Asian J. Med. Biol. Res. 2021, 7 (3), 249-259; doi: 10.3329/ajmbr.v7i3.56135

Asian Journal of Medical and Biological Research
ISSN 2411-4472 (Print) 2412-5571 (Online)
www.ebupress.com/journal/ajmbr

Article

Evaluation of an antimalarial herbal mixture and each extract for DNA and chromosomal mutations in Swiss albino mice and *Allium cepa* cells

Akinboro Akeem[1]*, Rufai Muhamed Akinlabi[2], Ogunbameru Aanuoluwapo Dorcas[1], Oladosu Bolaji Esther[1] and Taiwo Modinat Opeyemi[1]

[1]Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria
[2]Zoology Department, Osun State University, Osogbo, Osun State, Nigeria

*Corresponding author: Akinboro Akeem, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. Phone: +2348134727845; E-mail: aakinboro@lautech.edu.ng

Received: 13 August 2021/Accepted: 19 September 2021/ Published: 30 September 2021

Abstract: Toxicological evaluation of herbal medicines is necessary because of possible adverse effects that may be associated with their consumption. This study screened antimalarial herbal recipe (containing leaves of *Azadirachta indica* and stem-bark of *Alstonia boonei*) and its individual plant's extract for DNA and chromosomes mutation potentials following the DNA fragmentation and *Allium cepa* assays. Superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) activity of the recipe and each extract was determined. The kinds of phytochemicals present in them were determined using the FTIR technique. Water extracts of *A. indica*, and *A.boonei* at all the tested doses caused significantly lower DNA fragmentations than those of the controls. However, at 25.0% and 50.0% recipe, there was no significant difference in the percentage fragmented DNA compared to the positive control (0.05% sodium azide). Cell division was significantly inhibited by the extracts and recipe, chromosomal aberrations were not dose dependently induced and were significantly lowered than that caused by sodium azide (positive control). The individual extracts and their recipe significantly inhibited Root growth. However, 12.5% recipe promoted root growth that was not significantly different from that of distilled water (negative control). SOD and CAT activities of each of the extracts and their recipe were dose dependent and significantly higher than those of the controls. Water extract of *A. indica* significantly suppressed generation of malondialdehyde compared to water extract of *A. boonei* and recipe as well as the control. The individual extracts and their recipe contained phenolic phytochemicals. The obtained results show that extract of *A. indica*, *A. boonei* and their recipe have good antioxidant properties with strong mitodepressive and root growth inhibitory effects except at 12.5% recipe. However, *A. indica* extract seems to have least cyto-muta-genotoxic effects than water extract of *A. boonei* and the recipe in mice and *A. cepa* cells.

Keywords: DNA fragmentation; *Azadirachta indica; Alstonia boonei;* chromosomes aberrations

1. Introduction

The use of plants with medicinal properties either singly (monoherb) or in combination with other plants materials (polyherbs) or non-plant materials to prevent and treat diseases is a long time practice of about 60, 000 years ago. Today, natural products and plants having therapeutic values are increasingly been sought for throughout the world and have become important sources of orthodox medicines because they are rich in bioactive phytochemicals (Haidan *et al*., 2016; Olorunnisola *et al*., 2021; Garg*et al*., 2021). Over 50% of orthodox medicines is derived from plants. In the tropical and sub-tropical parts of the world, malaria is widely spread and it affects about 1 million people worldwide yearly, out of which 700,000 are children (Omoya and

Oyebola, 2019). About 30 – 50% and 25% infant morbidity and mortality, respectively are caused by malaria in Nigeria. The resistance to synthetic antimalarial orthodox drugs necessitates the increased use of herbal medicines, coupled with readily availability of medicinal plants most often with no or very low cost. *Azadirachta indica* (Juss) and *Alstonia boonei* (De Wild) belonging to the families Meliaceae and Apocynaceae, respectively, are the foremost medicinal plants employed for treatment of malaria and some other diseases in Nigeria. Their ethnopharmacological importance is perhaps based on their richness in phytochemicals with antimalarial properties. The leaf extract of *A. indica* contains phytochemicals such as flavonoids, glycosides, limonoids, coumarins and sterols which have schizontocidal and gametocidal effects (Afolabi *et al.*, 2021). The stem-bark of *A. boonei* is known to contain phytochemicals that have therapeutic properties such as antimalarial, analgesic, antidiabetic, antimicrobial, antirheumatic and anti-inflammatory (Adotey *et al*., 2012). Herbal medicines preparations can be made from either one part of these plants or parts of the two plants can be combined to form a recipe. Traditionally, herbal medicines are usually prepared by combining more than one plant part to make recipe which is believed to have diverse therapeutic effects because such contains numerous therapeutic phytochemicals. The use of herbal medicines to prevent and treat diseases should be done with some levels of toxicological consciousness due to the fact that some plants extracts are associated with toxicity. Plant extracts and herbal products have been reported to have mutagenic, genotoxic, carcinogenic teratogenic effects (Akinboro and Bakare, 2007; Akinboro *et al.*, 2011a, 2011b; Sponchiado *et al.*, 2016; Akinboro *et al.*,2017; Babamale *et al.*, 2017; Akinboro *et al.*, 2020b). Herbal medicines prepared with more than one plant should always be subjected to genetic toxicological assays to establish that both the individual plant extract and the recipe itself (mixture of extracts) are safe for consumption.

This study therefore aimed at investigating possible effects of individual leaves extract of *Azadirachta indica* and stem- bark extract of *Alstonia boonei* and their recipe (mixture of the two extracts) as a commonly used antimalarial herbal medicine on cell division, cellular DNA integrity and chromosome structure in plant and animal test organisms.

2. Materials and Methods

2.1. Plant collection and identification

Fresh leaves of *Azadirachta indica* (A. juss) and stem - barks of *Alstonia boonei* (Di wild) were respectively collected from the botanical garden, and around the New Biology Laboratory, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State, Nigeria. They were duly identified and assigned voucher number LHO 582 and LHO 583 to *Azadirachta indica* and *Alstonia boonei*, respectively by a taxonomist, Professor A.T.J. Ogunkunle at the herbarium unit of Department of Pure and Applied Biology, LAUTECH, Ogbomoso, Nigeria.

