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Full Length Research Paper

ANTIOXIDANT AND ANTIMICROBIAL STUDIES OF MEDICINAL PLANT EXTRACTS OF AFRICAN BIRCH (*Anogeissus leiocarpus*), ZOGALE (*Moringa oleifera*) AND AFRICAN CUSTARD APPLE (*Anona sengalensis)*

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ABSTRACT

Ethanol extracts of three selected Nigerian medicinal plants-*Anogeissus leiocarpus* (African birch), *Moringa oleifera* (Zogale) and *Anona senegalensis* (African custard –apple) were assessed for antioxidant and antimicrobial properties. Phytochemical screening gave positive tests for the following bioactive compounds: tannins, flavonoids, cardiac glycosides, alkaloids, Saponin, anthraquinones and steroids. The antimicrobial sensitivity test of the plant extracts was determined *in vitro* using the agar well diffusion method against clinical isolates of *Bacillus subtilis, Escherichia coli, Candida albicans*, *Trichophyton rubrum* and *Trichophyton mentagrophytes* at concentrations from 400mg/ml to 50mg/ml, Gentamycin and Fluconazole were used as standards. The minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) of the extracts were determined. The antioxidant property was determined using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) antioxidant assay method . Absorbance readings were taken at the wavelengths of 492nm and 620nm. Ascorbic acid was used as positive control and Dimethyl sulfoxide (DMSO) as the negative control. The MIC for *A. senegalensis against* both *B. subtilis* and *E. coli* was 12mg/ml while *A. leocarpus* has an MIC of 50mg/ml for both microorganisms. The MIC for *M. oleifera* was 6.2 mg/ml against *B. subtilis* and 12.5 mg/ml against *E. coli*. The MBC for *A. leoicarpus was* 100mg/ml for both *B. subtilis* and *E. coli. A. senegalensis* exhibited an MBC of 25mg/ml for both *B. subtilis* and *E. coli while Moringa Oleifera* had an MBC 25mg/ml against *E. coli* and *12.5* mg/ml *against B. subtilis* showing a higher activity. At the lowest concentration of 0.625mg/ml, *A. leiocarpus* had absorbance of 0.37 and 0.41 at 492nm and 620nm wavelength respectively. *A. senegalensis* had absorbance of 0.18 and 0.17 while absorbance of 0.12 and 0.11 were recorded for *M. oleifera* at 492nm and 620nm wavelengths respectively. The absorbance of Ascorbic acid at 0.625 mg/ml was 0.5 at 492nm wavelength and 0.36 at 620nm wavelength. The result indicated that the activity of all the extracts were best at 620nm wavelength. The antioxidant activities *M. oleifera*- and *A. senegalensis* are comparable to that of ascorbic acid but ascorbic acid has a better antioxidant activity with an absorbance of 0.36 at the lowest

concentration 0.625mg/ml used. *A. leiocarpus* has a higher antioxidant activity when compared to ascorbic acid with absorbance of 0.37 and 0.41 at a concentration of 0.625mg/ml for 492 and 620nm wavelengths respectively. These results suggest that the ethanol extracts of the studied plants possess significant antimicrobial as well as antioxidant and radical scavenging activities and as, such make potential candidates as natural phytomedicines and chemoprophylactic agents.

Keywords: Medicinal plants, Extracts, Antioxidants, Antimicrobial

INTRODUCTION

Synthetic chemicals are widely used against microorganisms and they develop resistance to many of these antibiotics (Mukherjee *et al*., 2002). These antibiotics sometimes cause allergic reaction and suppress immunity. The use of plant extracts in the right concentration is less damaging to the human health and environment (Isman, 2000). The recent growth in the knowledge of free radicals and reactive oxygen species (ROS) in biology has resulted in a medical revolution that promises a new age of health and disease management (Aruoma, 2003).

