IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 13, Issue 5 Ser. III (May. 2020), PP 21-26
www.iosrjournals.org

Phytochemical composition and potential antimicrobial activity of extracts of two neglected seeds (*Mangiferaindica*L.and *Persea Americana* M.) in Benin

Amoussatou Sakirigui[1,2*], EléonoreYayi Ladekan[1], Louis Fagbohoun[1,2], KamirouChabi Sika[3], Fidèle Assogba[1] and Joachim Djimon Gbenou[1,2]

[1]*Laboratory of Pharmacognosy and Essential Oils, Faculty of Sciences and Techniques/University of Abomey-Calavi (UAC) Cotonou, Republic of Benin.*

[2]*Aromatic, Food and Medicinal Plants Research Laboratory, Faculty of Sciences and Techniques, UNSTIM/Abomey Republic of Benin.*

[3]*Laboratory of Biology and Molecular Typing in Microbiology, Faculty of Sciences and Techniques/University of Abomey-Calavi, Cotonou, Republic of Benin.*

***Corresponding author**: Faculty of Sciences and Techniques, UNSTIM, box: 72, Natitingou, republic of Benin;E-mail: samoussatou@yahoo.fr; Tel: +229 95321069*

Abstract:

***Background**:Mangiferaindica*L. *and Persea Americana* M. *are two fruit trees very well-known and appreciated by the people of Benin for their fruits. The seeds from the fruits of these plants, despite their growing interest in pharmacology, are considered as environmental waste not yet recovered. This work aims to determine the phytochemical composition and the antimicrobial activity of the ethanolic extract of these two neglected seeds in Republic of Benin.*

***Materials and Methods**:50 g of powder of P. Americana and M. indicaseeds were separately crushed and recovered in 500 ml of ethanol 96°C to obtain their extracts. The qualitative and quantitative phytochemical screening was performed to determine the different phytochemical groups. Antimicrobial activity was performed on eleven bacteria to determine their inhibitory diameter zone, the Minimum Inhibitory Concentrations and the minimum Bactericidal Concentration.*

***Results:** Qualitative and quantitative phytochemical analysis of the ethanolic extract revealed that avocado seeds extract contained alkaloids: 2.14 ± 0.012 mg, flavonoids: 5.33 ± 0.064 mg, reducing compounds: 0.84 ± 0.027 mg and phenolic compounds 3.22 ± 0.035 mg. That of mango seeds also included alkaloids: 2.14 ± 0.012 mg of flavonoids, 5.33 ± 0.064 mg of reducing compounds 1.72 ± 0.019 mg and phenolic compounds (7.68 ± 0.027 mg). Antimicrobial tests indicated a larger inhibition diameter (25mm)and shown a lower minimum inhibitory concentration of (0.325 mg / ml) on certain bacteria with the two extracts. The mango extract had a bactericidal effect (1.25 mg / ml) on Staphylococcus epidermidis T22695.*

***Conclusion:** The extracts of the two seeds, rich in chemical compounds and having interesting antimicrobial properties could constitute sources of active principles in the development of antimicrobial agents.*

***Key Word**:Mangiferaindica, Persea Americana, seeds, phytochemical composition, Antimicrobial tests.*

Date of Submission: 18-05-2020 Date of Acceptance: 03-06-2020

I. Introduction

Due to report of increasing developments of drug resistance in human pathogen as well as undesirable side effects of certain antimicrobial agents, it is necessary to search for new agents that are better, cheaper and without side effect for treating infectious diseases especially in developing countries[1]. A wide variety of plant / natural products are used in the treatment of infections.

