Estimation of Phytochemical Compounds and Antimicrobial Studies of Different Solvent Extracts of *Garcinia kola* against Some Pathogens

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Abstract: This study was aimed to evaluate the *in vitro* antimicrobial activities of different solvent extracts of seeds of *Garcinia kola* (Clusiacea) on some pathogenic bacteria and fungi. The seed was extracted using n-hexane, ethyl acetate, honey, and vinegar as solvents. The *in vitro* antimicrobial activities of the extracts on *Candida albicans, Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923) were assayed using the disc diffusion method. The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Minimum Fungicidal Concentration (MFC) were evaluated using standard microbiological techniques. Phytochemical components of all the solvent extracts were determined and High-Performance Liquid Chromatography (HPLC) of the organic solvent extracts were further analysed. All the extracts exhibited varying degrees of concentration-based antimicrobial activities against the tested pathogens. The plant exhibited varying degrees of MIC/MFC with values ranging from 100 mg/mL (for n-Hexane extract against *E. coli* ATCC 25922) for organic solvents, followed 50 µg/mL obtained for honey extract (against *E. coli* ATCC 25922) and the least MIC value (12.5 µg/mL) was obtained for ethyl acetate (against *S. aureus* ATCC 25923 and *C. albicans*, respectively), the combination of solvent extract (against *C. albicans*) and vinegar (against *E. coli* ATCC 25922and *C. albicans*, respectively). The maximum MBC value was obtained for n-Hexane extract (100 µg/mL) and Honey extract (50 µg/mL) against *E. coli* ATCC 25922, while the least MBC value (25 µg/mL) was obtained for the combination of solvent extracts against *S. aureus* ATCC 25923 and C. *albicans*, respectively. Maximum MFC (50 µg/mL) was obtained for n-Hexane, vinegar, and the combination of solvent extracts and the least was n-Hexane. Saponin, tannin, phenolic, flavonoid, coumarin, anthocyanin, steroid, glycosides, triterpenes, phlobatannins amino acids, and alkaloids were randomly present in all the solvent extracts. The HPLC detector measured 13 compounds in the n-hexane extracts, 3 of which were identified at different concentrations Luteolin (96.94 mg/mL), Apigenin (4.43 mg/mL) and quercetin (4.06 mg/mL). A total of 14 compounds were detected in the ethyl acetate extracts, 6 of which were identified as Gallic acid (25.65 mg/mL), theobromine (17.31), caffeic acid (17.02), catechin (16.09), quercetin (8.99), and epicatechin (11.08 mg/mL). This study provides information on the antimicrobial activities and components of *G. kola* seeds. Also, it contributes to the development of alternative therapeutic agents against the pathogens tested in this study.

Key Words: Clusiacea, *G. kola*, Luteolin, Phlobatanin, Theobromine

INTRODUCTION

A superfluity of agents with proven antimicrobial properties such as methicillin, commonly used to treat diseases associated with a wide range of microorganisms like *S. aureus*, are becoming increasingly inactive (Marbach *et al*., 2017; Sullivan *et al*., 2017). Thus, alternative approaches towards mitigating the resistance of infectious agents, and with minimal to no side effects would play a significant role in the health care system of developing countries.

Since antiquity, a surfeit of medicinal plants serves as natural chemotherapeutic treasures against various ailments and diseases (Dhama *et al.*, 2013; Kumar *et al.*, 2013). To date, drug discovery research has since ancient times been the source of drug development from medicinal plants (Butler, 2004). The therapeutic benefits of medicinal plants outweigh that of their synthetic counterparts, this may be because they are readily available, more affordable, and have minimal to no side effects (Idu *et al*., 2007).

Garcinia kola, commonly known as bitter kola belongs to the family Clusiacea/Guttiferae (Adesuyi *et al*., 2012) and it is mostly grown in the South-Western and South-Eastern part of Nigeria where it is locally called *Orogbo* in Yoruba, *Aku ilu* in Igbo and *Namijin goro* in Hausa (Adegboye *et al*., 2008).

Extracts of various parts such as the seeds, roots, and barks (Nazıroğlu *et al.,* 2014) are used extensively in traditional African medicine (Xu *et al.*, 2013) due to their multifaceted health benefitsto treat various abdominal related ailments including diarrhea, respiratory conditions, and bronchitis (Jayaprakasha *et al.,* 2006; Adegboye *et al.,* 2008).The seeds have a bitter astringent and resinous taste and an after-sweet taste (Adesuyi *et al*., 2012).

Importantly, standardization, a crucial prerequisite towards ensuringthe quality of medicinal plants must be accomplished through processes such as phytochemical and pharmacognostic studies. Thus, studies on the constituents of plants are of utmost importance and the advantage in methodologies for separation technologies such as High-Performance Liquid Chromatography (HPLC) will subsequently expand the capacity for separation of plant chemical constituents (Pauli, 2006).

G. kola is important in some ceremonial celebrations in Nigeria including naming ceremonies and weddings. It is also a valuable raw material in the pharmaceutical industry (Mazi *et al*., 2013). Several authors have reported the antibacterial and antifungal activities including wound healing properties of Garcinia plants (Hemshekhar *et al.,* 2011; Adesuyi *et al.,* 2012; Ekene and Erhirhie, 2014). It has been reported to possess aphrodisiac properties and it is commonly used in the treatment of abdominal conditions (Odebunmi *et al.,* 2009).