Free radicals and antioxidants have become commonly used terms in modern discussions of disease mechanisms (Aruoma, 2003). Antioxidants are the compounds, that can safely interact with free radicals and terminate chain reactions induced by these free radicals before vital molecules or organs are damaged. They are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage. (Young *et al.*, 2000) Antioxidant compounds act as "free radicals scavengers" by preventing and repairing damages caused by reactive oxygen species (ROS) and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases (Valko *et al*.,2006). The physiological role of antioxidant compounds is to scavenge for free radicals (Surai, 2002). Active oxygen (hydroxyl, peroxylradicals and singlet oxygen) is highly toxic and an important causative agent of many diseases including cancer, heart disease, cataract and congestive disorders. Plant-derived antioxidants exert their effects by enhancing the levels of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase or by lowering the levels of lipid peroxides in the blood or liver (Usoh *et al*., 2005). High antioxidant phytochemicals are being researched for from plants to combat cancer cell. In this study, the antioxidant and antimicrobial properties of three plant extracts are investigated.

MATERIALS AND METHODS

Plant Material and Plant Extract

The plant materials employed in this study are *Anona senegalensis* (African custard apple), *Anogeosus leiocarpus* (African birch) *and Moringa oleofera* (Zogale). Their leaves were collected from some parts of Jos-North between June and July 2016, after they were dried and pulverized into powder. Fifty grams (50g) each of plant powder was weighed into 500ml conical flasks and was soaked in 70% ethanol. This was left to stand for 24h and shaken for 3h on a mechanical shaker. The filtrates were evaporated to dryness using a rotary evaporator and a drying cabinet. Percentage yield of the extract was determined and extract transferred into a stirrer sample container and preserved in the refrigerator. The phytochemical screening, sensitivity of test organisms and the antioxidant assay were carried out.

Antimicrobial Activity Test

The tests was carried out using Nutrient agar for bacterial strains and saboureaud dextrose

agar for fungal strains using agar well diffusion method as described by Valgas *et al*. (2007), Magaldi *et al*. (2004). Twenty milliliters (20ml) nutrient agar was poured into sterile Petri dishes arranged on the bench. The melted sterile agar was allowed to cool to about 45° c and then, 0.2ml of the overnight culture at 10^{-2} dilution of the test organism was added to each 20ml melted agar and mixed by rotating in the palm. It was poured into sterile Petri dishes and allowed to set firmly. A No.5 cone borer was dipped in alcohol and flamed, then allowed to cool, it was then used to cut or bore five holes in each plate by sterilizing the bores between the plates. About 0.2ml of each of the prepared extract was separately pipette into the cups. The plates were left at room temperature on the bench for an hour to allow for diffusion. They were them incubated overnight at 37°c and the zones of inhibitions were measured and recorded. Four micrograms (4µg/ml) and 40mg/ml of each of the Gentamycin and Fluconazole were used as standard.

Determination of the Minimum Inhibitory Concentrations (MIC)

A broth microdilution method was used to determine the minimum inhibitory concentration (MIC), as described by Valgas *et al*, (2007), Magaldi *et al.* (2004). Twelve grams (12.5g) nutrient both and SDA broth was weighed on foil paper and transferred into 2 separate conical flask. Five hundred Milles (500ml) of distilled water was added to dissolve the content of the conical flask and then thoroughly shaken. Five Milles (5ml) each was dispensed into test tubes covered with cotton wool and wrapped with aluminum foil. For double strength medium, 12.5g was weighed and dissolved in 250mls distilled water, 5ml was dispensed in each of the test tubes and covered with cotton wool and aluminum foil. It was sterilized in the autoclave for 15 minutes at 121°c before it was removed and allowed to cool completely. The solution of the extract under investigation was diluted serially with the growth media such that the concentration to half in each test tube in series, that is, 5ml of the solution of the extract was added to 5ml of double strength medium and mixed by shaking. Using a freshly sterile pipette, 5ml was transferred to tube two which contained single strength medium. This too was mixed with the extract by shaking and the procedure was repeated for up to six tubes for each of the organisms per extract. Finally, about 0.1 ml inoculums of the test organism was added. It was then incubated overnight (24 hours) at 37°c and observed for growth and recorded.

Determination of minimum bacteriocidal concentration (MBC) and minimum fungicidal concentration (MFC)

Freshly prepared sterile nutrient agar and sabauraud dextrose agar was poured separately into sterile Petri dishes and allowed to set firmly. A loopful of the mixture in the tube not showing growth was transferred to the agar in the plates and incubated for 24hours at 37^0c and observed.