2.2. Preparation of plant extracts

The collected plant parts (leaves of *A. indica* and stem – bark of *A. boonei*) were washed with clean water, thereafter air dried, before they were individually ground using 'Wing electric blender'(Malaysia). One hundred grams of the powder of each plant part was weighed on a Mettler weighing balance, while the recipe contained 50 g each of *A. indica* and *A. boonei*. The powder of each plant and that of the recipe in separate glass jars was added with five hundred milliliters of distilled water and then placed inside a water bath set at 64°C for 2 hours 15 minutes. The single extract and recipe were allowed to cool down before sieving each through a Whatman filter paper (No 1) and stored in a refrigerator at 4ºC for further use (Akinboro *et al.*, 2017; Akinboro and Jimoh, 2021a).

2.3. Oral feeding of animals

Fifty-five female albino mice were acclimatized for 1 week at the animal house of Department of Pure and Applied Biology, LAUTECH. They were fed with commercially formulated feed and clean water *ad libitum* throughout the period of the experiment. The animals were weighed and grouped into 5 mice per treatment and control groups. Every three groups were administered (0.1 ml per 10 g b/w) with each of the water extract of *A. indica*, *A. boonei* and the antimalarial recipe at 25.0%, 50.0% and 100.0% doses once per day for 48 hours (Akinboro and Soremekun, 2020a). Distilled water and sodium azide (0.05%) served as the negative and positive controls, respectively.

2.4. DNA fragmentation assay

This assay was carried out spectrophotometrically as previously described by Wu *et al*. (2005). One gram of liver tissue from a mouse treated with each of the extract and recipe was treated in 10 ml of Tris-

Ethylenediaminetetra acetic buffer. The absorbance of the reaction mixture was read at 620 nm. The percent fragmented DNA was calculated according to the formula below:

$$\text{Fragmented DNA (\%)} = \frac{\text{Absorbance of supernatant}}{\text{Absorbance of pellet+Absorbance of supernatant}} \times 100$$

2.5. *In vivo* antioxidant tests

2.5.1. Lipid peroxidation (malondialdehyde generation)

The amount of thiobarbituric acid (TBA) reactive substances (TBARS) produced during lipid peroxidation was determined as previously described by Ohkawa *et al.* (1979). Briefly, 0.4ml of the homogenized liver tissue was mixed with 1.6ml of 0.1 MTris-KCl buffer prepared at pH 7.4. To this, 0.5 ml of 30% Trichloroacetic acid (TCA) was added followed by 0.5ml of 0.75% TBA. The mixture was then placed in a water bath at 80^0C for 45 minutes and then cooled on ice before spinning at 3000 x g for 15 minutes. The absorbance of the reaction mixture was read in a spectrophotometer at 532 nm against distilled water which served as a reference blank. The amount of malondialdehyde molecules generated in the treated animals was determined with the molar extinction coefficient of 1.56 x $10^5 M^{-1}cm^{-1}$, using the formula below:

$$\text{MDA (}\mu\text{M/mg protein)} = \frac{\text{Absorbance} \times \text{Volume of mixture}}{E_{532nm} \times \text{Volume of Sample} \times \text{mg Protein}} \times 100$$

2.5.2. Superoxide dismutase test

The amount of superoxide dismutase present in the sample of liver cells of the animals administered with individual extract of *A. indica*, *A. boonei* and recipe was determined according to the previously described method (Mistra and Fridovich, 1972; Adekunle, 2012). Briefly, the homogenized sample of liver tissue was diluted in 10 fold dilutions, after which 0.2ml of the diluted sample was added to 2.5ml of 0.05M carbonate buffer (pH 10.2). Freshly prepared adrenaline (0.3ml) at 0.3mM was added to the mixture and was thoroughly mixed before equilibrated in the spectrophotometer. The reaction of the mixture was monitored for 150 seconds at 30 seconds intervals setting the absorbance at 480nm. The SOD activity was calculated as stated below:

SOD Activity = (Absorbance x Volume of mixture) / (ε_{480nm} x Sample Vol x mg protein)
= Unit/mg protein

Molar extinction of SOD at 480 nm (ε_{480nm}) = 525 $M^{-1}cm^{-1}$

2.5.3. Catalase test

Catalase activity of *A. indica*, *A. boonei* extracts and the recipe was determined as previously described by Tadayuki *et al*. (2013). Four milliliters of 0.2 M H_2O_2 solution (800 µmoles) was mixed with 5ml of 0.01 M Phosphate buffer (pH 7.0) in a flat bottom flask of 10 ml capacity. The reaction mixture was added with 1 ml of enzyme preparation, and subsequently with 2 ml of dichromate acetic acid at room temperature after every 1 minute. The decomposition of H_2O_2 by catalase was determined using the equation below:

$$H_2O_2 \text{ remained} = \frac{\text{Change in absorbance/minute}}{0.171}$$

H_2O_2 consumed = 800 - H_2O_2 remained
$K_0 = H_2O_2$ Consumed
Kat f = K_0/mg protein (ml)

2.5.4. Determination of total protein

Each of the test samples and calibrator (25 µl) was treated with 1 ml of Biuret solution, while the blank sample was 1 ml Biuret solution. After incubation of the mixtures at 37^oCfor 5 minutes, the absorbance of the sample and calibrator were read against the blank using a spectrophotometer set at 540 nm (Caguioa *et al.*, 2019). The total protein concentration was determined using the formula:

$$\text{Total protein}\left(\frac{\text{g}}{\text{dl}}.\right) = \frac{\text{Abs. of Sample}}{\text{Abs. of Standard}} \text{X 7 (Calibrator Conc.)}$$

Where Abs = Absorbance
Conversion factor (g/dL) X 144.9

2.6. *Allium cepa* assay

Onions were purchased from wazo market, Ogbomoso. They were sun dried for two weeks to reduce moisture content so as to facilitate root growth. The onions were descaled carefully using a razor blade, leaving intact the primordial root ring (Akinboro *et al.*, 2020b; Akinboro *et al.*, 2021a, 2021b). The onions were rinsed with distilled water and wiped with tissue paper.

