IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
**Research Article**
January 2022 Vol.:23, Issue:2


# Valorization of Neglected Bioactive Substances Through a Comparative Study of Their Phytochemical Compositions and Antimicrobial Properties with Those of *Garcinia kola*

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

**Amoussatou Sakirigui[1,2,5]*, Kabirou Chabi Sika[3], Allali Eugène Koffi[4], Fidèle Assogba[1], Franck Yovo[2], Eléonore Yayi Ladékan[1], Joachim Djimon Gbénou[1], Georges Coffi Accrombessi[5]**

*[1] Laboratory of Pharmacognosy and Essential Oils, Faculty of Sciences and Techniques/University of Abomey-Calavi (UAC) Cotonou, Republic of Benin.*
*[2] Kaba Research Laboratory in Chemistry and Applications (LaKReCA), University of Technical Sciences, Engineering and Mathematics (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.*
*[4] College of Agroforestry, Agrovalorisation Laboratory, Department of Biochemistry and Microbiology, Jean Lorougnon GUEDE University, Côte d'Ivoire. [5]Laboratory of physics organic chemistry and synthesis (LaCOPS), University of Abomey-Calavi (UAC), Faculty of Sciences and Technologies (FAST) / Department of Chemistry. Republic of Benin.*

**Submitted:** 24 December 2021
**Accepted:** 31 December 2021
**Published:** 30 January 2022

HUMAN JOURNALS
www.ijppr.humanjournals.com

**Keywords:** Avocado and mango seeds, *Garcinia kola* seed, ethanolic extracts, phytochemical composition, antimicrobial study

**ABSTRACT**

Avocado and mango seeds make up 20-60% of the total weight of each fruit. Unfortunately, these substances are very little exploited because of their ignorance by the population. It is in the optics of valuing these seeds that a comparative study between these seeds and the *Garcinia kola* seed was undertaken. These studies were carried out on the ethanolic extracts of each of the seeds. Determination and comparison of the phytochemical composition of the extracts from the three seeds revealed that the extracts from the two neglected seeds had some additional compounds than that of cola seed and quantitatively, their values were higher than those of kola seeds. The antimicrobial study revealed higher inhibition diameters with cola seed with a maximum value of 35 mm against *Proteus vulgaris*. But the determination of the minimum inhibitory concentrations showed a more pronounced activity of the mango seed followed by the avocado seed with a minimum value of 0.3125 mg/ml against several germs. *Garcinia kola* seed has been shown to have a bactericidal effect on over 90% of the bacteria used. The bactericidal effect in the cases of the other two seeds was noted in 20% of the bacteria. The richness in phytochemicals compounds, inhibitory and bactericidal activity noted in mango and avocado seeds were sometimes more pronounced than those noted in *Garcinia kola* seeds. These two seeds, available and often considered as waste, therefore neglected, could be the source of active ingredients entering into the formulation of antimicrobial phytodrugs.

## INTRODUCTION:

The whole world pays a heavy price for infectious diseases. The case of poor countries is even more alarming. Infectious diseases remain major cause of ill health among poor people. Almost 3 billion people live on less than US $ 2 a day, and they continue to be at the greatest risk for these diseases.[1] The search for less expensive, accessible and non-toxic remedies is important in developing countries and even around the world. In this context, natural bioactive substances are the most requested.[2] In other substances, the many virtues of *Garcinia kola* are known in the literature.[3] It remains a very coveted seed by the population because of its many known biological properties. These include its anti-inflammatory, antioxidant, antitussive, antidiabetic, antimicrobial activities etc.[4,5,6,7] These seeds are also known for strengthening the immune system.[3] The literature also refers to the numerous antibacterial properties of this substance.[8,9,10,11] This renewed interest for these seeds is driving up its price on the Beninese market. In the present work, we had focused our study on two neglected substances and very little known by the population for their different interesting biological properties. These are the *Persea Americana* L. and *Mangifera indica* L. seeds. The many properties of avocado and mango seeds are described in the literature.[12-15]Its refers their antioxidant, antimicrobial, anticancer, anti-inflammatory properties.[16-21] But the two seeds still remain unexploited in the Republic of Benin and sometimes even pose a very crucial seasonal environmental problem. To enhance these two seeds, a comparative study of the chemical composition, as well as the determination of the antimicrobial properties of the three seeds (*garcinia kola*, avocado and mango) will be carried out simultaneously. This study is all the more important given that we are always surrounded by microbial germs and valuing these seeds in this fight would be a great alternative.