Owing to the rising use of medicinal plants in developing countries, the preparation of extracts from medicinal plants using various solvents requires special attention. Also, the cultural disagreement on the safety and use of alcohol in herbal preparations requires the need for alternative solvents. Hence, there is a need to surpass this cultural disquiet by promoting the use of safer solvents in herbal preparations. Vinegar is the end product of the conversion of ethyl alcohol in raw materials like wine, malted barley, alcohol, fruits, and cider, to acetic acid by the microbe, Acetobacter (Ebner *et al*., 1995). Other resulting compounds of vinegar conversion are organic acids, flavonoids, polyphenols, vitamins, and minerals (del Campo *et al*., 2008). Apart from the nutritional support, antioxidant defense, and blood pressure-lowering potentials of vinegar, the content of the organic acids can also eliminate harmful intestinal bacteria (Nazıroğlu *et al.,* 2014).

Despite the well-known health benefits and healing potentials of organic acids (e.g. acetic acids, alginic acids, citric acids, etc.) and a series of scientific reports have proven antimicrobial activities of *G. kola* seeds using various organic solvents. Also, the synergistic potentials of vinegar with medicinal plants on microbes require more investigation. Hence, this study aimed to determine the effect of different solvents on the antimicrobial activities of *G. kola* seeds powder against some test pathogens.

MATERIALS AND METHODS

Test pathogens

Candida albicans, Escherichia coli ATCC 25922, and *Staphylococcus aureus* ATCC 25923 used in this study were obtained from the Microbiology Laboratory, Al-Hikmah University, Ilorin, Nigeria. The viability of the test pathogens was checked by incubating the bacterial strains on Nutrient agar for 24 hours at 37°C while the *C. albicans* strain was incubated on Sabouraud Dextrose agar for 48 hours at 28° C (Canli *et al.,* 2017). The organisms were further maintained on nutrient agar and SDA slants at 4°C.

Standardization of inocula

The inocula were further standardized after incubation by transferring each microbial strain into a sterile test tube containing 0.9% sterile saline solution, this was then adjusted to 0.5 McFarland standard to obtain an equivalence of 10^8 CFU/mL for bacteria and 10^7 CFU/mL for *C. albicans* (Ochei and Kolhatkar, 2008; Altuner *et al*., 2014).

Sample collection and preparation

The seeds of *G. kola* were purchased from herbs vendors in *Oja tuntun* (New market), Ilorin, Nigeria. The botanical identity of the sample was authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, where a specimen was deposited and voucher number ULH/002/2020/1217 was given for future reference. The seeds were shelled, shredded into tiny sizes, dried in the shade for two weeks, and pulverized using an electric miller (Master Chef Blender, Mode MC-BL 1980, China). Various extracts were prepared from the fine powder of the seeds by following the modified extraction method described by Nenaah and Ahmed (2011).

Preparation of extracts from the seeds of *G. kola*

Fifty grams(50g) of the powdered seed was separately extracted by maceration with 500 mL of n-hexane and ethyl acetate (1:10 w/v), respectively, for 48 hours with intermittent shaking at intervals. The resultant solution was filtered through Whatman Grade 1 filter paper, the filtrate was concentrated by evaporation using a rotary evaporator (Model RE Zhengzhou, Henan China) and subsequently dried in a water bath at 40^{o}C. The concentrated extract was weighed and stored in the refrigerator for further use. This extraction procedure was further repeated with honey and vinegar.

Preparation of Appropriate working Solutions

A quantity (1g) of the dried extract was transferred into a sterile tube and made up with 10 mL of 5% DSMO which was initially prepared by mixing 95 mL of distilled water with 5 mL of Dimethylsulphoxide. The stock solution was filtered through a 0.45 µm membrane filter before use, this represented 100 mg/mL concentration. A 2-fold serial dilution was further carried out from the stock solution into two other sterile tubes (labeled 50 mg/mL and 25mg/mLconcentrations).

Preparation of stock solutions and working concentrations of Apple cider vinegar and Honey extracts

To prepare 50% working concentrations of vinegar and honey samples, 50mL of honey and vinegar were individually mixed with sterile distilled water (1:1; v/v), respectively. A quantity of 20g sample of the honey extract was transferred into a sterile tube containing a 50 % honey solution, this represented a 200 mg/mL concentration of honey extract. Three working concentrations (100 mg/mL, 50 mg/mL and 25 mg/mL) were generated from the original (200 mg/mL) tubes using 2-fold serial dilution method. The same procedure was repeated for vinegar extract.

Sterility testing

Honey (w/v) and vinegar (v/v) solutionwere tested for sterility before use; this was achieved by plating the extracts on Mueller Hinton broth (MHA; Hi-Media) and Sabouraud Dextrose broth (SDB; Hi-Media) plates and incubated at 37°C for 24 h and 28°C for 48 h, respectively. Lack of cloudiness signified no microbial growth.