Sixty onion bulbs were grown in distilled water inside 100 ml beakers placed inside a cupboard for 24 hours in order to initiate root growth (Akinboro *et al*., 2017). Thereafter, the onions were transferred into different doses (100%, 50%, 25% and 12.5%) of each of the plants extracts and the controls for another 24 hours root growth in a cupboard. The negative and positive controls were distilled water and sodium azide (0.05%), respectively.

After 48 hours of root growth, roots from 4 onions were harvested and preserved in ethanol: glacial acetic acid (3:1) and stored at 4°C in a refrigerator for microscopic evaluation. The remaining six onions per dose were placed on fresh extracts, recipe and controls to continue their root growth for another 24 hours.

After 72 hours of root growth, root lengths from the onions were cut from the base and measured with a meter rule. The percentage root growth relative to that of the negative control was calculated according to the formula below:

$$\% \text{ root growth } = \frac{\text{Average root length at a dose of an extract}}{\text{Average root length of negative control}} \times 100$$

2.6.1. Slide preparation

The fixed roots tips in ethanol: acetic acid (3:1) were rinsed in distilled water inside a petri dish to cleanse them of the fixative. They were then hydrolyzed inside 1N hydrochloric acid in a petri dish, then placed in an oven set at 60˚C for 3minutes.The hydrolyzed roots were treated for slides preparation as previously described (Akinboro *et al.*, 2020b, Akinboro and Jimoh, 2021a; Akinboro *et al.*,2021b).

The prepared slides were observed under a binocular light microscope (PEC, Medical, USA) using immersion oil objective lens. A total number of 5,000 cells were counted and observed for stages of mitosis and chromosomal aberrations in each dose. Photomicrographs of the dividing stages and aberrations were taken with the help of a digital camera fixed on the microscope. The mitotic index of each treatment and control was calculated with the formula below:

$$\text{Mitotic Index } = \frac{\text{Number of dividing cells}}{\text{Total number of cells counted}} \times 100$$

2.7. FTIR analysis of antimalarial extracts and recipe

Each of the samples of *A. indica*, *A. boonei*, and recipe was transformed to Potassium bromide powdered tablets and then placed on SmartTR™ Attenuated Total Reflectance (ATR) accessory at room temperature (25°C). The prepared tablets were scanned using the Nicolet iS10 FTIR spectrophotometer (Thermo Fisher Scientific Inc, Madison, USA) coupled with deuterated triglycine sulfate (DTGS) detector and potassium bromide (KBr)/Germanium as abeam splitter. The spectra were scanned at wave-numbers of 4000-650cm-1 while connected to the software OMNICver.9.7. Data were recorded in three replicates using absorbance mode to facilitate quantitative analysis (Siregar *et al.*, 2018).

2.8. Statistical analysis

The obtained data in terms of DNA fragmentation, mitotic index, chromosomal aberrations and root growth were summarized to mean, standard error and percentage, and then analyzed using ANOVA in the SPSS programme (version 17.0). Duncans multiple range comparison test was performed to determine the significant differences between the means of treatments and controls ($p < 0.05$).

3. Results

DNA fragmentation activity of the water extract of *A. indica* and the antimalarial recipe was inversely proportional to the doses, unlike the water extract of *A. boonei* which induced DNA fragmentations in ascending order of the doses (Figure 1). The percentage of fragmented DNA recorded at the selected doses except at 25.0% and 50.0% of the recipe was significantly different ($p < 0.05$) from that induced by the positive control.

The water extract of *A. indica*, *A. boonei* and recipe significantly inhibited cell division in the roots of *A. cepa* as compared to the negative control (Figure 2). However, this effect was not dose- dependent. Individual water

extract of *A. indica* and *A. boonei* caused highest percentage mitotic index (MI) at 100.0% and 50.0%, respectively. The least percentage of MI induced by the recipe was obtained at 25.0%.

Chromosomal aberrations (CA) such as anaphase bridge, sticky chromosomes and micronucleus were induced by the individual water extracts and their recipe. However, they were not dose – dependent. The highest CA was induced at 12.5% and 50.0% doses of water extract of *A.boonei* and *A. indica* (Figure 3).

Root growth of *A. cepa* was significantly inhibited by the water extract of *A. indica*, *A. boonei* and recipe at the selected doses except 12.5% recipe which induced a slightly higher but not significantly different ($p > 0.05$) root length from that recorded for the negative control. Water extract of *A. boonei* inhibited the root growth in this study better than extract of *A. indica* and the recipe (Figure 4).

Super oxide dismutase activity of the water extract of *A. indica*, *A. boonei* and recipe was dose dependent and it was significantly different ($p < 0.05$) only at 100.0% dose of the individual extracts and recipe (Figure 5). The effect of the individual extract of *A. indica*, *A. boonei* and recipe on induction of catalase was significantly higher ($p < 0.05$) at all the selected doses in this study than the controls (Figure 6). The best catalase activity was recorded at100.0% recipe, however, this was not significantly different ($p > 0.05$) from the activity of water extract *A. boonei* at 50.0% and 100.0%. The ability of the extracts and recipe to generate malondialdehydes as products of lipid peroxidation of cell membrane showed that water extract of *A. indica* possessed the best inhibitory activity which was significantly different ($p < 0.05$) from that of *A. boonei* extract, recipe at the selected doses, and controls (Figure 7).

The bonds in the phytochemicals of *A. indica* were detected at 3289.4 cm^{-1}, and 1638.2 cm^{-1} corresponding to hydroxyl and carbonyl functional groups. The extract of *A. boonei* revealed bonding at 3283.1 cm^{-1}, 1638.2 cm^{-1} representing hydroxyl and carbonyl functional groups. The recipe showed bonds at 3270.7cm^{-1}, 1538.2 cm^{-1} and 1015.7 cm^{-1} corresponding to hydroxyl functional group, carbonyl functional group and carbon – carbon single bond (Figures 8, 9 and 10).