There have been several reports on the pharmacological effects and suitability of medicinal plants as phyto-therapies for diseases[2,3,4,5]. Among these plants, there are *Mangiferaindica* L. and *Persea Americana*M. which caught our attention. The numerous pharmacological properties of the leaves of these two plants are described in the literature[6,7,8,9,10,11]. But in Republic of Benin, these plants are mainly known and appreciated by the population for their very appetizing and nourishing fruits. The fully ripened fruits are used for table purpose. Despite the fact that mango and avocado seeds contain Phyto-constituents, the seeds are considered to be a waste and pose a real environmental problem in the country. Generally, plant parts (seeds, leaves, bark, fruits and stems) also contain bioactive agents. As proof, seeds of mango have a broad range of medicinal properties, such as antimicrobial, antiviral, antifungal, anti-inflammatory, anti-diarrhoeal, antioxidant, as well as

immunomodulatory[12,13,14,15,16,17]. The biological activities such as antioxidant, antihypertensive, fungicidal, larvicidal, hypolipidemic, amoebicidal and giardicidal activities of the seeds of avocado had also reported[18,19,20,21,22,23,24]. No study on the two seeds in Benin has been undertaken with a view to their valorization. The objective of this work is to make a study of the phytochemical composition and the antimicrobial activity of the two seeds with a view to their valorization in the antimicrobial fight in Benin.

II. Material And Methods

Extract Preparation: Ethanol is a very little toxic solvent and according to some authors allows to extract the maximum of chemical compounds[25]. That is what motivated us to take an interest in the ethanolic extract of the two organs.

Mango and avocado fruits were deseeded by removing the fleshy cover. The resultant seeds were eachwashed with clean tap water, crushed into smaller pieces with the help of manual grater. Thereafter, the seeds were air dried for 3 weeks under regular turning to enhance even drying. The dried leaves samples and seeds were separately grounded into fine powder using a mechanical grinder. The cold ethanol extraction was adopted for extraction.

50 g of powder of *P. Americana* and *M. indica*seeds were separately crushed and recovered in 500 ml of ethanol 96°C. After agitation and homogenization, the mixture is filtered on Wathman paper and the filter is concentrated in a rotary evaporator at a temperature between 55°C and 60°C with help of vacuum pump to obtain the extract. The dry watery triturated extract obtained was stored in a refrigerator at 4°C.

Phytochemical Screening: The qualitative phytochemical screening was performed based on colouring or precipitation reactions. It is made directly on the ethanolic extract of *P. Americana*and *M. indica*seedsaccording to Houghton, Raman method and Simo et al. methods[26,27]. Quantitative phytochemical tests were carried out according to the method of Harbon and Umeaku et al.[28,29].

Antimicrobial activity assessment methods: Eleven references strains such as *Escherichia coli* ATCC 25922, *Staphylococcus aureus*ATCC 29213, *Staphylococcus epidermidis*T22695, *Pseudomonas aeruginosa*ATCC 27853, *Proteus mirabilus*A24974, *Micrococcus luteus*ATCC10240, *Proteus vulgaris* A25015, *Streptococcusoralis*, *Enterococcus foecalis*ATCC 29212, *Salmonella typhi R 30951401* and*Escherichia coli O157* were used.

Sensitivity test: It was done according to the disc method inspired from the one described by Bauer et al., (1996). Brieflt, 1 ml of pre-culture of 18-24 h (10^6 UFC/ml) enabled planting a box of Petri dishes containing agar Mueller Hinton by flood. After seeding, the sterile Whatman paper discs (5 mm de diameter) were deposited with sterile pince. These discs have been carefully impregnated with 30 µl of plant extract (20 mg/ml. The dishes were kept for 15-30 min at room temperature before incubation at 37°C. The inhibition zones diameters were measured after 24 to 48 hours using a ruler graduated[30]. For each extract, the experiment was performed induplicate.

Determination of the Minimum Inhibitory Concentration (MIC): The MIC has been determined by macrodilution method with Visual assessment of the growth of microorganisms[31]. Briefly, nine concentrations (10 000, 5 000, 2 500, 1 250, 625, 312.5, 156.25, 78.12 and 39.06 µg/ml) was performed in screw tube. To 1 ml of the above concentrations was added 1 ml of the bacteria inoculum (10^6 UFC/ml). After 24 h of incubation turbidity tubes was examined relative to the control tube containing distilled water and the inoculum (10^6 UFC/ml).

