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Assessment of the antimicrobial and antioxidant activities of the extracts of *Garcinia kola*, *Garcinia epunctata* and *Acacia kamerunensis* plants used for oral hygiene in Ghana

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A B S T R A C T

Chewing sticks and chewing sponges derived from plants stems and twigs are commonly used alone or in combination with conventional brushing for routine dental hygiene in Ghana. To examine whether phytochemical attenuation of microbial growth and radical scavenging activities accounts for observed dental hygiene efficacy, methanol and water extracts of three plant species used traditionally for the preparation of chewing sticks (*Garcinia kola* and *Garcinia epunctata*) and for the preparation of chewing sponge (*Acacia kamerunensis*) were assessed for their antimicrobial and antioxidant activities *in vitro*. Phytochemical analysis revealed the presence of saponins in all plant extracts. With the exception of the water extract of *Acacia kamerunensis* that failed to inhibit the growth of *E. faecalis* and *K. pneumonaie* in broth dilution assays, all other extracts exhibited positive inhibitory activity against all the test microorganisms. Microbial growth inhibition efficacy varied but were lower than that of the standard drugs (Ciprofloxacin-antibacterial and Fluconazole-antifungal) and that of conventional mouthwash (Chlorhexidine). Extracts showed varied concentration dependent 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity with IC_{50} values that were significantly lower than that of Ascorbic acid. The results imply that locally used chewing stick and chewing sponges derived from the test plants have constituents that inhibits microbial growth *in vitro* and that also exhibits antioxidant activities *in vitro*.

Capsule Summary: The antioxidant and antimicrobial activities of the water and methanol extracts of *Garcinia kola*, *Garcinia epunctata* and *Acacia kamerunensis* used for oral hygiene in Ghana were assessed. The results imply that locally used chewing stick and chewing sponges derived from the test plants have constituents that inhibit microbial growth *in vitro* and that also exhibit antioxidant activities *in vitro*.

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INTRODUCTION

Chewing sticks produced from plant stems and chewing sponges derived from plant twigs are widely used traditional means of oral hygiene in both rural and urban West Africa (Tutu et al., 1979; Akpona et al., 2009). In Ghana, chewing sticks and sponges are used either in addition to routine conventional tooth brushing or instead of brushing by those who lack access to conventional oral hygiene measures. Past attribution of the hygienic efficacy of chewing sticks and sponges has been linked to mechanical agitation of dental tissues leading to the physical removal of microbial flora and plague-forming bacteria from the oral cavity (Hardie and Ahmad, 1995). Phytochemical-based attenuation of microbial growth in the oral cavity might be an additional alternative mechanistic route that underlies the improvement of oral health from the use of chewing sticks and chewing sponges but few studies have attempted to examine this possibility (Addai et al., 2002; Akande and Hayashi, 1998).

Palm alcohol extracts of the pulverized root-bark of *Acacia kamerunensis* are used ostensibly as analgesic and as anti-microbial agent for the routine relieve of pain caused by carious teeth (Addo-Fordjour et al., 2008). The chewing stems of *Garcinia kola* are reportedly phytochemical-rich showing the presence of saponins, tannins, flavonoids and alkaloids (Okwu and Ekeke, 2003). Stem-bark extracts of *Garcinia kola* are used in ethnomedicinal practices in Africa to relax the smooth muscle of the uterus and of the intestine (Okwu and Ekeke, 2003) and as efficacious treatment of cough, constipation and parasitic infections (Ofusori et al., 2008).

Although antimicrobial activities of the roots of *Garcinia kola* has been reported (Taiwo et al., 1999), prior studies has neither been undertaken to assess the antimicrobial activities nor to examine the antioxidant activities of the ready-to-use chewing stems of *Garcinia kola*, *Garcinia epunctata* and ready-to-use chewing sponges of *Acacia kamerunensis in vitro*. Given the high use prevalence of *Garcinia kola*, *Garcinia epunctata* and *Acacia kamerunensis* for oral hygiene in Ghana, understanding their complete mechanistic mode of teeth cleansing and oral heath action will be beneficial to public health educational campaigns.