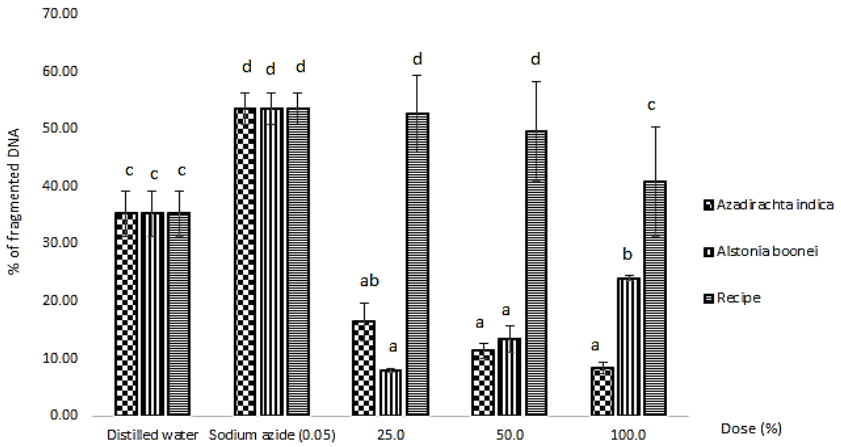

Figure 1. DNA fragmentation activity of water extract of *A. indica*, *A. boonei* and Recipe in mice liver cells.

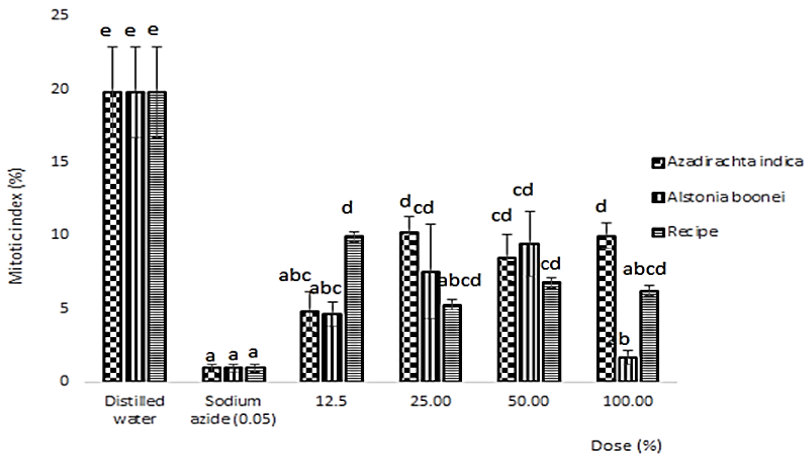

Figure 2. Effect of water extract of *A. indica*, *A. boonei* and Recipe on *Allium cepa* mitosis.

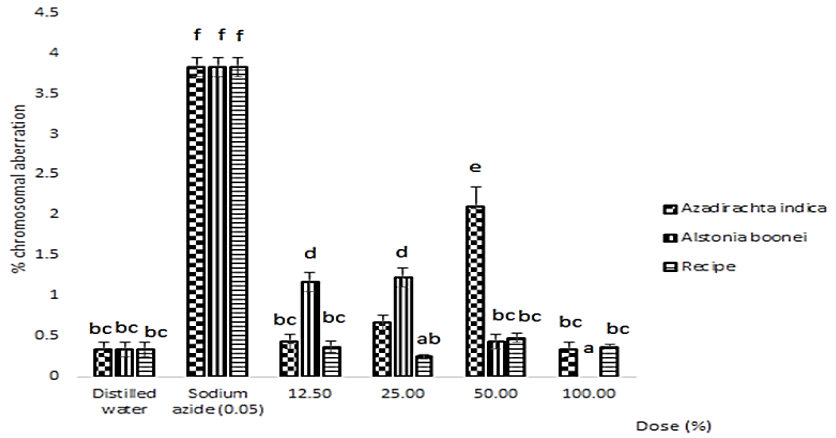

Figure 3. Effect of water extract of *A. indica*, *A. boonei* and Recipe on chromosomes of *Allium cepa* cells.

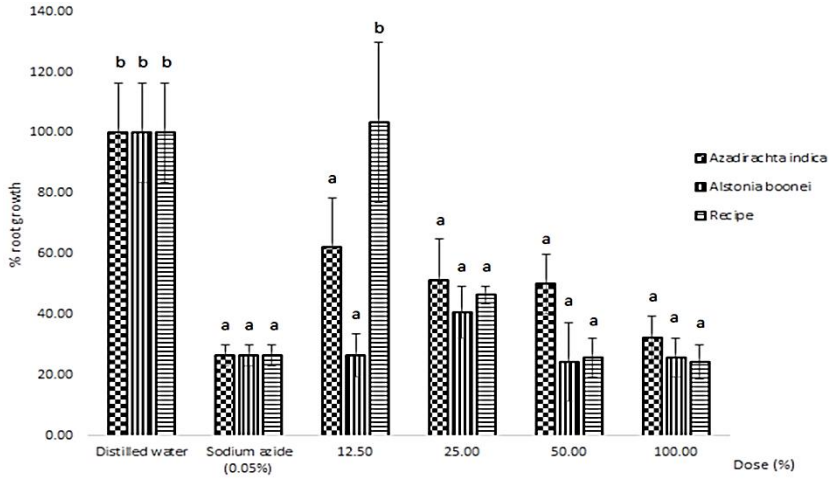

Figure 4. Effect of water extract of *A. indica*, *A. boonei* and recipe on root growth of *A. cepa.*