Determination of antioxidant activity

The antioxidant activity was evaluated using MTT Antioxidant Assay. Stock solutions of the extracts were prepared at 10 mg/ml, and 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma-Aldrich Chemical Co., UK) at 1 mg/ml. The extracts were reconstituted in Dimethyl sulphoxide (DMSO) and distilled water in a ratio 1:9, and diluted four-fold to give a 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, and 0.625 mg/ml concentrations. The MTT was prepared by dissolving in distilled water. Stock solutions of standards, ascorbic acid and Vitamin E were also prepared and serially diluted. The procedure is as described by Liu and Nair (2010) and Muraina *et al*. (2009), with slight modifications. Fifty microlitres (50 µl) of

MTT was pipette into 2 ml capped-vials. Fifty microlitres (50 µl) of the extract was added to the vial and vortexed for 1 min. Thereafter, One hundred microlitres (100 µl) of DMSO (1:9 H_2O) was added to each vial and vortexed again. The reaction mixture was incubated at 37°C for 3 hours. Each reaction mixture was pipette into 96-well plates, in addition to a blank (DMSO, 1:9 H_2O + MTT). Absorbance was read at wavelengths of 492 nm and 620 nm

RESULTS AND DISCUSSIONS

Phytochemical Analysis of the Extracts of the Plant Species

The results of the phytochemical analysis are shown in Table 1. The results revealed that bioactive compounds such as tannins, flavonoids, and cardiac glycosides were present in the plant extracts in large quantity, while anthraquinones occurred moderately and Saponin in traces in *Anogeossous lieocarpus* extracts. A high amount of alkaloids were present in Moringa oleifera and a lesser amount was present in *Anona senegalensis*. The percentage yields for the *A. leiocarpus, Moringa oleifera,* and *Anona senegalensis* are 24.5%, 25% and 23.2 % respectively.

This was calculated as:

$$\text{Percentage Yield} = \frac{W1-W2}{z} \times 100$$

W_1= Weight of the container

W_2= Weight of final extract and container.

z = Weight of crude sample

Table1: Phytochemical analysis of the extracts of the plant species

Biochemical components	*A. leiocarpus*	*M. oleifera*	*A. senegalensis*
Steroids	+	-	+
Alkaloids	-	+++	+
Saponin	+	-	-
Tannins	+++	+++	+++
Flavonoids	+++	+++	+++
Anthraquinones	++	-	-
Cardiac glycosides	+	++	++

Antimicrobial activity of the extract

The antimicrobial sensitivity test of the plant extracts was determined *in vitro* against clinical isolates. The results were determined at concentrations between 50 mg/ml to 400 mg/ml, Gentamycin (4 µg/ml) and Fluconazole (40 mg/ml) were used as the standard drugs. The minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) of the extracts were determined. .While *Escherichia coli* and *Bacillus subtilis* isolates were sensitive to all the test extracts, the fungi and dermatophyte isolates were resistant. *Anogeissus leiocarpus* showed good activity against *Bacillus subtilis* and *Escherichia coli* at concentrations between 50 and 400mg/ml *with an average* zones of inhibition ranging from 15.50±0.50 to 20.50±0.50 as compared with the standard at 20.25±0.25 recorded for *Bacillus subtilis*, while the range was between 14.75±0.50 to 20.50±0.50 compared to the standard at 22.50±0.50 was recorded for *Escherichia coli* (Table 2). The zones of inhibition for *Anogeissus leiocarpus* were all lower than that of the standard at all concentrations for both organisms except for the result of *Bacillus subtilis* at a concentration of 400mg/ml. The activity of *Moringa oleifera and Anona senegalensis* were seen to be similar and they showed a higher antibacterial activity as compared to that of *Anogeissus leiocarpus* at the concentration between 50mg/ml to 400mg/ml. These extract inhibited the growth of the test bacteria with an average zone of inhibition diameters ranging from 24.50±0.50 to 33.50±0.50 for both test organisms. *Moringa oleifera and Anona senegalensis* exhibited higher activity as compared to the standard except for the inhibitory activity recorded for *Moringa oleifera* against *Escherichia coli* which was at par with the standard at 50mg/ml. The combination of all three extracts of *A. senegalensis, A. leiocarpus* and