Determination of the Minimum Bactericidal Concentration (MBC): The MBC was determined by solid medium culture of all of the tubes from the MIC to high concentrations. These dishes were incubated at 37 ° C for 24 h. The highest dilution that yielded no bacterial growth on solid medium was taken as MBC[32].

Data treatment and analysis: The spreadsheet Microsoft Excel version 2013 has been used for the capture and encoding the data. Minitab (version 17) software was used for the variance analysis (ANOVA). Finally a structuring of the medium was made which allowed us to compare and identify the excerpt (s) most active on the various parameters through Student Newman and Keuls (SNK) test on the threshold of 5% of significance.

III. Results

Performance extraction: For 50 g of avocado seeds powder, 5.56 g of ethanolic extract were obtained. This extract was therefore obtained with a yield of 11.12%. That of the mango seeds was obtained with a yield of 10.33%.

Qualitative and quantitative composition of extracts: The phytochemical screening of the extracts of mango and avocado seeds had qualitatively shown the presence of Reducing compound, Alkaloids, Flavonoids, phenolic compounds consisting of catechic and gallic tannins, Saponins and Terpenoids in the two extracts (table1). Free Anthracenics and Leuco-anthocyanins were present in the avocado seeds and absent in the mango seeds. Mucilages and O-heterosides are present in the extract of mango seeds and absent in the

extract of avocado seeds. Other compounds such as Anthocyanins, Quinonics compound, Cartenoids, are not present in the two extracts (table no 1).

The quantitative analysis of the two extracts shows that the mango nuts are richer in alkoids (9.66 ± 0.22 mg) and flavonoids (6.86 ± 0.20 mg) than avocado nuts (alkoids: 2.14 ± 0.012 mg and flavonoids: 5.33 ± 0.064 mg). Contrary to the first observation, avocado nuts are richer in reducing compounds (1.72 ± 0.019 mg) and phenolic compounds (7.68 ± 0.027 mg) (table no 1) than mango nuts (reducing compounds: 0.84 ± 0.027 mg ; phenolic compounds 3.22 ± 0.035 mg). These values can also depend on the type of extract produced.

Table no 1: Phytochemical constituents of *P. Americana* and *M. indica*seeds(mg/100g)

Compounds	AS/QLA	AS/QNA	MS/QLA	MS/QNA
Reducing compound	+	1.72 ± 0.019	+	0.84 ± 0.027
Alkaloids	+	2.14 ± 0.012	+	9.66 ± 0.22
Flavonoids	+	5.33 ± 0.064	+	6.86 ± 0.20
Tanins catechic	+	7.68 ± 0.027	+	3.22 ± 0.035
Tanins gallic	+		+	
Anthocyanins	-		-	
Leuco-anthocyanins	+		-	
Quinonics compound	-		-	
Saponin	+		+	
Coumarin	-		-	
Terpenoids	+		+	
Mucilages	-		+	
Cartenoids	-		-	
Free Anthracenics	+		-	
O-heterosides	-		+	

(+) = Presence; (-) = Absence; **AS/QLA**: Avocado seeds /Qualitative analysis; **AS/QNA**: Avocado seeds/Quantitative analysis; **MS/QLA**: Mango seeds/Qualitative analysis; **MS/QNA**:Mango seeds/Quantitative analysis.