## 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.[22] In addition, *Garcinia kola* seeds are consumed in the Republic of Benin by maceration in this solvent. That is what motivated us to take an interest in the ethanolic extract of the three seeds.

Mango and avocado fruits were deseeded by removing the fleshy cover. The resultant seeds and peeled *garcinia kola* seed were each washed 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 seeds were separately grounded into fine powder using a mechanical grinder. Cold ethanol extraction was adopted for extraction.

50 g of powder of *P. americana*, *M. indica and G. kola* seeds were separately crushed and recovered in 500 ml of ethanol 96°C. After agitation and homogenization, the mixture is filtered on Whatman 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 extracts of each seed 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*, *M. indica and G. kola* seeds according to Houghton and Raman method, (1998) and Simo et al., (2019).[23;24] Quantitative phytochemical tests were carried out according to the method of Harborn, (1973) and Umeaku et al., (2018).[25,26]

**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).[27]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.[28] For each extract, the experiment was performed in duplicate.

**Determination of the Minimum Inhibitory Concentration (MIC)**

The MIC has been determined by the microdilution method with Visual assessment of the growth of microorganisms.[29] 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.[30]

**Data treatment and analysis:**

The spreadsheet Microsoft Excel version 2013 has been used for the capture and encoding the data.

**RESULTS AND DISCUSSION**:

**Performance extraction**

From 50 g of *Garcinia kola* seeds powder, 4.42 g of ethanolic extract were obtained. This extract was therefore obtained with a yield of 8.84%. From 50 g of avocado seeds powder, 5.56 g of ethanolic extract were obtained. This extract was obtained with a yield of 11.12%. That of the mango seeds was obtained with a yield of 10.33%. The extraction solvent being the same, it appears that the seeds avocado have a higher extraction yield than that of those of mango. The lowest extraction yield was obtained with *Garcinia kola* seeds. According to this study, it turned out that the extract from the most coveted and popular seed was obtained with the lowest extraction yield.

**Qualitative and quantitative composition of extracts**

The phytochemical screening of the extracts had qualitatively shown the presence of Reducing compound, Alkaloids, Flavonoids, phenolic compounds consisting of catechic and gallic tannins in the three seeds (table-1). Saponin and Terpenoids are present in avocado and

mango extracts and absent in *G. kola*seed extract. Quinonics compound are present in the extract of *G.kola* and absent in extract of mango and avocado seeds (Table-1). On the one hand, Free Anthracenics and Leuco-anthocyanins were present in the avocado seeds and absent in the mango seeds (table-1). On the other hand, Mucilages and O-heterosides are present in the extract of mango seeds and absent in the extract of avocado and kola seeds. Other compounds such as Anthocyanins, Quinonics compounds, Cartenoids, are not present in the three extracts (table-1). Similar qualitative compositions have been noted in Kaur's work on mango seeds in 2010.[31] A comparison of the three extracts shown that extracts from mango and avocado seeds are richer in phytochemical compounds than extract from *G. kola*seeds. There were nine families of compounds in the extract of mango and avocado seeds against six families of compounds in the extract of kola seeds. The neglected ethanolic extract seedsare therefore very rich in phytochemical compounds during this work.

This 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, ours screening was carried out on the latter instead of the powder.