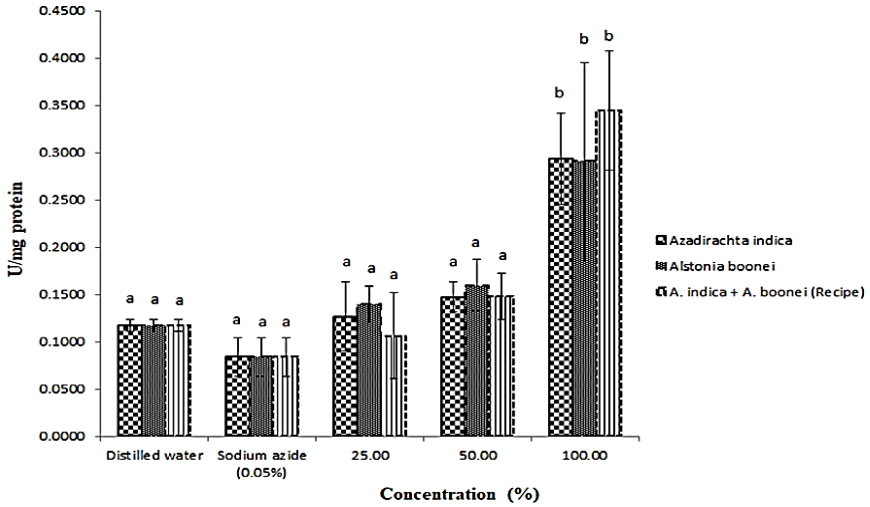

Figure 5. Superoxide dismutase activity of water extract of *A. indica*, *A. boonei* and recipe.

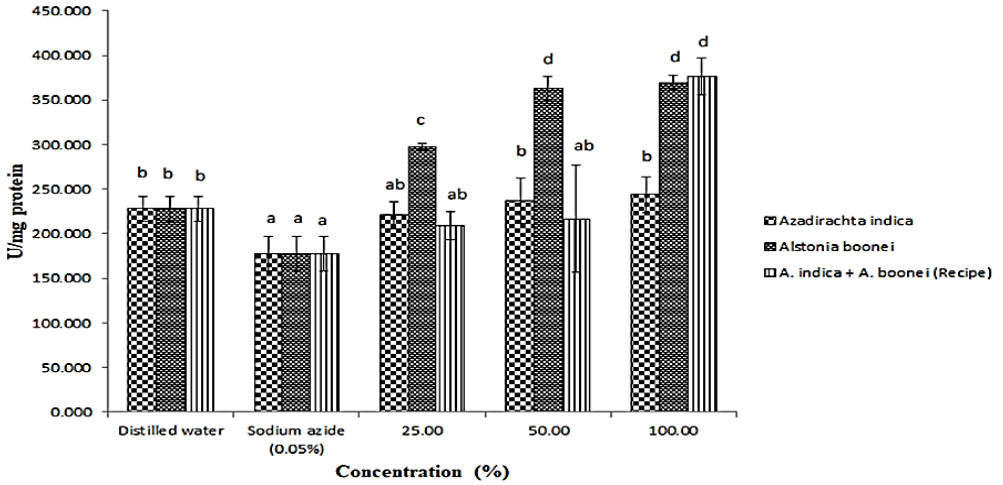

Figure 6. Catalase activity of water extract of *A. indica*, *A. boonei* and recipe.

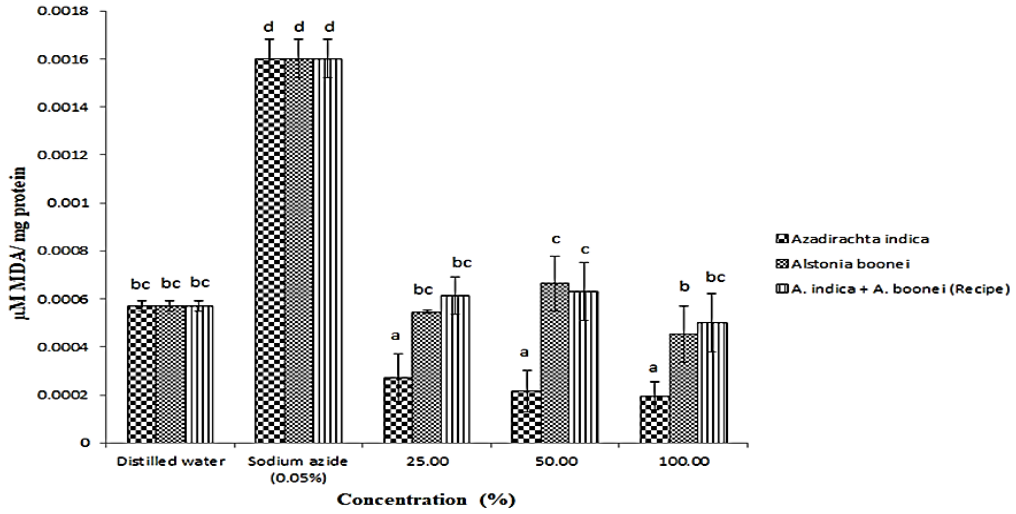

Figure 7. Malondialdeyde suppression activity of water extract of *A. indica*, *A. boonei* and recipe.

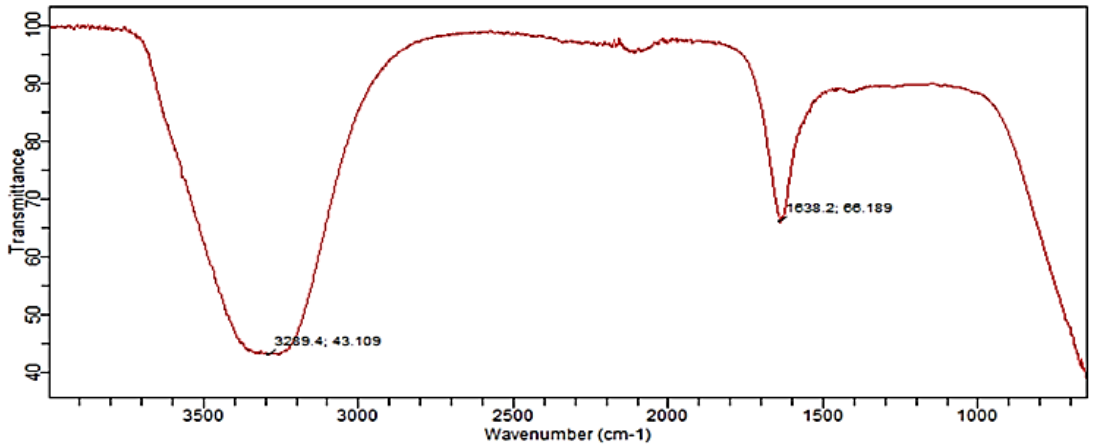

Figure 8. FTIR spectra of functional groups detected in the water extract of *A. indica.*

