while its roots are used in the treatment of sterility, mental illness, syphilis, leprosy and as an aphrodisiac [4]. The oil extract of *A. Indica* seeds, have also been reported to elucidate antimicrobial activity depending on its protein and carbohydrate content [4]. In a study conducted by [5], the oil extract from the fruits of *Khaya senegalensis* was found to possess

some activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The oil has potential as a cosmetic product due to its easy emulsification, good spreadability, consistency, emollient property and being easily washed off from the skin. Similarly, [6] observed that the ethanol extracts of *Khaya Senegalensis* leaves have antimicrobial activity against *S. aureus, S. typhi, E. coli* and *C. albicans* at different concentration and can be used for the treatment of diarrhea, syphilis, pyrexia and malarial fever [7]. Phytochemical screening of both plants extracts revealed the presence of secondary metabolites such as saponins, cardiac glycoside, tannins, alkaloids and anthraquinones [6]. This study therefore evaluates the possibility of synergistic or antagonistic activities of *Khaya Senegalensis* and *A. indica* oil against microbial isolates.

## Materials and methods

### *Equipment and materials used*

Syringes (10 ml, 5 ml and 1 ml), Beakers, conical flasks, Pestle and mortar, Nutrient agar, autoclave, wire loop, Cotton wool, filter paper, Hexane, distilled water, Ethanol, Bunsen burner, Forceps, refrigerator, foil paper, hand gloves, Levofloxacin as standard (positive), universal sterile bottles and Cork borer.

### Sample Collection and Preparation

*Azadirachta indica* and *Khaya senegalensis* oil were obtained locally from the Uba central market in Askira Uba Local Government Area of Borno State, Nigeria. Tween 80 was used as an anionic surfactant in this study.

### Isolates Collection and Identification

The following organisms; *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae* were obtained from the Microbiology department of the University of Maiduguri Teaching Hospital, identified using Microgen kits and were stored in agar slants in a refrigerator pending the time the isolates were used.

### Antimicrobial screening

The antimicrobial screening was carried out as described by [8]. Nutrient agar was prepared by dissolving 28g in 1 litre of distilled water in a conical flask and then covered with aluminium foil. The media was boiled to dissolution and then sterilized at 121°C for 15 minutes in an autoclave. The media was allowed to cool to 45°C and 20ml of the sterilized media was poured into the sterilized petri dish and allowed to cool and solidify. The plates were dried aseptically at 37°C in an oven for 30 minutes and are to be used for well diffusion method. The plates were labelled with the test organisms and the microbes were spread evenly over the surface of the media with the aid of a glass spreader.

Serial dilution (concentration) of *A. indica* and *K. Senegalensis* oils were prepared individually and in combination aseptically in a sterile bottle. The oils (9ml each) were mixed with 1ml of Tween 80, to allow for easy dissolution into the agar while 2ml was also added with admixture (18ml) of the two oils combined. Further diluted to 1:100 was also done for all the oils.

A standard cork-borer of 6mm in diameter was used to cut a well at the centre of each inoculated plate and the agar removed from the well. 0.2ml of the oils was then introduced into the well created at the centre of each of the plates. The bacteria plates were incubated at 37°C for 24 hours and observed for the zone of inhibition of growth. The zones were measured with a transparent ruler and the results were recorded in millimetres.

### Determination of Activity index of oil

The activity index, which is a proportionality representation of the activity of the oils, was calculated thus;

$$\text{Activity index (AI)} = \frac{\textit{mean zone of inhibition of the oil}}{\textit{zone of inhibition for standard antibiotic}}$$

## Results and discussion

Table 2 and Table 3 showed the antibacterial activity of the crude extracts of *Khaya senegalensis* and *Azadirachta indica* respectively. *Khaya senegalensis* oil had increased activity on the organism tested (Table 2) as compared to *Azadirachta indica* (Table 3) which is only active against *Staphylococcus aureus.* Serial dilutions of *Khaya Senegalensis* oil with sterile water exhibited activity similar to the crude oil extract which indicates that the activity is unaffected by dilution except for *Escherichia coli* which shows no activity at the tested concentration. This activity conforms to the investigation by [5] in which the oil was found to possess some activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Table 4 shows the activity of crude mixed extracts of *Khaya senegalensis* and *Azadirachta indica* on the microorganisms tested. Higher activities were seen at ratio KA 3:1 and KA 2:2. The highest noted activity was seen with *S. aureus* with 15mm zone of inhibition.