The quantitative analysis revealed that mango seeds were mostly rich in alkoids (9.66 ± 0.22 mg) and flavonoids (6.86 ± 0.20 mg). Then there was a not-insignificant value of phenolic compounds (3.22 ± 0.035). Reducing compounds were present in very small quantities in this extract (0.84 ± 0.027) (table 1). The quantitative analysis of avocado seeds indicate the strong presence of phenolic compound (7.68 ± 0.027 mg). The amount of flavonoids (5.33 ± 0.064 mg) in the extract is also interesting. Alkoids: 2.14 ± 0.012 mg, reducing compounds (1.72 ± 0.019 mg) remain the lowest amounts noted in this extract (table-1). Surprisingly, the lowest values of phytochemical compounds are noted in the extract of the seed of *G. kola*. These values remain slightly higher for phenolic compounds (3.96 ± 0.01) and for reducing compounds (1.65 ± 0.03) (table-1). In summary, after the quantitative analysis, the values noted for mango and avocado seeds extract remained the highest. We always insisted that these quantities may vary depending on the nature of the extraction performed. This is justified since other authors have reported varying quantitative values depending on the nature of their extracts.[32] The direct study on the powder could also lead to other values. Even the climate, the nature of the soil, and the season are all parameters that could influence the chemical composition of a plant.[33] But these results are promising for a possible biological study on the two very highly neglected seeds in the Republic of Benin.

**Table-1**: **Phytochemical constituents of *P. Americana, M. indica,* and *G. kola* seeds (mg/100g)**

| Compounds | AS/QLA | AS/QNA | MS/QLA | MS/QNA | GKS/QLA | **GKS**/QNA |
|---|---|---|---|---|---|---|
| Reducing compound | + | 1.72 ± 0.019 | + | 0.84 ± 0.027 | + | 1.65 ± 0.03 |
| Alkaloids | + | 2.14 ± 0.012 | + | 9.66 ± 0.22 | + | 2.11 ± 0.02 |
| Flavonoids | + | 5.33 ± 0.064 | + | 6.86 ± 0.20 | + | 2.16 ± 0.04 |
| Tanins catechic | + | 7.68 ± 0.027 | + | 3.22 ± 0.035 | + | 3.96 ± 0.01 |
| Tanins gallic | + | | + | | + | |
| Anthocyanins | - | | - | | - | |
| Leuco-anthocyanins | + | nd | - | | - | |
| Quinonics compound | - | | - | | + | nd |
| Saponin | + | nd | + | nd | - | |
| Coumarin | - | | - | | - | |
| Terpenoids | + | nd | + | nd | - | |
| Mucilages | - | | + | nd | - | |
| Cartenoids | - | | - | | - | |
| Free Anthracenics | + | nd | - | | - | |
| O-heterosides | - | | + | nd | - | |

(+) = 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. **GKS/QLA:** *Garcinia kola* seeds/Qualitative analysis; **GKS/QNA:** *Garcinia kola* seeds/Quantitative analysis; **nd**: not determined.

**The extracts inhibitory diameter zone with the reference strains**

Figure 1 shows the inhibitory activity of avocado, mango and kola 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 three extracts tested showed a pronounced antagonistic effect by inhibiting the growth of the pathogenic strains tested (Figure-1). *Garcinia kola* indicated an effect on 100% of bacteria while extracts from avocado and mango seeds indicated an inhibitory effect on 90% of bacteria (Figure-1). The largest inhibition diameter (35 mm) was obtained against *P.vulgaris* strain with *G. kola* extract. In all cases, the inhibition diameter of *G. kola* remained the highest with the

exception of *Staphylococcus aureus* ATCC 29213 where a larger inhibition diameter is noted with the extract of avocado seed (Figure-1). The second most active extract is from the mango seed with the exception on a few cases. The smallest inhibition diameters are obtained with avocado seed extract. But this extract remains more active than that of the mango seed in certain cases (*Staphylococcus aureus* ATCC 29213, *Proteus vulgaris* A25015, *Escherichia coli* ATCC 25922). We deduced that the inhibitory activities determined by the inhibition diameters of the extracts of avocado and mango seeds were similar. This activity was similar to that of the seed of *G. kola*. Moreover, the antimicrobial activities of avocado and mango seeds are widely described in the literature.[34] Those of *G. kola*are not only described in the literature but also known by population.[35,36] We retain from this section that the seeds of mango and avocado could present a significant biological interest approaching that of the seed of *G. kola*.