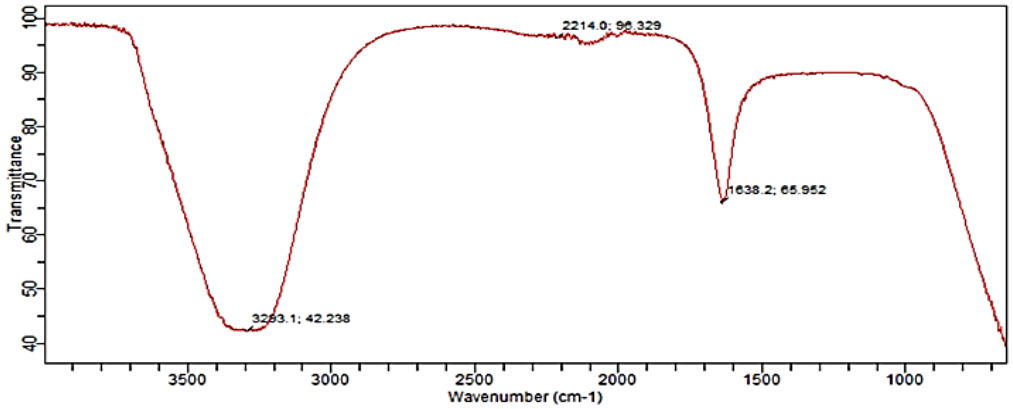

Figure 9. FTIR spectra of functional groups detected in the water extract of *A. boonei.*

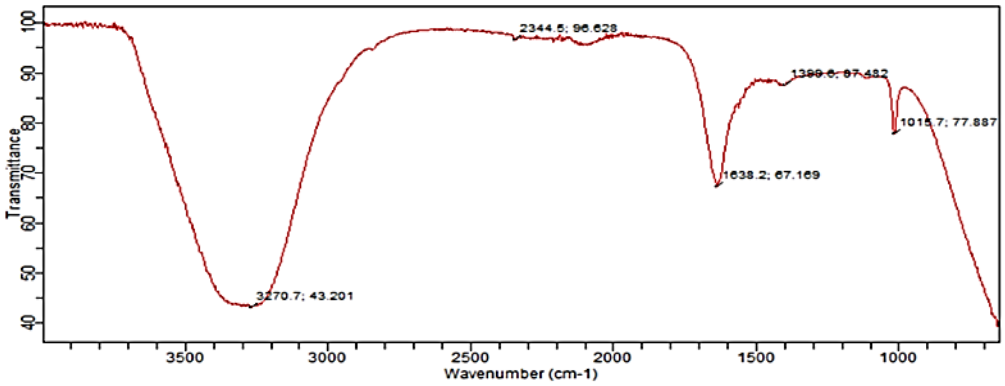

Figure 10. FTIR spectra of functional groups detected in the antimalarial herbal recipe.

4. Discussion

Safe consumption of herbal medicines by man is highly necessary, and it has to be established for their continuous ethnopharmacological uses. Plant extracts in herbal medicines are necessary to be screened for toxicity on cellular organelles and cell division individually and in combination so as to remove any of the recipe's constituents that has significant toxic effects rather than expected to be therapeutically effective.

The individual water extract of *A. indica*, and *A. boonei* could be said to be non-mutagenic since the percentage DNA fragmentation induced by each of them was significantly lowered than that caused by distilled water (negative control), or had weak mutagenic activity having recorded significantly lowered percentage DNA fragmentation when compared to that induced by sodium azide (positive control). However, the recipe was more mutagenic than its two individual extracts as it induced DNA fragmentations that were singnificantly similar to that induced by sodium zide (positive control). Sodium azide is a mutagen capable of damaging DNA molecule when metabolized to an organic metabolite of azide called azidoalanine which may cause point mutation during DNA replication (Salim *et al.,* 2009; Srivastava *et al.,* 2011). The hydroxyl functional group detected in the extracts and the recipe suggests their richness in polyphenolic or phenolic phytochemicals which are known to possess good antioxidants properties. Extracts from leaves of *A. indica* and stem-bark of *A. boonei* were reported to contain phenolic and other kinds of phytochemicals (Akinmoladun *et al.,* 2007; Awodele *et al.,* 2010). The non mutagenic effect of the individual extracts of *A. indica* and *A. boonei* could be connected to the presence of antioxidants which are known to have good protective effect on DNA molecule thereby preventing the nucleic acid from undergoing mutation caused either through an inducing agent or spontaneously.In contrast,antioxidants may become pro-oxidants which readily cause DNA damage through free radicals generation in form of reactive oxygen species (Hussin *et al.,* 2021). Free radicals are most often implicated in molecular damages inflicted on lipids, proteins and nucleic acids (Lin *et al.,* 2013).The higher level of antioxidant activity of the recipe especially at 100% dose could be responsible for its induction of more DNA fragmentations than the individual extracts. Polyphenols and flavonoids act as antioxidants capable of scavenging free radicals andredox active metals ions chelation as well as indution of cellular antioxidant

protection and repair. In terms of chromosomes aberrations indcued, the extracts individually, and the recipe had weak genotoxic effects on *A. cepa* cells based on the induction of chromosomal aberrations in a non-dose dependent manner, and which are significantly lower compared to that of the postive control (0.05% sodium azide). Similar less genotoxic effect of stem-bark and leaves extracts of *A. boonei* was earlier reported in Swiss albino mice following the bone marrow micronucleus assay (Babamale *et al.*, 2017). Likewise, leaf extract of *A. indica* had earlier been reported to cause various chromosomal aberrations in *A. cepa* cells and point mutations in the cells of *Salmonella typhimurium* TA 98 and TA 100 strains (Akaneme and Amaefule, 2012; Katabale *et al.*, 2017).