The extracts inhibitory diameter zone with the reference strains: Figure no1 shows the inhibitory activity of avocado and mango seeds extracts on the eleven bacteria tested. The sensitivity test performed with extracts on these bacteria varies from one strain to another depending on the type of extract. The observed variation is very significant ($p < 0.001$). The two extracts tested showed a pronounced antagonistic effect by inhibiting the growth of 90% of the pathogenic strains tested (Figure no 1). The largest inhibition diameters were obtained with the avocado seeds extract. The largest inhibition diameter (25 mm) was obtained against *P.vulgaris* strain and the following inhibition diameter (24 mm) against *Staphylococcus aureus* ATCC 29213 strain. The largest inhibition diameter obtained with mango seeds extract is (21 mm) against *Enterococcus faecalis* ATCC29212 strain. This extract is also active on *Proteus vulgaris* A25015 and *Staphylococcus epidermidis* T22695 (20 mm) and on *Staphylococcus aureus* ATCC 29213 (19.5 mm) (Figure no 1). The smallest inhibition diameter for the two extracts is (0 mm) against the same strain *Salmonella typhi* R.

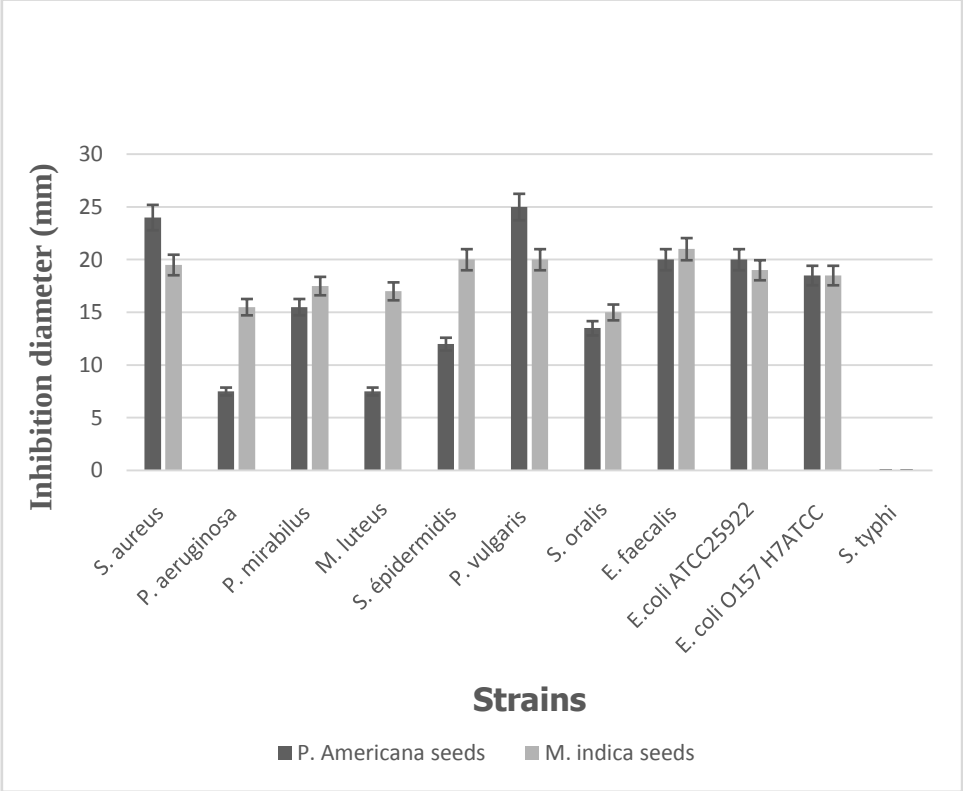

Figure no 1: Diameters of inhibition of *P. Americana* and *M. indica*seedsextracts

Minimum Inhibitory Concentrations (MIC) of *P. Americana* and *M. indica* seeds extracts: The two extracts inhibit the proliferation of most pathogenic bacteria with variable minimum inhibitory concentrations (MIC) ($p < 0.05$). According to our results, the smallest (0.3125 mg / ml) was obtained with the two extracts. It was obtained against *Staphylococcus epidermidis* T22695, and *Proteus vulgaris* A25015 with the extract of mango seeds and against *Streptococcus oralis*, *Escherichia coli* ATCC25922 and *Micrococcus luteus* with the extract of avocado seeds. Apart from the intermediate MIC of 0.625 mg / ml obtained against 6 bacteria with the two extracts, the largest MIC is 1.25 mg / ml obtained against 4 bacteria with the extract of mango seeds. The largest MIC obtained with avocado seeds is 2.5 mg / ml against *Staphylococcus epidermidis* T22695 strain.