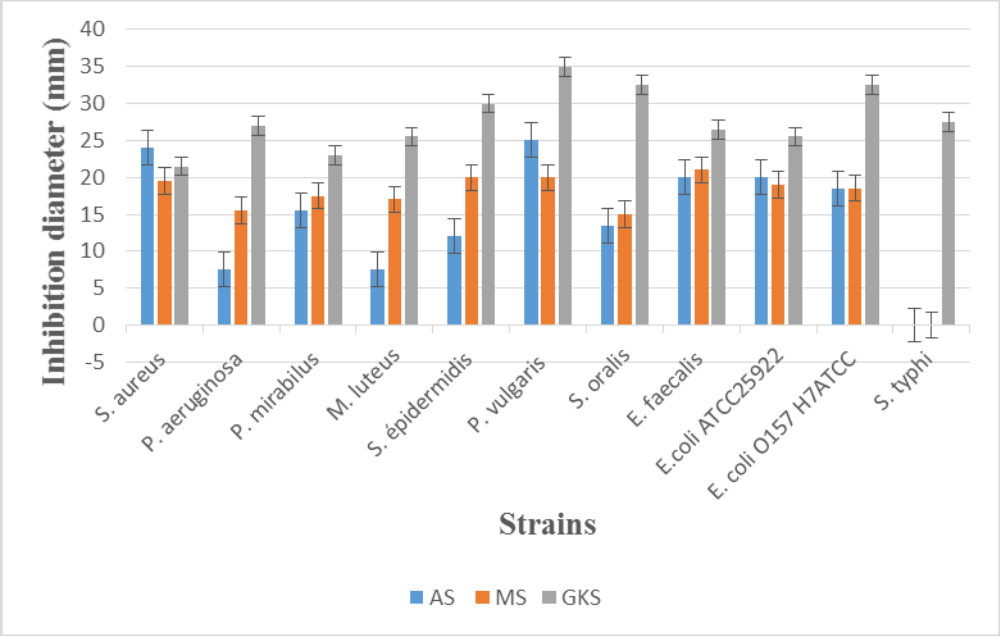


**Figure-1: Diameters of inhibition of *P. Americana, M. indica,* and G. kola seeds extracts**

**Minimum Inhibitory Concentrations (MIC) of *P. Americana, M. indica* and *G. kola* seeds extracts**

The three extracts inhibited the proliferation of most pathogenic bacteria with variable minimum inhibitory concentrations (MIC). According to our results, the smallest (0.3125 mg/ml) was obtained with two extracts (avocado and mango seeds) (table-2). With mango's seeds extract, it was obtained against two bacteria (*Staphylococcus epidermidis* T22695, and *Proteus vulgaris* A25015). The smallest values in the case of avocado seeds were obtained

against three bacteria (*Streptococcus oralis*, *Escherichia coli* ATCC25922 and *Micrococcus luteus)*. The smallest value obtained with *G. kola* seed is 0.625 mg/ml against *Staphylococcus aureus*only. From table 2, the smallest values were noted on more bacteria with the avocado seed. The same values were obtained with mango seed on fewer strains. Kola seeds remained those with the weakest inhibitory powers. The inhibitory activity determined during this work is related to ethanolic extracts. Other extracts of the same seeds made with other solvents could influence the results obtained during our work. The antibacterial activities of the three extracts have been proven by the literature [34;35], but this work remains a great advance insofar as the work showed that the activities of mango and avocado seeds could exceed that of cola seed very well-known and appreciated by the population. Above, extracts from avocado and mango seeds were found to be richer in phytochemicals than kola seed extract. The more pronounced inhibitory activities noted at the level of these two seeds would be due to the presence of the different phytochemical compounds in the different extracts. Noted that mango's seed represents 20 to 60% of the total weight of the fruit.[37] The valorization of these seeds in the implementation of antibacterial products could constitute a great asset, as well in the field of phytotherapy as in the economic field.