Antibacterial and Antifungal susceptibility testing of the crude extracts

Susceptibility testing of the sterile crude extract was done for all the microbial isolates by the adapted Kirby Bauer disc diffusion method (Parekh and Chanda, 2007). Following the Clinical and Laboratory Standards Institute (CLSI) guideline, 0.1 ml of the respective standardized inoculum of each test organism was uniformly streaked onto sterile Mueller Hinton Agar (Hi-Media) plates for the bacteria strains and Sabouraud Dextrose Agar (SDA) for the fungal strains. The plates were allowed to dry, subsequently, discs impregnated with 20 µL of the extracts were aseptically placed onto the inoculated agar surface and gently pressed with the applicator to ensure contact of the discs with the medium. The crude extracts were allowed to pre-diffuse from the discs into the agar medium for 1 hour on the bench before incubation at 37 °C for 24 hours (for the bacterial strains) and 48 hours at 28 °C (for

the fungal strain). A control was set up; involving the use of 50% vinegar, honey, a combination of honey and vinegar (1:1; v/v), and 5% DMSO against the test pathogens. Clear zones around the discs were measured (mm) and recorded as the zones of inhibition (Jorgensen *et al*., 1999).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration

The lowest concentration (Minimum Inhibitory Concentration) of the solvent extracts that will inhibit any visible growth of the bacterial and fungal strains in Mueller Hinton broth and Sabouraud dextrose broth, respectively, was determined by 2-fold dilutions method as previously described by Altuner *et al*. (2010), while the Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were subsequently carried out from the tube with MIC:

Tubes labeled 1 -7, representing 0.195 – 100 µg/mL were set up for the 2-fold tube dilution assay which was achieved by transferring 1 mL of the solvent extracts into 1 ml of Mueller Hinton broth, further transfers were made from tube 1 into the next and up to the seventh tube. Subsequently, 1 ml of the 24 h-culture of the test pathogen was inoculated into each test tube and vortexed for uniform mixing before incubation at 37°C for 24 h for bacterial strains and 28°C for 48 h for the fungal strain. This procedure was done in triplicates for all the solvent extracts and the test organisms. After incubation, the tube with no turbidity was taken, recorded as the MIC, and used for MBC by subsequent plating microbial suspension from such plates on MHA and SDA plates, for bacterial and fungal isolates, respectively. The plates were further incubated and checked after 24 hours and 48 hours for presence or absence of growth. MBC and MFC were taken as the lowest concentration of the extract that exhibited no microbial growth on the agar plates.The action of an antibacterial agent on bacterial strains can be characterized by two parameters namely MIC and MBC. According to the ratio, MBC/MIC, the antibacterial activitycan be apperceived. If the ratio MBC/MIC=1 or 2, the effect is bactericidal but if the ratio MBC/MIC=4 or 16, the effect is bacteriostatic.

Phytochemical screening

The preliminary phytochemical screening of the extracts was carried out for saponins, flavonoids, terpenoids, steroids, coumarins, alkaloids, tannins (Amadi *et al*., 2004).

HPLC assay

Preparation of sample for HPLC was achieved by macerating 1 g of the fine *G. kola* seeds powder with 10 ml HPLC grade n-hexane (1:1; v/v, 10.0 mL), this was centrifuged for 10 minutes at 3000 rpm and filtered through 0.45 µm membrane filter to get rid of free unextractable components before use. This process was repeated for ethyl acetate extracts. The filtrates of plant extracts were thus preserved at 4°C for further HPLC assay.

The n-hexane and ethyl acetate extracts were individually screened on a modular Shimadzu (Nexeramx) HPLC system comprising of an LC-10AD pump and equipped with a CTO-10A column oven, an SPD-10A UV-DAD detector, a CBM-10A interface, and an LC-10 Workstation. An LC-18 column (250 mm x 4.6 mm ID x 5 mm) from Ubondapak, Bellefonte (USA) was employed at a column temperature of 40 ^{o}C. Separations were done in the isocratic mode, using acetonitrile: water (40:60; v/v) at a flow rate of 1.0 mL/min with an injection volume (“loop”) of 10 µL, UV detector was set at 254 nm.

RESULTS

All the solvent extracts of *G. kola* displayed concentration-dependent zones of inhibitions against the test pathogens. The highest (16.00 mm) inhibition zonewas exhibited by the combined solvent extract (honey mixed with vinegar) against *C. albicans* at 100 mg/mL concentration, while the least (7.00 mm) inhibition zone was observed at 25 mg/mL by n-Hexane extract against *E. coli* ATCC 25922.

As for the control (5% DMSO), the highest (7.50 mm) inhibition zone was against *C. albicans* while the least (6.50 mm) inhibition zone was observed against *E. coli* ATCC 25922 (Figure 5).

The traditional solvent (combination of vinegar with honey) extracts of *G. kola* (Figure 4) gave the highest (20.00 mm) inhibition zone at 100 mg/mL concentration against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. The least inhibition (7.00 mm) zone was observed at 25 mg/mL by Honey extract against *E. coli* ATCC 25922.