Table no 2: Minimum inhibitory concentrations (mg/ml) of the extracts on the studied reference strains

STRAINS	*S. aureus* ATCC 29213	*P. aeruginosa* ATCC 27853	*P. mirabilus* A24974	*M. luteus*	*S. épidermidis T22695*	*P. vulgaris A25015*	*S. oralis*	*E. faecalis ATCC29212*	*E. coli ATCC25922*	*E. coli O157 H7ATCC*	*S. typhi R 30951401*
MS	1.25	1.25	1.25	0.625	0.3125	0.3125	1.25	0.625	0.625	0.625	0
AS	1.25	1.25	1.25	0.3125	2.5	0.625	0.3125	0.625	0.3125	1.25	0

MS: Mango seeds; **AS**: Avocado seeds.

Minimum Bactericidal Concentration (MBC) (mg / ml) of *P. Americana* and *M. indica* seeds: The two extracts tested have a weaker bactericidal effect against the bacteria used. The bactericidal effect was noted on two microorganisms (table no 3). The lowest minimum bactericidal concentration (1.25 mg / ml) was obtained with the mango seeds extract against two bacteria (*Staphylococcus epidermidis* T22695, *Enterococcus faecalis* ATCC29212). With the extract of avocado seeds, two values were noted, one of (1.25 mg / ml) obtained against *Enterococcus faecalis* ATCC29212 and the other of (10 mg / ml) was obtained against *Staphylococcus epidermidis* T22695 (table no 3).

Table no 3: Minimum Bactericidal Concentrations (mg/ml) of extracts with reference strains

STRAINS	*S. aureus* ATCC 29213	*P. aeruginosa* ATCC 27853	*P. mirabilus* A24974	*M. luteus*	*S. épidermidis T22695*	*P. vulgaris A25015*	*S. oralis*	*E. faecalis ATCC29212*	E. coli ATCC25922	*E. coli O157 H7ATCC*	*S. typhi R 30951401*
EL	0	0	0	0	1.25	0	0	1.25	0	0	0
ES	0	0	0	0	10	0	0	1.25	0	0	0

MS: Mango seeds; **AS**: Avocado seeds.

IV. Discussion

The extraction solvent being the same, it appears that the seeds avocado have a higher extraction yield than that of those of mango. The phytochemical composition may vary depending on other extraction solvents. It can also vary if it is made directly on the powder. The compounds responsible for the desired activity being those present in the extract, the screening was carried out on the latter instead of the powder. The phytochemical composition are slightly different from those observed in other work which had used other solvents during their work[33,34,35]. This can also be explained by the fact that the extracts studied are different, or even by the difference in certain natural parameters such as the nature of the soil, the climate, the season etc. [36].

For the antimicrobial activity, the extract of avocado seeds remains more active than the extract of mango seeds on certain microbes. But the two activities are not significantly different. The inhibition diameters observed shown that the extracts are fairly active on most of the strains studied. These values compared to those of the literature are very interesting[37]. This can be explained by the fact that the phytochemical composition of the two extracts is not very different from one extract to another. Avocado seeds are richer in alkaloids and flavonoids, while mango seeds are richer in phenolic and reducing compounds. The alkaloids and flavonoids would be responsible for the activity observed with the extract of avocado seeds, and the phenolic and reducing compounds for the activity observed with mango seeds. The antimicrobial activities of these various compounds present in the two extracts have been described in the literature[16,38,39,40,41]. Alkaloids, flavonoids, phenolic and reducing compounds have been described as very good antimicrobial agents. But we can also consider a synergistic effect between the different compounds. In general compared to the literature, the observed MIC values indicate that the two seeds have good activities. The activities of the two seeds have also been described in the literature, but with other extracts obtained from other solvents[16]. The lower concentration being obtained with more germs in avocado seeds, we could also remember that the latter have a higher activity than that of mango seeds. Mango seeds have a more pronounced bactericidal effect on bacteria than avocado seeds. In addition, the two extracts have an effect on the same bacteria, except that the mango seeds have been shown to be more active on *Staphylococcus epidermidis*. These bacteria would be more sensitive to the compounds present in large quantities in mango seeds (phenolic and reducing compounds). These observations are in perfect correlation with those observed above. Both extracts inhibit *Enterococcus faecalis* ATCC29212 with the same value and the MIC of the mango seeds extract on *Staphylococcus epidermidis* T22695 is smaller than the MIC of the avocado seeds extract (Table no 2).