M. oleifera showed minimal activity at the various concentrations and inhibited the bacterial isolates while the fungi were all resistant. The average zones of inhibition diameter for the combined extracts of *A. senegalensis, A. leiocarpus* and *M. oleifera ranges* between 12.50±0.50mm and 20.50±0.50mm for both *Escherichia coli and Bacillus subtilis* against the standard at 22.5±0.50 mm for both organisms. The MIC for *A. senegalensis against* both *B. subtilis* and *E. coli* was 12mg/ml (Table 3) while *A. leocarpus* has an MIC of 50mg/ml for both microorganism (Table 2). The MIC for *M. oleifera* is 6.2 mg/ml against *B. subtilis* and 12.5 mg/ml against *E. coli* (Table 3). The MBC for *A. leoicarpus* is 100mg/ml for both *B. subtilis* and *E. coli* (Table 5). *A. senegalensis* exhibited an MBC of 25mg/ml for both *B. subtilis* and *E. coli* (Table 7) *while Moringa oleifera has an* MBC 25mg/ml against *E. coli* and *12.5* mg/ml *against B. subtilis* showing a higher activity (Table 6).

Table 2: Minimum inhibitory concentration for *A. leiocarpus*

Concentration (mm/ml)	100	50	25	12.5	6.25	3.125	1.563
B. subtilis	-	-	+	+	+	+	+
E. coli	-	-	+	+	+	+	+

Key: Negative (no growth), + Positive (growth)

Table 3: Minimum inhibitory concentration for *A. senegalensis*

Concentration (mm/ml)	100	50	25	12.5	6.25	3.125	1.563
B. subtilis	-	-	-	-	+	+	+
E. coli	-	-	-	-	+	+	+

Key: Negative (no growth), + Positive (growth)

Table 4: Minimum inhibitory concentration for *M. oleifera*

Concentration (mm/ml)	100	50	25	12.5	6.25	3.125	1.563
B. subtilis	-	-	-	-	-	+	+
E. coli	-	-	-	-	+	+	+

Key: Negative (no growth), + Positive (growth)

Table 5: Minimum bactericidal concentration for *A. leiocarpus*

Concentration (mm/ml)	100	50	25	12.5	6.25	3.125	1.563
B. subtilis	-	+	+	NT	NT	NT	NT
E. coli	-	+	+	NT	NT	NT	NT

Key: Negative (no growth), + Positive (growth), NT =Not tested

Table 6: Minimum bactericidal concentration for *M. oleifera*

Concentration (mm/ml)	100	50	25	12.5	6.25	3.125	1.563
B. subtilis	-	-	-	-	+	NT	NT
E. coli	-	-	-	+	+	NT	NT

Key: Negative (no growth), + Positive (growth), NT =Not tested

Table 7: Minimum bactericidal concentration for *A. senegalensis*

Concentration (mm/ml)	100	50	25	12.5	6.25	3.125	1.563
B. subtilis	-	-	-	+	+	NT	NT
E. coli	-	-	-	+	+	NT	NT

Key: - Negative (no growth), + Positive (growth), NT =Not tested

Antioxidant activity of the extracts of the plant species

The lowest concentration of the extracts to which antioxidant capacity can be detected is the highest dilution at which the formation of bluish-purple colouration disappears. Weak absorbance = poor antioxidant activity. This implies that, the lowest concentration with a higher absorbance value is an indication of high antioxidant activity. The experiment compared the antioxidant activity of the three extracts with that of the standards, vitamin E and ascorbic acid (positive control) and DMSO as the negative control. All three extracts exhibited good antioxidant activity at the wavelengths of 492nm and 620 nm. Vitamin E which is a known antioxidants, did not record any activity and this could probably be due to the fact that it does best at the recommended wavelength of 570nm as against the wavelengths used for this experiment. Also, it is possible that Vitamin E