The water extract of *A. indica*, *A. boonei* and their recipe was cytotoxic. This was evident in the significant reduction of percentage of dividing cells (mitotic index) obtained at tested doses compared to the negative control. However, the toxic effect on mitosis of *A. cepa* was weak in that this was not doses-dependent. This kind of mitotic inhibition caused by the extracts and their recipe is mitostatic which can be reversed when the affected cells are no more in contact with the extracts. Mitodepressive effect of aqueous extract of *A. indica* and nine other plants extracts had been reported (Priyanka *et al.*, 2019). Mitotic inhibition during cell division may occur due to supression of DNA synthesis, or distruption of events taking place at G2 thereby preventing entry into the M-pahse of the cell cycle. Previous study has attributed cytotoxicity of plants extracts and a polyherbal formulation (HF8 extract) to bioactive compounds such as flavonoids, phenols, terpenoids and so on (Akinboro *et al.*, 2020b; Abutaha *et al.*, 2021). The significant reduction of percentage root growth of *A. cepa* exposed to the indvidual extracts of *A. indica*, *A. boonei* and their recipe at the selected doses except 12.5% of the recipe as compared to the obtained root growth with distilled (negative control) implies toxicity. This corroborates the effect of these plants extracts on cell division. Root elongation is a function of cell division. It is expected that when there is an inhibtion of cell division, there is going to be reduciton of root growth leading to short or reduced root lengths. The recipe was found to promote root growth at 12.5% and this indicated that it was not toxic at this dose like the negative control. Similar root growth inhibition and promotion in *A. cepa* was recorded for the evaluation of water extract of *Lawsonia inermis* (Akinboro *et al.*, 2020b). Toxicity of phytochemicals to plants roots may cause short radicle and coleoptile enlongation, necrosis of root tips, lack of root hairs as well as root hairs discolouration. Water extract of *A. indica* had the least toxicity to the root cells probably because of its best anti-lipid peroxidation activity in this study causing the least amount of malondialdehyde molecules at 100.0%. The more there is lipid peroxidation of the cell membrane the more is permeability of such cell to exogenogenous materials thereby loosing its cellular contents and finally lead to adverse effect on the physiology of such cells.

5. Conclusions

This study has shown that the antimalarial herbal recipe had higher mutagenic effect than the individual leaves extract of *A. indica* and stem-bark of *A. boonei*. However, both the extracts and recipe were not significantly genotoxic. The antimalarial recipe and each of its constituent extracts were mitodepressive. The recipe at 12.5% promoted root growth while at other doses of the extracts and recipe there was a significant root growth inhibition. The extracts and recipe possess good superoxide dismutase and catalase activities, however, leaves extract of *A. indica* showed the best activity against the generation of malondialdehyde molecules through cell membrane lipid peroxidation. Phenolic phytochemicals, which have hydroxyl functional group, were detected in the extract of *A. indica*, *A. boonei* and recipe. Water extract of *A. indica* seems to be safer than stem – bark extract of *A. boonei* and the antimalarial recipe in this study.

Conflict of interest

None to declare.

Authors' contributions

Dr. Akeem Akinboro planned and supervised the research work that yielded the results presented for publication in this journal. He also wrote the manuscript.
Dr. Muhamed Rufai Akinlabi encouraged and facilitated the publication of this manuscript.
Miss. Ogunbameru Aanuoluwapo Dorcas was involved in carrying experiments in the laboratory
Miss. Oladosu Bolaji Esther was involved in carrying experiments in the laboratory
Miss. Taiwo Modinat Opeyemi was involved in carrying experiments in the laboratory.

References

Abutaha NM, MF Farooq, M Al-Zharani, A Alotaibi, MAW Cordero, A Bepari and S Alarifi, 2021. Cytotoxic activity and toxicity study of HF8, a poly-herbal formulation. J. King Saud-Uni. Sci., 33: 101377.

Adekunle AS, 2012. Oxidative Stress in Biochemical, Seminological and Histological Alterations Due to Acute Administration of Intramuscular Artemether in Mice. Researcher, 4: 9 – 17.

Adotey JPK, EA Genevieve, OB Yaw and AA Frederick, 2012. A Review of the Ethnobotany and Pharmacological Importance of *Alstoniaboonei* De Wild (Apocynaceae). ISRN Pharmacol., 2012: 587160.

Afolabi OJ, IA Simon-Oke and OI Oladokun, 202. Antiplasmodial Activity of Ethanolic Extract of Neem Leaf (*Azadirachta indica*) in Albino Mice Infected with *Plasmodium berghei*. Int. Arch. Clin. Pharmacol, 7: 1-7.

Akaneme FI and CC Amaefule, 2012. Evaluation of the cytotoxicity and genotoxicity of aqueous leaf extracts of *Azadirachta indica* A. Jussusing the Allium test. J. Med. Plant. Res., 6: 3898-3907.

Akinboro A and AA Bakare, 2007. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. J. Ethnopharmacol., 112: 470475.

Akinboro A, BM Kamaruzaman, A Mohd-Zaini and AS Othman, 2011a. Mutagenic and antimutagenic potentials of fruit juices of five medicinal plants in *Allium cepa* L: Possible influence of DPPH free radicals scavengers. Afr. J. Biotechnol., 10: 10520-10529.

Akinboro A, BM Kamaruzaman, A Mohd- Zaini, FS Shaida and AS Othman, 2011b. Antioxidants in aqueous extract of *Myristica fragrans* (Houtt.) suppressed mitosis and cyclophosphamide – induced chromosomal aberrations in the *Allium cepa* L. cell. J. *Zhejiang Univ.* Sci. *B*, 12: 915-922.

Akinboro A, OT Adedosu, FO Famurewa, T Olowe and OO Oyewole, 2017. Antioxidant, Proximate and Cyto-genotoxic Evaluation of Anti-typhoid fever herbal recipe in *Allium cepa* cells. GJBB, 6: 24-29.