This study presents the *in vitro* anti-microbial activities of the water and methanolic extracts of *Garcinia kola* and *Garcinia epunctata* (commonly used Ghanaian chewing sticks) and *Acacia kamerunensis* (commonly used Ghanaian chewing sponge) against a panel of microbes that includes *E. coli*, *B. subtilis*, *S. aureus*, *S. pneumonia*, *P. aeruginosa*, *S. typhi*, *E. faecalis*, *K. pneumonaie* and *C. albicans*. The study results suggest that aqueous extracts of *Garcinia kola*, *Garcinia epunctata* chewing sticks and *Acacia kamerunensis* chewing sponge have phytochemical constituents that likely inhibits microbial growth in the oral cavity and that also potentially confers anti-oxidant properties to oral tissues.

MATERIAL AND METHODS

Chemicals

All reagents used were of analytical grade. Nutrient broth, nutrient agar and Sabouraud broth were obtained from Sigma Chemical Co. (St. Louis, MO, USA). DPPH (2, 2-Diphenyl-2-picrylhydrazyl) and ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Ethanol and Methanol were obtained from Merck Chemical Supplies (Damstadt, Germany).

Culture and maintenance of microorganisms

The panel of microbial specimen used for the assessment of antimicrobial activity were acquired from ATCC (USA), stored and maintained as previously described (Mensah et al. 2016).

Collection and preparation of samples

Ready-to-use *Garcinia kola* and *Garcinia epunctata* chewing sticks and *Acacia kamerunensis* chewing sponge were purchased from the central market of Kumasi, Ghana. Purchased specimens were identified by a botanist at the Department of Applied and Theoretical Biology of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Voucher specimen of all species was deposited at the School of Botany of the KNUST. Prior to extraction, the chewing sticks were pulverized and all samples were air dried, and then stored in an air tight container.

Phytochemical extraction

Soxhlet apparatus was filled with 50 g of pulverized samples. A 500 mL water or a 500 mL methanol was added to fresh samples of each species in the soxhlet and extraction was performed for 3 h. Extraction solvents was removed at 45^{o}C on a rotavap. Extracts were then stored frozen at -20 ^{o}C until needed.

Diagnostic TLC

The assessment of the number of individual chemical compounds present in each extract was performed using a TLC protocol previously described (Mensah et al., 2014; Mensah et al. 2016). The retention factor (Rf) for each observed spot was computed as the ratio of the distance moved by the spot to the distance moved by the solvent.

Basic phytochemical screening

Evaluation of extracts phytochemical constituents that encompasses alkaloids, terpenoids, steroids, flanonoids, tannins, coumarins, saponins, anthraquninones and glycosides were performed using a modification of the protocols of Trease and Evans (1984) previously described (Mensah et al., 2016).

Antimicrobial assay

Table 1: Thin layer chromatography (TLC) report and phytochemical contents extracts

Plant species	**Number of spots from TLC and Rf values**		**Phytochemicals present in extract**
	Aqueous extracts	Methanol extracts	Aqueous extracts
G.kola	One Rf: 0.506	Two Rf: 0.602, 0.403	Alkaloids and Saponins
G. epunctata	One RF: 0.494	Two Rf:0.321, 0.214	Saponins
A. kamerunensis	One Rf: 0.129, 0.194	Three Rf: 0.105, 0.210, 0.298	Saponins and Flavonoids

Table 2: Broth dilution for Aqueous extracts

Plant species	Concentration (mg/ml)							
	Organisms	**128**	**64**	**32**	**16**	**8**	**4**	**2**	**1**
A. kamerunensis	*E. coli*	-	-	+	+	+	+	+	+
	B. subtilis	-	-	+	+	+	+	+	+
	S. aureus	-	-	+	+	+	+	+	+
	S. pneumoniae	-	-	+	+	+	+	+	+
	C. albicans	-	-	+	+	+	+	+	+
	P. aeruginosa	-	+	+	+	+	+	+	+
	S. typhi	-	+	+	+	+	+	+	+
	E. faecalis	+	+	+	+	+	+	+	+
	K. pneumonaie	+	+	+	+	+	+	+	+
		128	**64**	**32**	**16**	**8**	**4**	**2**	**1**
G. kola	*E. coli*	-	-	-	+	+	+	+	+
	B. subtilis	-	-	-	+	+	+	+	+
	S. aureus	-	-	-	-	-	-	+	+
	S. pneumoniae	-	-	-	-	-	+	+	+
	C. albicans	-	-	-	-	-	-	+	+
	P. aeruginosa	-	-	-	-	-	-	+	+
	S. typhi	-	-	-	-	-	-	+	+
	E. faecalis	-	-	-	-	-	-	+	+
	K .pneumoniae	-	-	-	+	+	+	+	+
		128	**64**	**32**	**16**	**8**	**4**	**2**	**1**
G. epunctata	*E. coli*								
	B. subtilis	-	-	-	-	+	+	+	+
	S. aureus	-	-	-	-	-	-	+	+
	S. pneumoniae	-	-	-	-	+	+	+	+
	C. albicans	-	-	-	-	-	-	+	+
	P. aeruginosa	-	-	+	+	+	+	+	+
	S. typhi	-	-	+	+	+	+	+	+
	E. faecalis	-	-	+	+	+	+	+	+