did not mix well with water when the stock solution was being prepared. DMSO did not show any activity. At the lowest concentration of 0.625mg/ml, *A. leiocarpus* had absorbance of 0.37 and 0.41 at 492nm and 620nm wavelength respectively. *A. senegalensis* had absorbance of 0.18 and 0.17 while absorbance of 0.12 and 0.11 were recorded for *M. oleifera* had at 492nm and 620nm wavelengths respectively. The absorbance of Ascorbic acid at 0.625 mg/ml was 0.5 at 492nm wavelength and 0.36 at 620nm wavelength. The result indicated that at the activity of all plant extracts was best at 620nm wavelength. The activities *M. oleifera* and *A. senegalensis* are comparable to that of ascorbic acid but ascorbic acid has a better antioxidant activity with an absorbance of 0.36 at the lowest concentration 0.625mg/ml used. *A. leiocarpus* has a higher antioxidant activity as compared to the positive control, ascorbic acid with an absorbance of 0.37 and 0.41 at a concentration of 0.625mg/ml for 492 and 620nm wavelengths respectively. Figure 1 indicates that ascorbic acid has a better result at wavelength 620nm while *A. leocarpus* did better at both 492 nm and 620 nm wavelength. At concentration of 0.625 mg/ml, the absorbance of *A. leocarpus* at both wavelengths which are 0.37 and 0.41 at 492 nm and 620 nm as compared to that of ascorbic acid 0.25 and 0.36 respectively at the same concentration. Thus *Anogeissus leoicarpus* has a higher antioxidant activity than ascorbic acid. Figure 2 shows that the activity of *Anona Senegalensis* at both wavelengths is comparable. The antioxidant activity of the extract is also comparable to that of ascorbic acid but ascorbic acid performed better. Figure 3 above shows that the extract of *M. oleifera* has a good antioxidant activity but the activity of ascorbic acid shows better absorbance of 0.36 as against 0.11 at a concentration of 0.625mg/ml and at a wavelength of 620 nm.

Table 8: Absorbance of reaction mixtures at 492 nm and 620 nm obtained after reaction between extracts (10 mg/ml, 5 mg/ml and 2.5 mg/ml) and MTT (1mg/ml) at 37 °C

Extract concentration (mg/ml)	**Absorbance 492 nm**	**Absorbance 620 nm**
Anogeissus leoicarpus		
10	0.96	0.62
5	0.76	0.57
2.5	0.66	0.65
1.25	0.52	0.57
0.625	0.37	0.41
Anona senegalensis		
10	1.21	0.97
5	0.67	0.65
2.5	0.39	0.31
1.25	0.25	0.21
0.625	0.18	0.17
Moringa oleifera		
10	0.50	0.47
5	0.29	0.29
2.5	0.21	0.22
1.25	0.17	0.16
0.625	0.12	0.11
Ascorbic acid		
10	0.07	0.08
5	0.07	0.08
2.5	0.09	0.10
1.25	0.09	0.11
0.625	0.25	0.36
Vitamin E		
10	0.06	0.06
5	0.07	0.06
2.5	0.06	0.05
1.25	0.06	0.05
0.625	0.06	0.05
DMSO		
1	0.07	0.07
2	0.05	0.06
3	0.05	0.05
4	0.05	0.05
5	0.05	0.05

Also the absorbance of ascorbic acid at the wavelength 492nm, which is 0.25 is better than that of *M. oleifera* at the same wavelength. In Figure 4, the activity of the three plant extracts shows that the antioxidant

activity of *A. senegalensis* and *M. oleifera* are comparable. while *A.leiocarpus* showed better activity than the other two extracts. Figure 1-6 shows the activity of the test extracts.

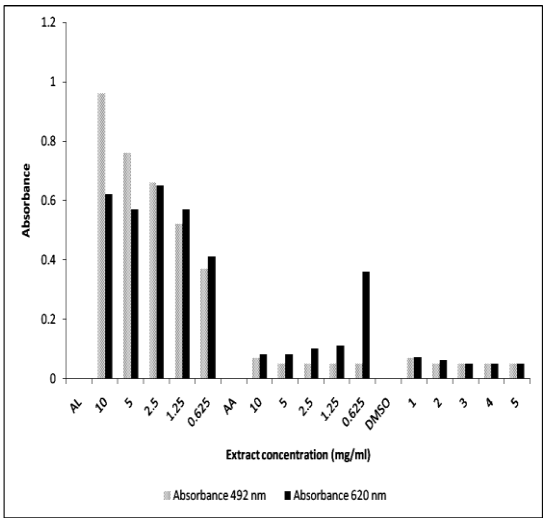

Figure 1: Comparing absorbance of *A. leiocarpus* with that of Ascorbic acid obtained at 492 nm and 620 nm after reaction between Extracts (10 mg/ml, 5 mg/ml,2.5 mg/ml, 1.25mg/ml and 0.625mg/ml) and MTT(1mg/ml) at 37 °C