As for the control agent, the highest inhibition zone was exhibited by the combination of Vinegar with Honey against *E. coli* ATCC 25922 (8.00 mm) while the least inhibition zone (6.0 mm) was observed by Honey extract against *C. albicans* (Figure 5).

The maximum MIC value (50 µg/mL) was obtained for n-Hexane extract against *E. coli* ATCC 25922, while the least MIC value (12.5 µg/mL) was obtained for ethyl acetate (against *C. albicans*) and combination of the solvent extract (against *S. aureus* ATCC 25923 and *C. albicans*, respectively. The maximum MIC value (50 µg/mL) was obtained for both honey extract (against *E. coli* ATCC 25922) and the combination of honey with vinegar extract (against *C. albicans*) while the least MIC value (12.5 µg/mL) was obtained for vinegar against *E. coli* ATCC 25922 and *C. albicans*, respectively.

The maximum MBC value (100 µg/mL) was obtained for n-Hexane extract against *E. coli* ATCC 25922, while the least MBC value (25 µg/mL) was obtained for ethyl acetate and the combination of solvent extractagainst *S. aureus* ATCC 25923 and *C. albicans*, respectively.

The Maximum MFC (50 µg/mL) was obtained for n-Hexane and the combination of solvent extract (Figure 1) while the least (25.00 mm) was by ethyl acetate extract.

The preliminary phytochemical analysis of the solvent extracts (Table 1) showed that all the traditional and organic extracts of *G. kola* seeds contained saponin, tannin, flavonoid, terpenoids, and alkaloids. The n-Hexane extract containedsaponin, tannin, phenolic, flavonoids, anthocyanin, steroid, glycosides, triterpenes, terpenoids, and alkaloids. The ethyl acetate extract contains saponin, tannin, phenolic, flavonoids, coumarin, anthocyanin, glycosides, triterpenes, terpenoids, phlobatannin, and alkaloids, while the honey extract contained saponin, tannin, phenolic, flavonoids, steroid, glycosides, terpenoids, and alkaloids. The vinegar extract contained saponin, tannin, flavonoids, coumarin, steroid, triterpenes, terpenoids, amino acids, and alkaloids.

The HPLC detector measured 13 compounds (Figure 6) in the n-hexane extracts of *G. kola* seeds powder, 3 of these compoundsrepresented by different peak heights were identified based on a comparison known as Luteolin (identified in the highest concentration, 96.94 mg/mL), Apigenin (4.43 mg/mL)and quercetin (4.06 mg/mL).

The HPLC detector measured 14 compounds in the ethyl acetate extracts of *G. kola* seeds powder (Figure 7), 6 of which were identified as Gallic acid (identified in the highest concentration, 25.65 mg/mL), theobromine (17.31), caffeic acid (17.02), catechin (16.09), quercetin (8.99), epicatechin (11.08 mg/mL)and quercetin (4.06 mg/mL).

DISCUSSION

Varying levels of bactericidal and fungicidal activities were observed in the current study and this was exhibited by the general appreciable range of zones of inhibition displayed by all the solvent extracts of *G. kola* seeds against the tested pathogens. Antimicrobial activities of the seeds of *G. kola* have earlier been reported by various researchers (Ezeifeka, 2004; Akinpelu *et al*., 2008). A study by Penduka and Okoh (2011) reported the inhibitory activities of n-Hexane and aqueous extracts of *G. kola* on vibrio isolates. Findings in the present study also share some similarity with the work of Adegboye *et al*. (2008) in which the crude

extract of *G. kola* exhibited *in vitro* antimicrobial activities against Gram-positive and Gram-negative bacteria. Activities of aqueous extracts of *G. kola* seed on Gram-negative bacteria had also been previously described by Ezeifeka *et al.* (2004), also, the reports of Penduka and Okoh (2011) corroborates the outcome of this study on the inhibitory effect of *G. kola* seed extracts on *C. albicans* which was again supported by the study of Badger-Emeka *et al.* (2018).

Generally, concentration of all the solvent extracts affected the zones of inhibition obtained. This observation is in concurrence with the previous report that the efficacy of most plant extracts was concentration-dependent (Agbaje *et al*., 2006; Akinnibosun *et al*., 2009). While higher zone of inhibition was obtained by the traditional than the organic solvent extracts, the zone of inhibition displayed by the organic solvent extracts of *G. kola* was higher than what was obtained by the control agents (when used individually). This may mean that the traditional solvents contained more constituents than the organic solvents. This is in disagreement with the report of Ijeh and Adedokun (2006) which stated that the concentration of the active components affects the activity of the substance.

It is noteworthy that the result obtained when the extractant was used singularly and in combination against the test pathogens signified their potential activities. Such synergistic activity was demonstrated with a better result (low MIC value) that was obtained with the combination of solvent extracts against the test pathogens. The low MIC is an indication of higher antimicrobial activity and this was consequently displayed by higher zones of inhibition recorded in this study. Previous reports also showed that a lower MIC value of 20 mg/mL showed greater growth inhibition (Akinnibosun and Itedjere, 2013).