**Table 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 |
| GKS | 0.625 | 2.5 | 2.5 | 1.25 | 1.25 | 1.25 | 1.25 | 2.5 | 2.5 | 1.25 | 5 |

**MS**: Mango seeds; **AS**: Avocado seeds; **GKS**: Garcinia kola seeds.

**Minimum Bactericidal Concentration (MBC) (mg/ml) of *P. Americana, M. indica* and *G. kola* seeds extracts**

The three extracts tested had a weaker bactericidal effect against the bacteria used. From Table 3, *garcinia kola* seeds remained the most active. They killed over 90% of germs with different concentrations. The smallest bactericidal concentration recorded was 1.25 mg/ml against three bacteria, namely: *P. mirabilus* A24974, *M. luteus*, *P. vulgaris* A25015 (table-3). Of the 11 atrains, the mango and avocado seeds each had a bactericidal effect on two bacteria (*S. epidermidis* T22695, *E. faecalis* ATCC29212). But the mango seed remained more active than the avocado seed. This activity is noted on the two germs (*S. epidermidis* T22695, *E. faecalis* ATCC29212) and remained stronger than that of the kola seed. Avocado seed was less active on *S. epidermidis* T22695 and more active on *E. faecalis* ATCC29212 compared to the activity of kola seed. Despite the bactericidal effect on fewer strains in mango and avocado seeds, the bactericidal concentrations of these two seeds remained lower. In places, they indicated more pronounced bactericidal effects than those of kola seeds.

**Table-3: Minimum Bactericidal 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 | 0 | 0 | 0 | 0 | 1.25 | 0 | 0 | 1.25 | 0 | 0 | 0 |
| AS | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 1.25 | 0 | 0 | 0 |
| GKS | 2.5 | 2.5 | 1.25 | 1.25 | 2.5 | 1.25 | 2.5 | - | 10 | 10 | - |

**MS**: Mango seeds; **AS**: Avocado seeds; **GKS**: Garcinia kola seeds

**CONCLUSION:**

At the end of this work devoted to the comparative study of the phytochemical composition and the antibacterial activity of mango, avocado, and cola seeds, it appears that the extracts of mango and avocado seed are slightly richer in phytochemicals than kola seed. In places, antimicrobial tests showed the pronounced activity of the three seeds. The very little

exploitation of avocado and mango seeds, not known for their biological interests by the Beninese population, could constitute a very good alternative in the fight against microbial and that at little cost.

**Acknowledgments**

I thank Doctor Kabirou Chabi Sika of the Laboratory of Biology and Molecular Typing in Microbiology, for his frank collaboration. I wish also to express my deep sense of gratitude to my Mentors, Professors Joachim Djimon Gbénou, Georges Coffi Accrombessi, and Mansourou Moudachirou. I am greatly obliged to them for inspiring me through their precious suggestions and scholarly guidance.

**Disclosure of conflict of interest**

The authors declare that they have no competing interests.