Comparing the activity index of the extracts, the highest seen activity is with *K. pneumoniae* at a dilution of 2:2 with 0.4 activity index. Generally as compared with the activity of *Azadirachta indica*, the combined extracts have a better activity against a wider range of organisms than when used alone.

The antagonistic activity was seen at the ratio of 1:3 *Khaya senegalensis: Azadirachta indica*, this shows that at the concentration tested, the combination gives a lower activity as with the activity of *Khaya senegalensis* alone. The agonistic activity was seen at 2:2 *Khaya senegalensis: Azadirachta indica*. At this combination, the extracts showed activity against the variety of the organisms tested.

**Table 1:** Mean Concentration of the oils used

| Code number | Azadirachta indica (ml) | Khaya Senegalensis (ml) | Tween 80 (ml) | Total (ml) |
|---|---|---|---|---|
| $AK_1$ | 4 | 0 | 1 | 5 |
| $AK_2$ | 3 | 1 | 1 | 5 |
| $AK_3$ | 2 | 2 | 1 | 5 |
| $AK_4$ | 1 | 3 | 1 | 5 |
| $Ak_5$ | 0 | 4 | 1 | 5 |

**Table 2:** Antibacterial activity of *Khaya Senegalensis* crude and diluted oil extracts

| Extract | Organism | Standard | Mean ± SD | Activity index |
|---|---|---|---|---|
| KS | *K. pneumonia* | 36 | 12.33 ± 0.58 | 0.34 |
| | *S. aureus* | 37 | 14.00 ± 1.00 | 0.38 |
| | *E. coli* | 37 | 14.00 ± 1.00 | 0.38 |
| 1:10 Dilution | *K. pneumonia* | 37 | 14.00 ± 1.73 | 0.38 |
| | *S. aureus* | 41 | 13.67 ± 2.51 | 0.33 |
| | *E. coli* | 43 | 0 | 0 |
| 1.100 dilution | *K. pneumonia* | 40 | 11.67 ± 0.58 | 0.29 |
| | *S. aureus* | 40 | 14.00 ± 2.00 | 0.35 |
| | *E. coli* | 42 | 0 | 0 |

**Key**: KS = *Khaya Senegalensis crude extract.*
Standard used is Levofloxacin 200µg

**Table 3:** Antibacterial activity of *Azadirachta indica*

| Extract | Organism | Standard | Mean ± SD | Activity Index |
|---|---|---|---|---|
| AI | *K. pneumonia* | 39 | 0 | 0 |
| | *S. aureus* | 39 | 11.67 ± 0.58 | 0.30 |
| | *E. coli* | 42 | 0 | 0 |
| 1:10 Dilution | *K. pneumonia* | 38 | 0 | 0 |
| | *S. aureus* | 38 | 0 | 0 |
| | *E. coli* | 42 | 0 | 0 |
| 1:100 Dilution | *K. pneumonia* | 42 | 0 | 0 |
| | *S. aureus* | 39 | 0 | 0 |
| | *E. coli* | 39 | 0 | 0 |