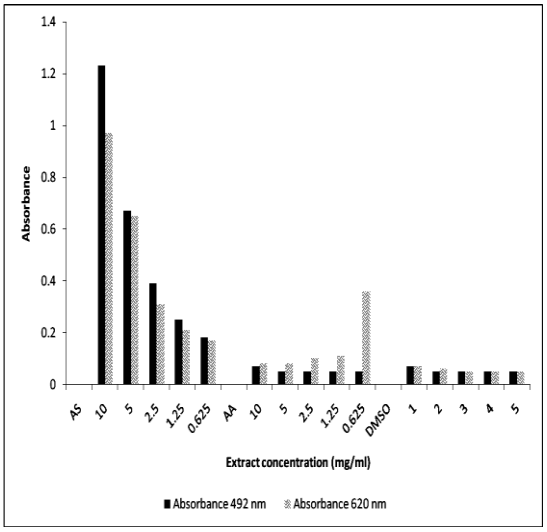

Figure 2: Comparing absorbance of *A. senegalensis* with that of Ascorbic acid obtained at 470 nm and 620 nm after reaction between extracts (10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml and 0.625mg/ml) and MTT (1mg/ml) at 37°C.

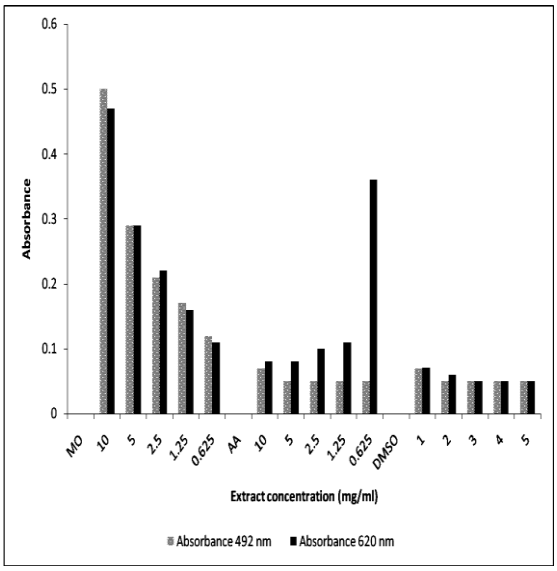

Figure 3: Comparing absorbance of *M. oleifera* with that of Ascorbic acid obtained at 470nm and 620nm after reaction between extracts (10 mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml and 0.625mg/ml) and MTT (1mg/ml) at 37°C.

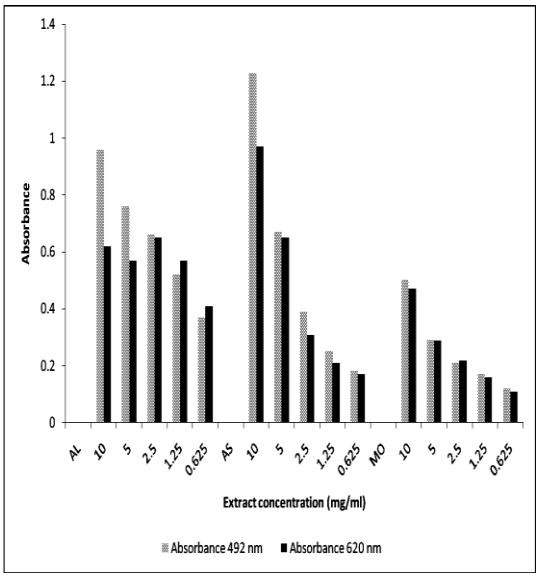

Figure 4: Comparing absorbance of all test extracts obtained at 470 nm and 620 nm after reaction between extracts (10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25mg/ml and 0.625mg/ml) and MTT (1mg/ml) at 37°C.