The activities exhibited by the use of the traditional solvents individually (against the test pathogens) was not as high as what was exhibited by the solvent extracts of *G. kola* seeds powder. In this study, vinegar extract was recorded as the second most effective agent after the combination of solvents (vinegar and honey) against the test pathogens. The antimicrobial activity of rice vinegar against *E. coli* O157:H7 was previously reported by Chang and Fang (2007), while the antimicrobial effect of vinegar (acetic acid) was also reported by Bornemeier *et al*. (1997) against *S. aureus*. In addition, earlier publications reported that the antimicrobial potentials of honey may be attributed to its composition of H_2O_2 (Bang *et al*., 2003) and its acidity. In agreement with our study, Lu *et al*. (2014) reported the activity of honey against *S. aureus*, while Lusby *et a*l. (2005) also reported a broad-spectrum antimicrobial activity of honey against some pathogens including *C. albicans.*

The type of solvent used in extraction during this study did not affect the phytochemical components (saponins, tannin, flavonoid, terpenoids, alkaloids, phenolic, flavonoids, anthocyanin, steroid, glycosides, triterpenes, coumarin, triterpenes, phlobatanin and amino acids), which were detected haphazardly in all the solvent extracts. Similar phytochemical compounds of *G. kola* seeds extracts have been reported in other scientific literatures (Esimone *et al*., 2007; Adegboye *et al*., 2008; Adejumo *et al*., 2011). Thus, the presence of phytoconstituents detected in the study may be responsible for the demonstrated antimicrobial effects. Okwu and Okwu (2004) reported the therapeutic effect of saponin and alkaloids.

The chemical components (Luteolin, apigenin, quercetin gallic acid, theobromine, caffeic acid, catechin, quercetin, epicatechin, and quercetin) identified at varying quantities are reported to have multifaceted health benefits, including antimicrobial efficacy. Quercetin, the compound identified in this study as the least concentration in n-hexane extract of *G. kola* has been reported to confer series of biological activities including antimicrobial, and anti-inflammatory activities.

Other flavonoid compounds detected (Luteolin and apigenin), Gallic acid, catechin, epicatechin, chlorogenic acid, caffeic acid, and p-coumaric acid have been reported to possess antimicrobial properties (Dávalos *et al*., 2005; Budak *et al*., 2011). Hence, this study reveals that honey and vinegar extracts of *G. kola* seeds have antimicrobial and antifungal potentials and can serve as an antimicrobial source. Also, the components of organic solvent fractions of *G. kola* justify its antimicrobial activities.

CONCLUSION

Findings in this study revealed the antimicrobial activities of n- hexane, ethyl acetate, honey and vinegar extracts of *G. kola* against the test pathogens. Importantly too, the study surpasses the safety and cultural concerns that may arise with the use of alcohol in traditional herbal preparations as it supports the use of honey and vinegar extracts of *G. kola* as herbal preparations for the treatment of infections relating to the test pathogens.

RECOMMENDATIONS

From the findings in this study, it is recommended that future work should be done on the active components and *in vivo* antimicrobial efficacy, and toxicological studies of the honey and vinegar extracts of this plant should be explored.

Acknowledgments

The author wishes to acknowledge the support of Mr. Bolu Ajayi of the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

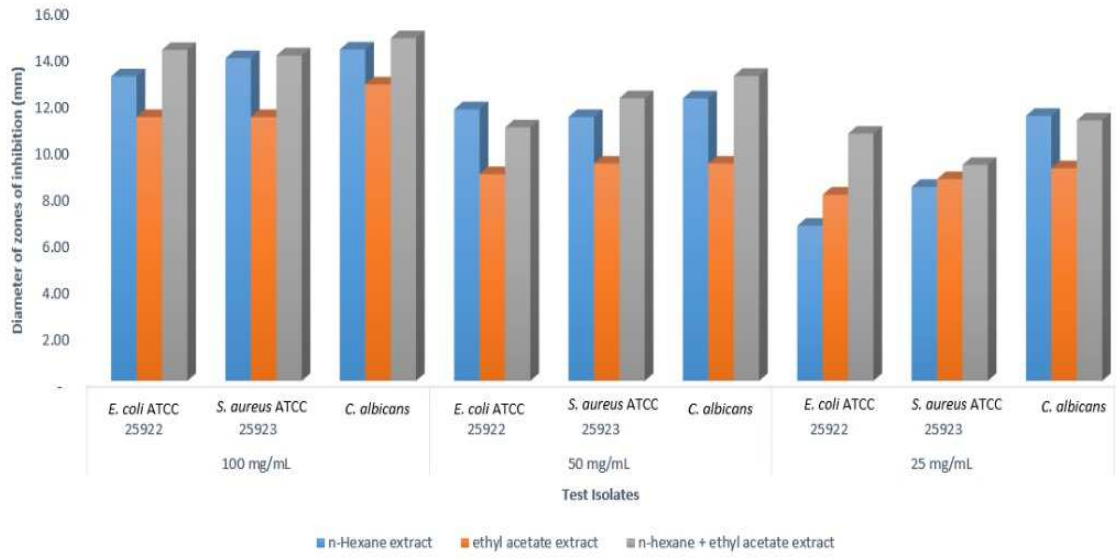


Figure 1: Mean values of diameter of zones of inhibition of the organic solvent extracts of *G. kola* on the test pathogens