**REFERENCES:**

1. World Health Organization. Global Report for Research on Infectious Diseases of Poverty. World Health Organization on behalf of the Special Programme for Research and Training in Tropical Diseases 2012.
2. Bassene E, Laurance A, Olschwang D, Pousset JL. Plantes Médicinales Africaines J Chromatogr A.1985:346: 428–430.
3. Ekene EN, Erhirhie EO.*Garcinia kola*: a review of its ethnomedicinal, chemical and pharmacological properties. Int J Cur Res Rev. 2014: 06 (11): 1-7.
4. Oloyede OI, Afolabi M. Antioxidant potential of *Garcinia kola* (Leaf). Acad Res Int.2012: 2 (2): 49–54
5. Buba CI, Okhale SE, Muazzam I. *Garcinia kola*: The phytochemistry, pharmacology and therapeutic applications, Int J Pharmacogn.2016: 3 (2): 67–81.
6. Omwirhiren EM, James SA, Abass AO. The phytochemical properties and antimicrobial potentials of aqueous and methanolic seed extract of Cola nitida (Vent) and Cola acuminata (Beauvoir) grown in South West, Nigeria. Saudi J. Med. Pharm. Sci. 2016: 2(12): 354-363.
7. Adaramoye OA, Adeyemi EO. Hypoglycaemic and pypolipidaemic effects of fractions from kolaviron, a biflavonoid complex from *Garcinia kola* in Streptozotocin-induced diabetes mellitus rats. J Pharm Pharmacol.2006: 58 (1): 121–128.
8. Akerele JO, Maureen OO, Ebomoyi I, Oboh IE, Uwumarongie OH. Antimicrobial activity of the ethanol extract and fractions of the seeds of *Garcinia kola*Heckel (Guttiferae), African Journal of Biotechnology. 2008 7 (2): 169-172.
9. Idu M, Erhabor JO, Towuru GE. Antimicrobial effects of the chloroform and ethanolic leaf extracts of Dacryodes Edulis (G. Don) H.J. Lam, *Garcinia Kola*Heckel and Chrysophyllum Albidum G. Don on some test isolates. Medical Science. 2013: 1(3): 63-66.
10. Amalu PC, Chukwuezi FO, Ugwu OPC. Antimicrobial Effects of Bitter Kola (*Garcinia Kola*) Nut on *Staphylococcus Aureus, Eschererichia Coli and Candida Alibicans*. IOSR-JDMS). 2014: 13(4) : 29-32.
11. Okonkwo EC, Nworie O, Nwuzo AC, Onwa AC, Udu-Ibiam EO, Oke B, Nwosu UO, MercyAN. Antimicrobial Effects of Bitter Kola (*Garcinia kola*) Nut on*Staphyloccous aureus, Escherichia coli*and *Candida albicans*. World Journal of Medical Sciences. 2017 : 14 (3): 89-96.
12. Jimenez P, Garcia P, Quitral V, Vasquez K, Parra-Ruiz C, Reyes-Farias M, Garcia-Diaz DF, Robert P, Encina C, Soto-Covasich J. Pulp, Leaf, Peel and Seed of Avocado Fruit: A Review of Bioactive Compounds and Healthy Benefits. Food Reviews International. 2021: 37(6): 1-38