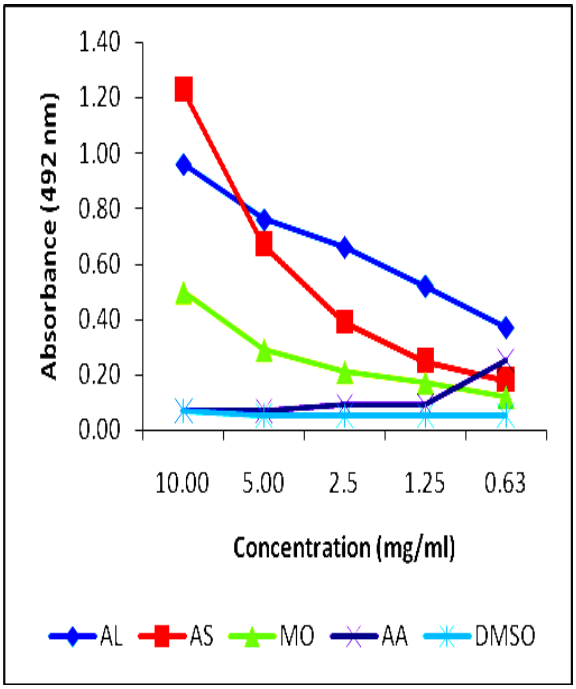

Figure 5: Absorbance of Test extracts at wavelength 620 with DMSO.

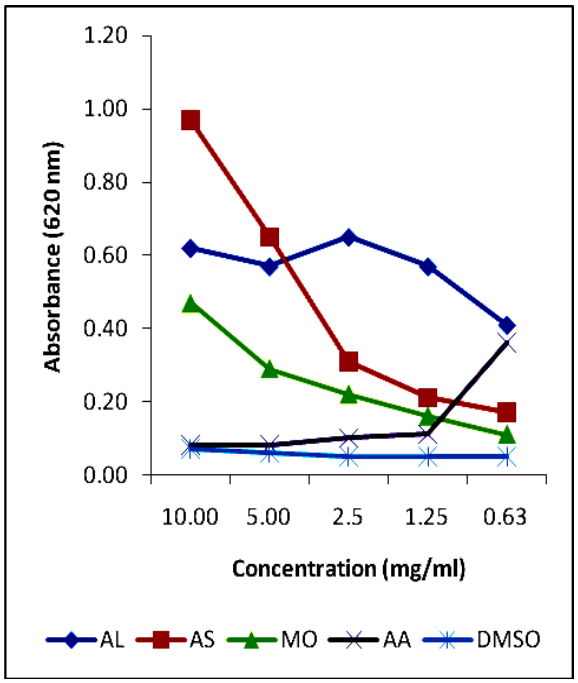

Figure 6: Absorbance of Test extracts at wavelength 620 with DMSO

CONCLUSION

The leaves of *Anogeissus leiocarpus, Anona senegalensis* and *Moringa oleifera* have shown antioxidant and antibacterial activity against all the test organisms. This reveals that, the plant contains some bioactive compound which is responsible for these activities. The result for this research has ascertained the claim of their use as complementary medicine or as traditional medicine. The plant extract could be an alternative source of antibiotics. Due to reported consequence of widespread and indiscriminate use of antibiotics during the past decade, resistant bacteria have evolved and weakened the anti microbial potential of many antibiotics as chemotherapeutic agents. This forms the bases for further investigation on purification and structural determination of the most promising constituents of these plants extracts for *in vivo* evaluation of these plants in animals and human studies to ascertain the possibility of usage as alternative antimicrobials (chemotherapeutic agents) and for the prevention and cure of cancers (chemo prophylactics). The phytochemical analysis reveals *Moringa oleifera* to contain high quantity of alkaloids. The chromatography of alkaloids should be carried out to see which of the compounds is responsible for the activity. The bioactive compounds responsible for antioxidant activity could also be harnessed and applied industrially for preservation of foods particularly oily foods to prevent oxidation and rancidity.

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