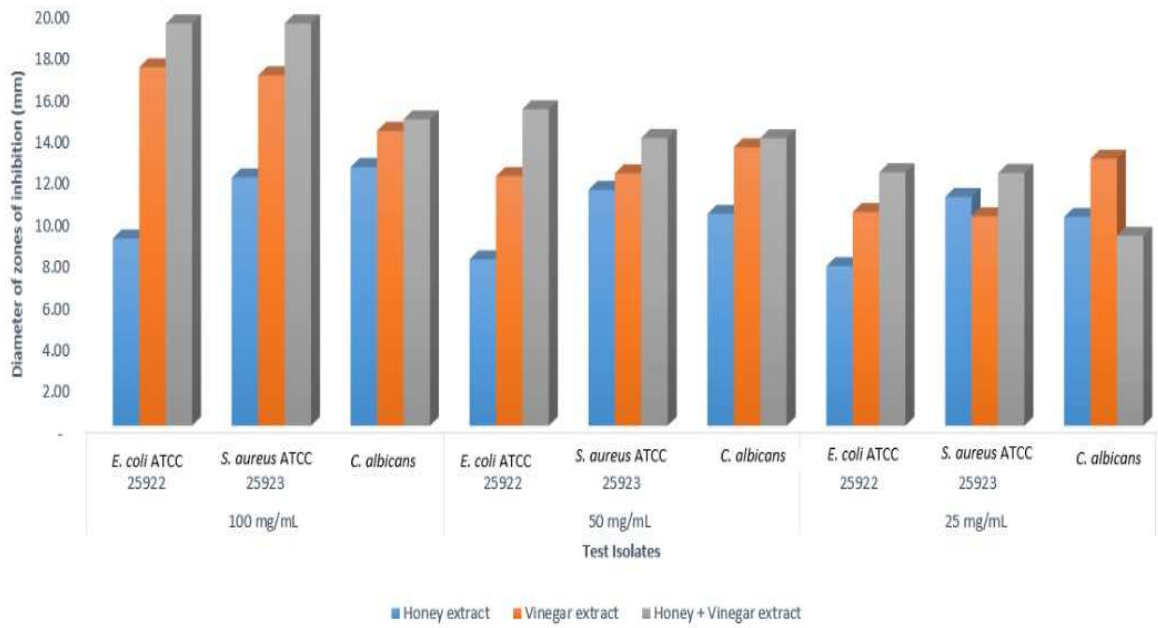


Figure 2: Mean values of diameter of zones of inhibition of the traditional solvent extracts of *G. kola* on the test pathogens

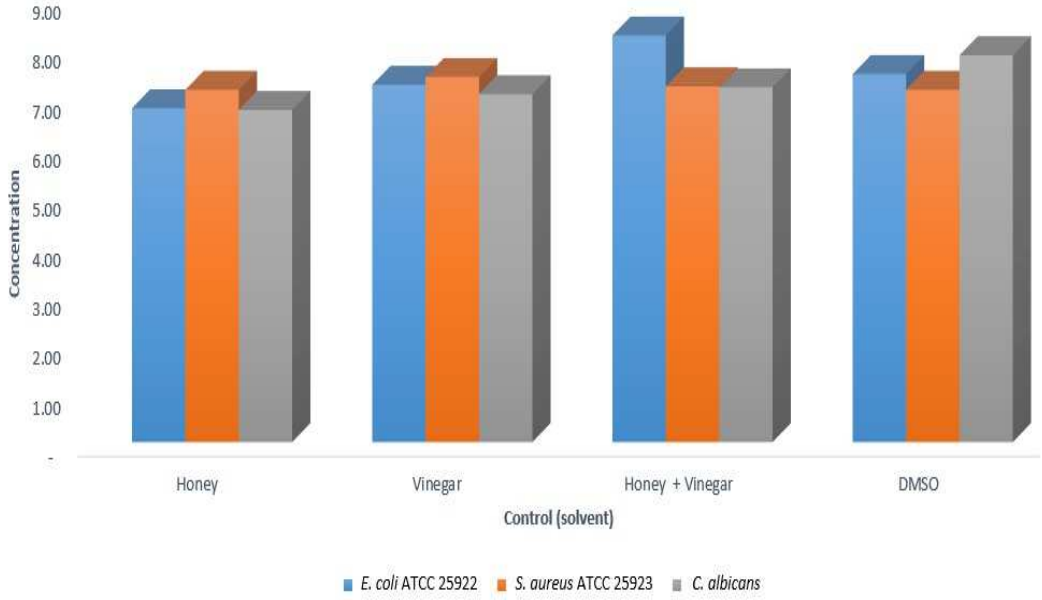


Figure 3: Mean values of diameter of zones of inhibition of the solvents (control) alone on the test pathogens