V. Conclusion

The phytochemical study carried out on *Mangiferaindica* L. and *Persea Americana*M. seeds, shown the presence of large chemical groups in different proportions in the extracts. The antimicrobial study reveals that the extracts of the two seeds inhibited the 11 bacteria tested by more than 90%. These two extracts also had a bactericidal power on two micro-organisms: *Enterococcus faecalis* and *Staphylococcus epidermidis*T22695. In light of these results, mango and avocado seeds could change from their status as environmental pollutants to the status of sources of active ingredients that may be involved in antimicrobial control.

References

[1]. Awad El-Gied AA, Joseph MRP, Mahmoud IM, Abdelkareem AM, Al Hakami AM, Hamid ME. Antimicrobial Activities of Seed Extracts of Mango (*Mangiferaindica*L.).Advances in Microbiology: 2012; 2: 571-576.
[2]. Marjorie MC. Plant Products as antimicrobial Agents. Clinical and Microbiology Reviews. 1999; 12: 564-582.
[3]. Ahmed I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants. Journal of Ethnopharmacology*2001*; 74(2*)*:113-123
[4]. Singh M, Khatoon S, Singh S, Kumar V, Rawat AK, Mehrotra S. Antimicrobial Screening of Ethno- bo-tanically Important Stem Bark of Medicinal Plants. Pharmacognosy Research. 2010; 2: 254-257.
[5]. Sofowora A. medicinal plants and traditional Medicine in Afric, John Wiley and Sons Ltd., New York, 1982.

[6]. Syeda VK, Ahmad A, Padhye S, Sarkar FH. Perspectives on Medicinal Properties of Mangiferaindica. Mini-Reviews in Medicinal Chemistry. 2012; 12(5): 412-425.

[7]. Tsabang N, Fokou PV, Tchokouaha LR, Noguem B, Bakarnga-Via I, Nguepi MS, Nkongmeneck BA, Boyom FF. Ethnopharmacological Survey of Annona- ceae Medicinal Plants Used to Treat Malaria in Four Ar- eas of Cameroon. Journal of Ethnopharmacology. 2012; 139(1): 171-180.

[8]. Doughari JH, Manzara S. In Vitro Antibacterial Activity of Crude Leaf Extracts of MangiferaindicaLinn. African Journal of Microbiology Research. 2008; 2: 67-72.

[9]. Nahak MM, TedjasulaksanaR,Sumerti NN. Ability difference of Beluntas Leaf (*Plucheaindica L*) ethanol extract and avocado leaf (*Perseaamericana Mill*) ethanol extract in Inhibiting caries-causing Streptococcus mutans Bacteria Growth. Bali Med. J. 2017; 6(3): 387-390.

[10]. Evbuomwan L, Inetianbor J. Antibacterial Activity of Persia americana Leaf Extracts Against Multidrug Resistant Bacterial Isolates. AASCIT Journal of Bioscience. 2017; 3(4): 29-34.

[11]. OwusuBoadi N, Saah SA, Mensah JK, Badu M, Addai-Arhinand S, Mensah MB. Phytoconstituents, antimicrobial and antioxidant properties of the leaves of *Persea Americana* Mill cultivated in Ghana. JMPR. 2015;9(36): 933- 939.