13. BahruTB, TadeleZ, AjebeEG, A Review on Avocado Seed: Functionality, Composition, Antioxidant and Antimicrobial Properties. Chemical Science International Journal. 2019: 27(2): 1-10.
14. QuintanaSE, SalasS, García-ZapateiroLA. Bioactive compounds of mango (*Mangifera indica*): a review of extraction technologies and chemical constituents, Journal of the Sciences of food and agriculture. 2021:101(15), 6186-6192.
15. Ganeriwala B, Gudaganatti KS, Bolmal UB.*Mangifera indica*(amra) and its seed kernel - an useful drug. Int. J. Ayur. Pharma Research, 2016: 4(11):78-83.
16. Hürku MM, Sarıaltın SY, Köroğlu A, Çoban, T. In vitro inhibitory potential of avocado fruits, Persea americana (Lauraceae) against oxidation, inflammation and key enzymes linked to skin diseases. Revista de Biología Tropical. 2021. 69(2), 472-481.
17. García-Vargas MC, Contreras MdM Castro E. Therapeutic Bio-Compounds from Avocado Residual Biomass. Proceedings. 2021: 79(4): 1-6.
18. Melgara B, Dias MI, Ciric A, Sokovic M, Garcia-Castello EM, Rodriguez-Lopezd AD, Barros L, Ferreir ICRF. Bioactive characterization of Persea americana Mill. by-products: A rich source of inherent antioxidants. Industrial Crops & Products. 2018:111: 212–218.
19. El-Gied AAA, Joseph MRP, Mahmoud IM, Abdelkareem AM, Al Hakami AM, Mohamed E. Hamid2*Antimicrobial Activities of Seed Extracts of Mango (Mangifera indica L.) Advances in Microbiology. 2012: 2: 571-576.
20. Raju NV, Sukumar K, Reddy GB, Pankaj PK, Muralitharan G, Annapareddy S, Sai DT, Chintagunta A. D. In-Vitro Studies on Antitumour and Antimicrobial Activities of Methanolic Kernel Extract of Mangifera Indica L. Cultivar Banganapalli. Biomed Pharmacol J. 2019;12(1): 357-362.
21. Choudhary P, Tushir S, Bala M, Sharma UR, Evaluation of antioxidant and antimicrobial potential of mango seed kernel, International Journal of Chemical Studies. 2020: 8(6): 2921-2924.
22. Umar M, Mohammed IB, Oko JO, Tafinta IY, Alika AA and Jobbi DY. Phytochemical Analysis and Antimicrobial Effect of Lemon Grass (Cymbopogon citratus) Obtained From Zaria, Kaduna State, Nigeria. Journal of Complementary and Alternative Medical Research. 2016: 1(2):1-8.
23. Houghton DJP, Raman A. Laboratory handbook for the fractionation of natural. Extracts Science. 1998; 199p.
24. 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.
25. Harbon JB phytochemical methods. London chapman and Hall Ltd., 1973: l88.
26. 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.
27. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. AJCP. 1996: 45: 493-496.
28. Adesokan AA, Akanji MA, Yakubu MT. Antibacterial potentials of aqueous extract of Enantiachlorantha stem bark. Afr. J. Biotechnol.2007: 6(22): 2502-2505.
29. Delarras C. Microbiology. 90 hours of practical work. GaétanMorien Publisher. 1998;169-178. ISBN: 291074907 X, 9782910749071
30. 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.
31. Kaur J, Rathinam X, Kasi M, MiewLeng K, Ayyalu R, Kathiresan S, Subramaniam S. Preliminary investigation on the antibacterial activity of mango (Mangiferaindica L: Anacardiaceae) seed kernel. Asian Pacific Journal of Tropical Medicine. 2010: 707-710.
32. Rakholiya K, Kaneria M, Chanda S,Physicochemical and Phytochemical Analysis of Different Parts of Indian Kesar Mango–A unique variety from Saurashtra Region of Gujarat. Pharmacogn. J. 2016: 8(5):502-506.
33. 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 (Pinus sylvestris L.) and

European beech (Fagus sylvatica L.) stands in northeastern Bavaria, Germany. Biogeochemistry. 2001:55: 103–143.
34. Sakirigui A, YayiLadekan E, Fagbohoun L, Chabi Sika K, Assogba F and Gbenou JD, Phytochemical composition and potential antimicrobial activity of extracts of two neglected seeds (MangiferaindicaL. and Persea Americana L.) in Benin. IOSR Journal of Applied Chemistry. 2020: 13(5): 21-26.
35. Sakirigui A, Nonviho G, Hounkpatin ASY, Chabi Sika K. Effects of teguments on phytochemistry and antimicrobial activities of Garcinia kola seeds, Journal of Pharmacognosy and Phytochemistry. 2020: 9(5): 21-26,
36. Dah-Nouvlessounon D, Baba-Moussa DF, Adjanohoun A, Sina, Noumavo HPA, Adoukonou-Sagbadja H, Diarrassouba N, N'tcha C, Anago FS, Baba-Moussa L. Phytochemical screening and biological activities of Garcinia kola (bark, leaves and seeds) collected in Benin African Journal of Microbiology Research. 2015: 9(28): 1716-1727a.
37. Ashoush IS, Gadallah MGE. Utilization of mango peels and seed kernels powders as Sources of phytochemicals in Biscuit. World Journal of Dairy & Food Science 2011;6:35-42.