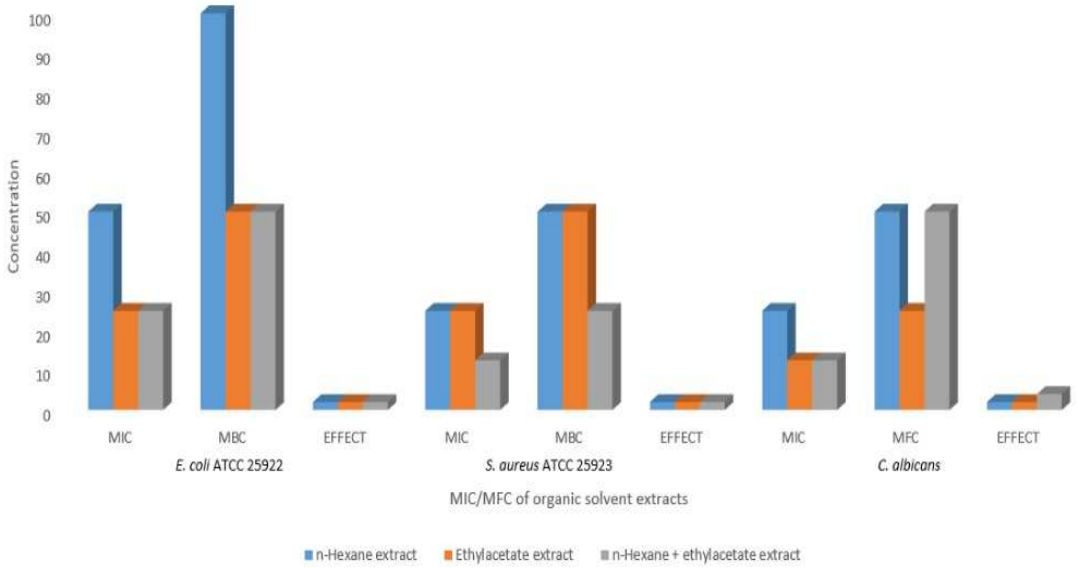


Figure 4: MIC, MBC and MFC values of organic solvent extracts of *G. kola* seeds

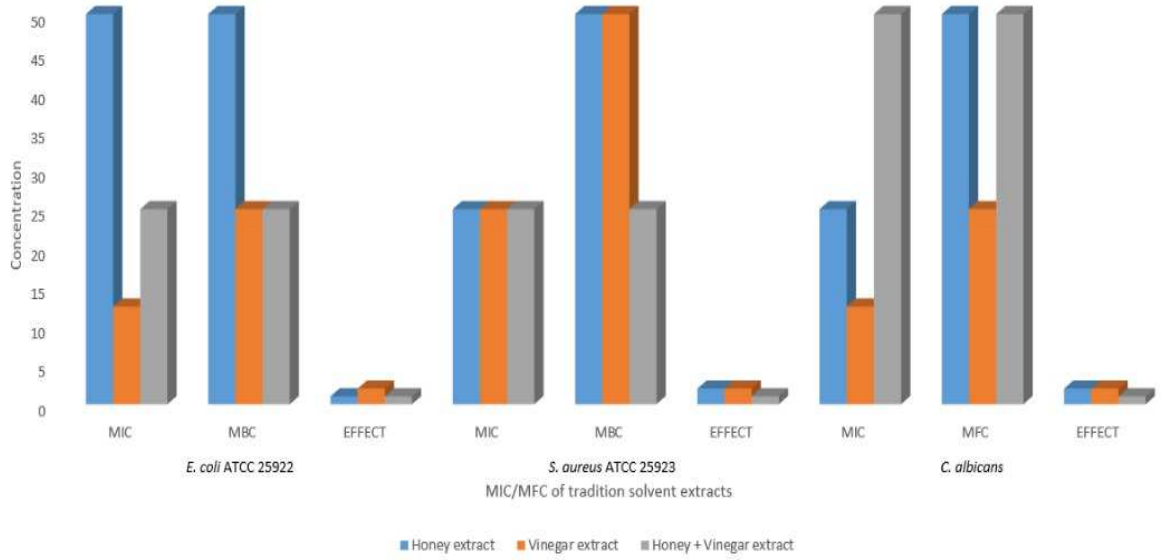
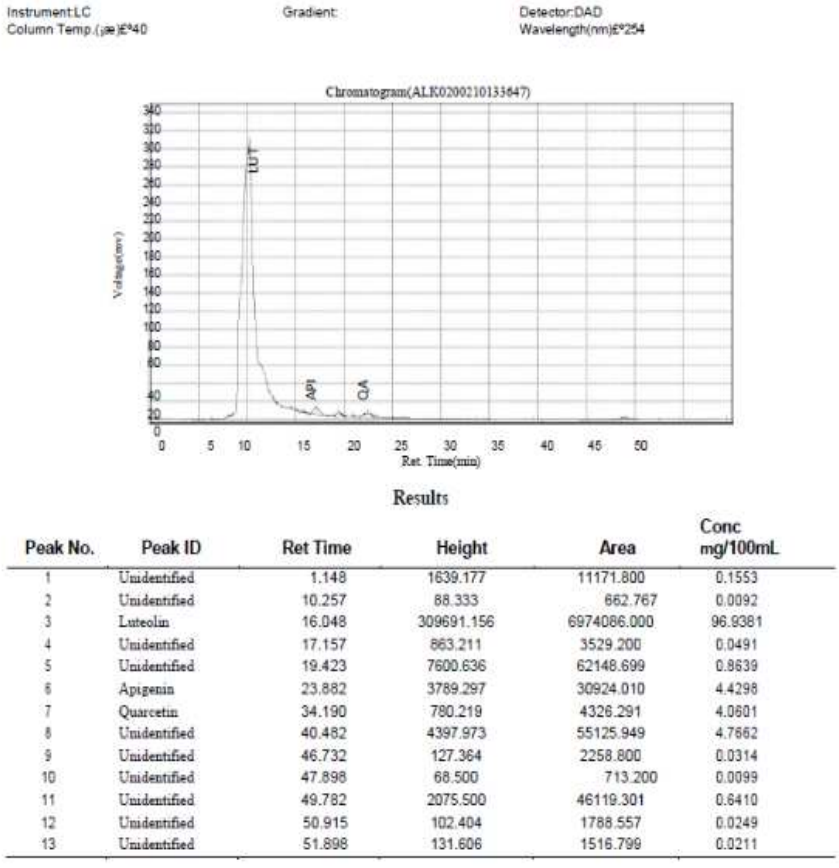