[12]. Talba AM, Suleiman MM, Raji MA, Oniye SJ. Phytochemical Screening and *In-vitro* Antibacterial Activity of *Mangiferaindica*(Mango) Kernel on *Aeromonascaviae.* IOSR Journal Of Pharmacy.2014; 4(10): 45-50.

[13]. Sairam K, Hemalata S, Kumar A, Srinivasan T, Ganesh J, Shankar M. Evaluation of anti-diarrhoeal activity in seed extracts of *Mangiferaindica*. J. Enthnopharm. 2003; 84:11-15.

[14]. Azhagesan G, Rajan S, Soranam R. Anti-salmonella activities of *Mangiferaindica*seed kernel aqueous extract. Advances in Applied Science Research. 2015; 6(5):75-80.

[15]. Vaghasiya Y, Patel H, Chanda S. Antibacterial activity of *Mangiferaindica*L. seeds against some human pathogenic bacterial strains, African Journal of Biotechnology. 2011; 10(70): 15788-15794,

[16]. Kaur J, Rathinam X, Kasi M, MiewLeng K, Ayyalu R, Kathiresan S, Subramaniam S. Preliminary investigation on the antibacterial activity of mango (MangiferaindicaL: Anacardiaceae) seed kernel. Asian Pacific Journal of Tropical Medicine. 2010; 707-710.

[17]. Kamble VA, Somkuwar1 DO, Wankhade SJ. Chemical composition, antioxidant and antisalmonella activity of *Mangiferaindica*L. flower and seed kernel extracts. International Current Pharmaceutical Journal. 2016; 5(10): 82-93.

[18]. Anaka ON, Ozolua RI, Okpo SO. Effects of the aqueous seed extract of *Persea Americana* mil (Lauraceae) on the blood pressure of sprague dawley rats. Afr. *J.* Pharm. Pharmacol. 2009; 3(10): 485–490.

[19]. Alhassan AJ, Sule MS, Atiku MK. Effects of aqueous avocado pear (*Perseaamericana*) seed extract on alloxan induced diabetes rats. GJMS. 2012; 2(1): 005–011.

[20]. Chia TWR, Dykes GA. Antimicrobial activity of crude epicarp and seed extracts from mature avocado fruit (*Perseaamericana*) of three cultivars. Pharm Biol. 2010; 48(7):753–756.

[21]. Adaramola B, Onigbinde A, Shokunbi O. Physiochemical properties and antioxidant potential of Persea Americana seed oil. Chemistry International. 2016; 2(3): 168-175.

[22]. Dennis CN, Wulandari S. Antibacterial effect of ethanol extract of the avocado seed (*Persea Americana* Mill.) as an alternative root canal Irrigants against *PorphyromonasGingivalis*(*In Vitro*). IJADS. 2017; 3(1): 89-93.

[23]. Kristanti CD, Simanjuntak FP, Dewi KP, Tianri SV, Hendra P. Anti-inflammatory and analgesic activities of avocado seed (*perseaamericana*mill.). Jurnalfarmasisainsdankomunitas. 2017;14 (2): 104-111.

[24]. Thanh Sang V, Phuong Uyen L, Dai Hung N. Free radical scavenging and anti-proliferative activities of avocado (Persea Americana Mill.) seed extract. Asian Pac J Trop Biomed. 2019; 9(3): 91-97.

[25]. Umar M, Mohammed IB, Oko JO, Tafinta IY, Alika AA and Jobbi DY. Phytochemical Analysis and Antimicrobial Effect of Lemon Grass (*Cymbopogoncitratus*) Obtained From Zaria, Kaduna State, Nigeria. Journal of Complementary and Alternative Medical Research. 2016; 1(2):1-8.

[26]. Houghton DJP, Raman A. Laboratory handbook for the fractionation of natural. Extracts Science. 1998; 199p.