Akinboro A, M Kamaruzaman and A Zaini, 2020a. Effects of rutin in *Myristicafragrans*Houtts.against cyclophosphamide-induced genotoxicity in sperm cells, on liver and kidney tissue, in Swiss Albino mice. American J. Physiol. Biochem. Pharmacol., 10: 7-17.

Akinboro A and SI Soremekun, 2020b. Cytogenotoxicity evaluation of water extract of *Lawsoniainermis* (Henna) following the *Allium cepa* root mitosis assay. *Science focus*: Int. J. Biol. Phys. Sci., 25: 61 – 70.

Akinboro A and A Jimoh, 2021a. Antigenotoxic potential of gel extract of *Aloe vera* against Sodium azidegenotoxicity in *Allium cepa* cells. J. Med. H*erbs* Ethnomed., 7: 1-5.

Akinboro A, NA Peter, MA Rufai and AO Ibrahim, 2021b. Evaluation of Asa River water in Ilorin, Kwara State, Nigeria for available Pollutants and their effects on mitosis and chromosomes morphology in *Allium cepa* cells. J. Appl. Sci. Environ. Manag., 25: 119-125.

Akinmoladun AC, EO Ibukun, E Afor, BL Akinrinlola, TR Onibon, AO Akinboboye, EM Obuotor and EO Farombi, 2007. Chemical constituents and antioxidant activity of *Alstonia boonei*. Afr. J. Biotechnol., 6: 1197-1201.

Awodele O, VO Osunkalu, O R Akinde, JA Teixeira da Silva, WO Okunowo, EC Odogwu and A Akintonwa, 2010. Modulatory Roles of Antioxidants against the Aqueous Stem Bark Extract of *Alstonia boonei* (Apocynaceae)-induced Nephrotoxicity and Testicular Damage. Int. J. Pharm. Biomed. Res., 2: 76-80.

Babamale OA, O AIyiola, SB Adeyemi, AF Sulaiman, AO Abdulkareem, AT Anifowoshe, OD Awe, D Ajani and US Ugbomoiko, 2017. Comparative studies of genotoxicity and anti-plasmodial activities of stem and leaf extracts of *Alstonia boonei* (De Wild) inmalaria-infected mice. Niger. J. Parasitol., 32: 192-197.

Caguioa JJO, JM Manundan and JD Rapanot, 2019. Measuring protein concentration of bovine albumin serum using spectrophotometric analysis: a magnesium chloride based biuret assay. Phl. Normal Univ. Coll. Grad. Studies Teacher Edu. Res., 1 - 3.

Garg AK, M Faheem and S Singh, 2021. Role of Medicinal Plant in Human Health Disease. *Asian* J. *Plant* Sci. Res., 11: 19-21.

Haidan YM Qianqian, YLi and P Guangchun, 2016. The Traditional Medicine and Modern Medicine from Natural Products. Molecules, 21: 559.

Hussin M, HO Maizatul, IR Elda Nurafnie, NS Shazlan, HM Farah, MS Lau and A Norizah, 2021. Phytochemical and in vitro genotoxicity studies of standardized *Ficus deltoidea* var. *kunstleri* aqueous extract. Plants, 10: 343.

Katabale MK, NM Esther and D Kariuki, 2017. Phytochemical screening, cytotoxic, genotoxic and mutagenic effects of the aqueous extract of *Azadirachta indica* leaves. Int. J. Herb. Med, 5: 39-44.

Lin KH, YY Yang, CM Yang, MY Huang, HF Lo, KC Liu, HS Lin and PY Chao, 2013. Antioxidant activity of herbaceous plant extracts protect against hydrogen peroxide-induced DNA damage in human lymphocytes. BMC Res. Notes, 6: 490.

Mistra HP and I Fridovich, 1972. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem, 247: 3170-3175.

Ohkawa H, N Ohishi and K Yagi, 1979. Assay for lipid peroxides in animal tissues thiobarbuturic acid reaction. Anal. Biochem., 95: 351-358.

Olorunnisola OS, PI Adegbola, BS Ajilore, OA Akintola and OS Fadahunsi, 2021.The role of poly-herbal extract in sodium chloride-induced oxidative stress and hyperlipidemia in male wistar rats. Medicines, 8: 25.

Omoya F and TF Oyebola, 2019.Antiplasmodialactivity of stem bark and leaves of *Alstonia boonei* (De Wild). J. Microbiol. Exp., 7: 241–245.

Priyanka, M Reena, K Pushpa and K Baidyanath, 2019. Cytotoxicity evaluation of aqueous extracts of medicinal plants on *Allium cepa* L. J.Biotechnol. *Biochem.*, 5: 35-49.

Siregar C, SMartono and A Rohman, 2018. Application of Fourier transform infrared (FTIR) spectroscopy coupled with multivariate calibration for quantitative analysis of curcuminoid in tablet dosage form. J. Appl. Pharm. Sci., 8: 151-156.

Sponchiado G, ML Adam, CD Silva, BS Soley, Cde Mello-Sampayo, DA Cabrini, CJ Correr and MF Otuki, 2016. Quantitative genotoxicity assays for analysis of medicinal plants: asystematic review. J. Ethnopharmacol., 178: 289–296.

Srivastava P, S Marker, P Pandey and DK Tiwari, 2011. Mutagenic effects of sodium azide on the growth and yield characteristics in wheat (*Triticum aestivum* L., em., Thell.). Asian J. Plant Sci, 10: 190-201.

Tadayuki I, T Akiko, S Shinya, O Ken-ichi, H Ippei, K Yuko, T Koji and M Yoshimitsu, 2013. A simple assay for measuring catalase activity: a visual approach. Sci Rep, 3: 3081.

Wu B, A Ootani, R Iwakiri, Y Sakata, T Fujise, S Amemori, F Yokoyama, S Tsunada and K Fujimoto, 2005. T cell deficiency leads to liver carcinogenesis in Azoxymethane-treated rats. Exp. Biol. Med., 231: 91-98.