AI- *Azadirachta indica* crude extract
Standard used is Levofloxacin 200µg

**Table 4:** Summary of the premixed and diluted oil extracts of *Khaya senegalensis* and *Azadirachta indica*

| Extracts | Ratio | Organism | Standard | Mean ± SD | Activity index |
|---|---|---|---|---|---|
| KA 3:1 | 3:1 | *K. pneumoniae* | 41 | 15.67 ± 0.58 | 0.38 |
| | 3:1 | *S. aureus* | 45 | 14.67 ± 2.31 | 0.33 |
| | 3:1 | *E. coli* | 43 | 12.67 ± 1.15 | 0.29 |
| 1:10 Dilution | 3:1 | *K. pneumoniae* | 40 | 0 | 0 |
| | 3:1 | *S. aureus* | 45 | 13.67 ± 2.08 | 0.30 |
| | 3:1 | *E. coli* | 43 | 0 | 0 |
| 1:100 Dilution | 3:1 | *K. pneumoniae* | 39 | 0 | 0 |
| | 3:1 | *S. aureus* | 39 | 12.33 ± 0.58 | 0.32 |
| | 3:1 | *E. coli* | 42 | 12.33 ± 0.58 | 0.29 |
| KA 2:2 | 2:2 | *K. pneumoniae* | 37 | 14.67 ± 1.53 | 0.40 |
| | 2:2 | *S. aureus* | 42 | 15 ± 0 | 0.36 |
| | 2:2 | *E. coli* | 39 | 13.33 | 0.34 |
| 1:10 Dilution | 2:2 | *K. pneumoniae* | 38 | 0 | 0 |
| | 2:2 | *S. aureus* | 39 | 0 | 0 |
| | 2:2 | *E. coli* | 39 | 0 | 0 |
| 1:100 Dilution | 2:2 | *K. pneumoniae* | 38 | 11.67 ± 1.15 | 0.31 |
| | 2:2 | *S. aureus* | 39 | 0 | 0 |
| | 2:2 | *E. coli* | 41 | 0 | 0 |
| KA 1:3 | 1:3 | *K. pneumoniae* | 40 | 0 | 0 |
| | 1:3 | *S. aureus* | 41 | 12.33 ± 1.15 | 0.30 |
| | 1:3 | *E. coli* | 40 | 13 ± 1 | 0.33 |
| 1:10 Dilution | 1:3 | *K. pneumoniae* | 39 | 0 | 0 |
| | 1:3 | *S. aureus* | 40 | 0 | 0 |
| | 1:3 | *E. coli* | 38 | 0 | 0 |
| 1:100 Dilution | 1:3 | *K. pneumoniae* | 38 | 0 | 0 |
| | 1:3 | *S. aureus* | 39 | 0 | 0 |
| | 1:3 | *E. coli* | 39 | 0 | 0 |

KA 3:1, KA 2:2 and KA 1:3 are the respective ratios of mixed oils of *Khaya senegalensis* (K) and *Azadirachta indica* (A).
Standard used is Levofloxacin 200µg

## Conclusion

It can be concluded that the extracts when combined give a higher activity than the crude extracts alone. Activity index which is a proportionality expression of the activity of the extracts alone is lower than that shown by the mixture of extracts. It can thus be said to be moderately synergistic against tested sorganism.

**References**


1. Abalaka ME, Adeyemo SO, Daniyan SY. Evaluation of The Antimicrobial Potentials of Leaf Extracts of Khaya senegalensis Journal of Pharmaceutical Research and Opinion. 2011; 1(2):48-51. http://www.innovativejournal.in/index.php/jpro
2. Ashafa AOT, Orekoya LO, Yakubu MT. Toxicity profile of ethanolic extract of *Azadirachta indica* stem bark in male Wistar rats. Asian Pacific Journal of Tropical Biomedicine. 2012; 2(10):811-817. doi:10.1016/S2221-1691(12)60234-2.
3. Egharevba HO, Odigwe Anselem C Abdullahi, Makailu Sabo, Simon O Koma, Joseph O Ibumeh. Phytochemical Analysis and Broad Spectrum Antimicrobial Activity of *Cassia occidentalis* L (whole plant) New York Science Journal. 2010; 3(10).
4. Audu JD, Peter PO Olorunfemi, N Njoku. Antimicrobial and pharmaceutical properties of Khaya senegalensis seed oil Journal of pharmacyand Bioresources. 2006; 3(1):19-24.
5. Asif M. Antimicrobial Potential of A. indica Against Pathogenic Bacteria and Fungi. Journal of Pharmacognosy and Phytochemistry. 2012; l(4):78-83.
6. Kuta FA, Tsado DJ, Garba SA, Saidu AN Antibacterial activity of the leaf and stem bark crude extracts of Khaya senegalensis. Medicinal and Aromatic Plant Research Journal. 2015; 3(1):9-15.
7. Suresh K, Kumar R, Khan A. Medicinal Plant Resources: Manifestation and Prospects of Life sustaining Healthcare System continental Journal of Biological Science. 2011; 4(1):19-29.
8. Zaruwa MZ, Manosroi J, Manosroi A, Gidado RSM. Some essential oils from plants and their medicinal uses and aroma therapy in the Northern part of Nigeria. In. Conference Journal of the eighth National Seminar on Pharmaceutical Biotechnology, Chiang Mai. Thailand. 2008, 357-363.