[27]. Simo M, Donati M, Siwe G, Majoumouo S, Chen Z, Toghueo R, Keumoe R, Jazet P, Sameza M and Fekam F. Antioxidant potential of fractions from the stem methanol extract of *Uvariacomperei*(Annonaceae). Int J Pharmacognosy.2020; 7(3): 76-82.

[28]. Harbon JB phytochemical methods. London chapman and Hall Ltd., 1973; l88.

[29]. Umeaku CN, Chris-Umeaku CI, Emmy-egbe IO, Ukoha CC, Uzor UC, Agbo UJ. Proximate, Phytochemical and Antibacterial Analysis of *Persea Americana* Obtained from Nigeria. Journal of Diseases and Medicinal Plants. 2018; 4(3): 89-95.

[30]. Adesokan AA, Akanji MA, Yakubu MT. Antibacterial potentials of aqueous extract of *Enantiachlorantha* stem bark. Afr. J. Biotechnol.2007; 6(22): 2502-2505.

[31]. Delarras C. Microbiology. 90 hours of practical work. GaétanMorien Publisher. 1998;169-178. ISBN: 291074907 X, 9782910749071

[32]. Farshori NN, Al-Sheddi ES, Al-Oqail MM, Musarrat J, Al-Khedhairy AA and Siddiqui MA. Anticancer Activity of *Petroselinum sativum* Seed Extracts on MCF-7 Human Breast Cancer Cells. Asian Pac J Cancer Prev. 2013;14(10): 5719-5723.

[33]. Omolara OO, Friday ON Chinelo MO. Comparative Study of the Constituents of the Fruits Pulps and Seeds of Canariumovatum, Persea Americana and DacryodesEdulis. Jordan Journal of Chemistry. 2017;12 (2): 113-125.

[34]. Egbuonu AC, Opara IC, Onyeabo C, Uchenna NO. Proximate, Functional, Antinutrient and Antimicrobial Properties of Avocado Pear (*Perseaamericana*) Seeds. J Nutr Health Food Eng. 2018; 8(1): 78–82.

[35]. DAKARE1 MA, AMEH DA, AGBAJI AS, ATAWODI SE. Chemical Composition and Antinutrient Contents of Yellow Maize, Raw and Processed Composite Mango (Mangiferaindica) Seed Kernel from Zaria, Kaduna State Nigeria, International Journal of Advanced Research. 2014; 2(7): 90-97.

[36]. Kaiser K, Guggenberger G, Haumaier L, Zech W. Seasonal variations in the chemical composition of dissolved organic matter in organic forest floor layer leachates of old-growth Scots pine (*Pinussylvestris*L.) and European beech (*Fagussylvatica*L.) stands in northeastern Bavaria, Germany. Biogeochemistry. 2001;55: 103–143.

[37]. Nounagon MS, Dah-Nouvlessounon D, N'tcha C, Akorede SM, SinaH.Phytochemical Screening and Biological Activities of *Combretumadenogonium*Leaves Extracts. ESJ. 2017; 13(30): 358-375.

[38]. Karthy ES, RanjithaP.Screening of antibacterial tannin compound from mango (*Mangiferaindica*) seed kernel extract against Methicillin resistant *Staphylococcus aureus* (MRSA), Elixir Pharmacy. *2011; 40C: 5251-5255.*

[39]. Karou1 D, Savadogo A, Canini A, Yameogo S, Montesano C, Simpore J, Colizzi V, Traore AS. Antibacterial activity of alkaloids from *Sidaacuta.* Afr. J. Biotechnol. 2006; 5 (2): 195-200.

[40]. Ngoupayo J, Assonfack FRM, Chelea M, Djiele NP and Ndelo J. Evaluation of the antimicrobial activity of tannin extracted from the barks of *Erythrophleumguineensis* (Caesalpiniaceae). J PharmacognPhytochem. 2016; 5(4): 287-291.

[41]. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. IJAA. 2005; 26(5):343-356.