Figure 5: MIC, MBC and MFC values of traditional solvent extracts of *G. kola* seeds

Table 1: Phytochemical constituents of the organic and traditional solvent extracts of *G. kola* seeds

Parameters	Organic solvents		Traditional solvent	
	n-Hexane extract	Ethyl acetate extract	Honey extract	Vinegar extract
Saponin	+	+	+	+
Tannin	+	+	+	+
Phenolics	+	+	+	-
Flavonoids	+	+	+	+
Coumarin	-	+	-	+
Anthocyanin	+	+	-	-
Steroid	+	-	+	+
Glycosides	+	+	+	-
Triterpenes	+	+	-	+
Terpenoids	+	+	+	+
Phlobatanin	-	+	-	-
Amino Acids	-	-	-	+
Alkaloids	+	+	+	+

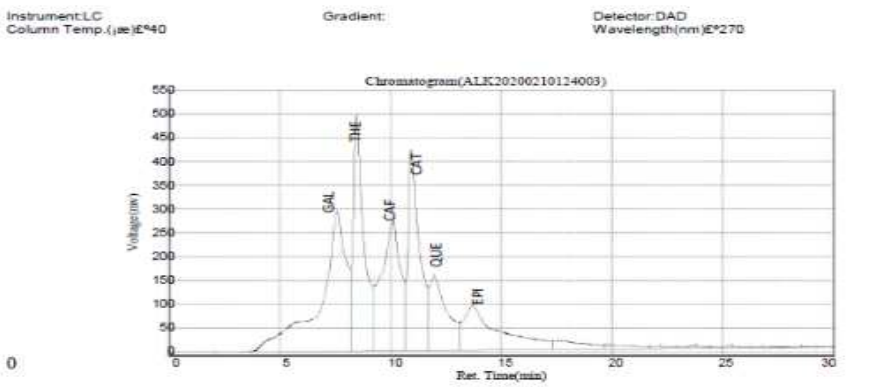
Key: + = Present
- = Absent



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc mg/100mL
1	Unidentified	1.148	1639.177	11171.800	0.1553
2	Unidentified	10.257	88.333	662.767	0.0092
3	Luteolin	16.048	309691.156	6974086.000	96.9381
4	Unidentified	17.157	863.211	3529.200	0.0491
5	Unidentified	19.423	7600.636	62148.699	0.8639
6	Apigenin	23.882	3789.297	30924.010	4.4298
7	Quarcetin	34.190	780.219	4326.291	4.0601
8	Unidentified	40.482	4397.973	55125.949	4.7662
9	Unidentified	46.732	127.364	2258.800	0.0314
10	Unidentified	47.898	68.500	713.200	0.0099
11	Unidentified	49.782	2075.500	46119.301	0.6410
12	Unidentified	50.915	102.404	1788.557	0.0249
13	Unidentified	51.898	131.606	1516.799	0.0211

Figure 6: Representative chromatogram of n-hexane extract of *G. kola* seeds



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc mg/100g
1	Gallic acid	7.515	289745.438	4883409.500	25.6495
2	Theobromine	8.690	491016.281	3295875.500	17.3112
3	Caffeic Acid	10.032	269365.969	3241580.500	17.0260
4	Catechin	12.190	418813.750	3065232.750	16.0998
5	Quercetin	13.398	154217.766	1712993.625	8.9973
6	Epicatechin	14.740	93766.445	2109856.750	11.0818
7	Unidentified	17.523	18154.287	362571.594	1.9044
8	Unidentified	23.965	7782.454	142749.219	0.7498
9	Unidentified	24.440	4030.784	56117.496	0.2948
10	Unidentified	24.757	4028.337	63364.758	0.3328
11	Unidentified	25.082	3255.931	59477.191	0.3124
12	Unidentified	25.365	1672.321	22911.545	0.1203
13	Unidentified	25.715	881.038	13465.985	0.0707
14	Unidentified	26.015	997.509	9375.831	0.0492

Figure 7: Representative chromatogram of Ethyl acetate extract of *G. kola* seeds

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