+ indicates microbial growth; - indicates no microbial growth

A modified protocol prescribed by Murray et al., 1999 was used for the broth dilution assay as described previously (Mensah et al, 2016). Ciprofloxacin and fluconazole and chlorhexidine (at different concentrations) were used as positive controls. MICs were expressed in mg/mL and were taken as the lowest extract concentrations that showed complete growth inhibition as represented by the last tube with no visible violet color from the addition of 0.1 mL of MTT.

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay

A modification of the protocol prescribed by Govindarajan et al., 2005 was used in the assessment of the antioxidant

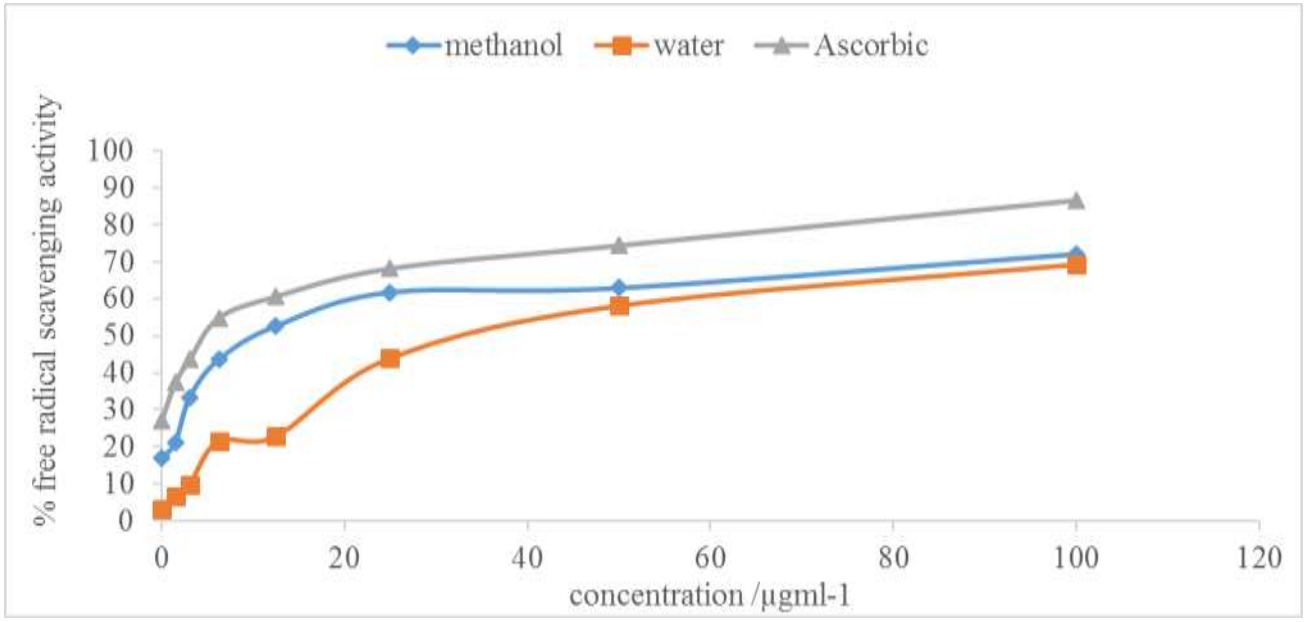

Fig 1: DPPH radical scavenging activity of methanol and water extracts of *A. kamerunensis* and control (Ascorbic acid).

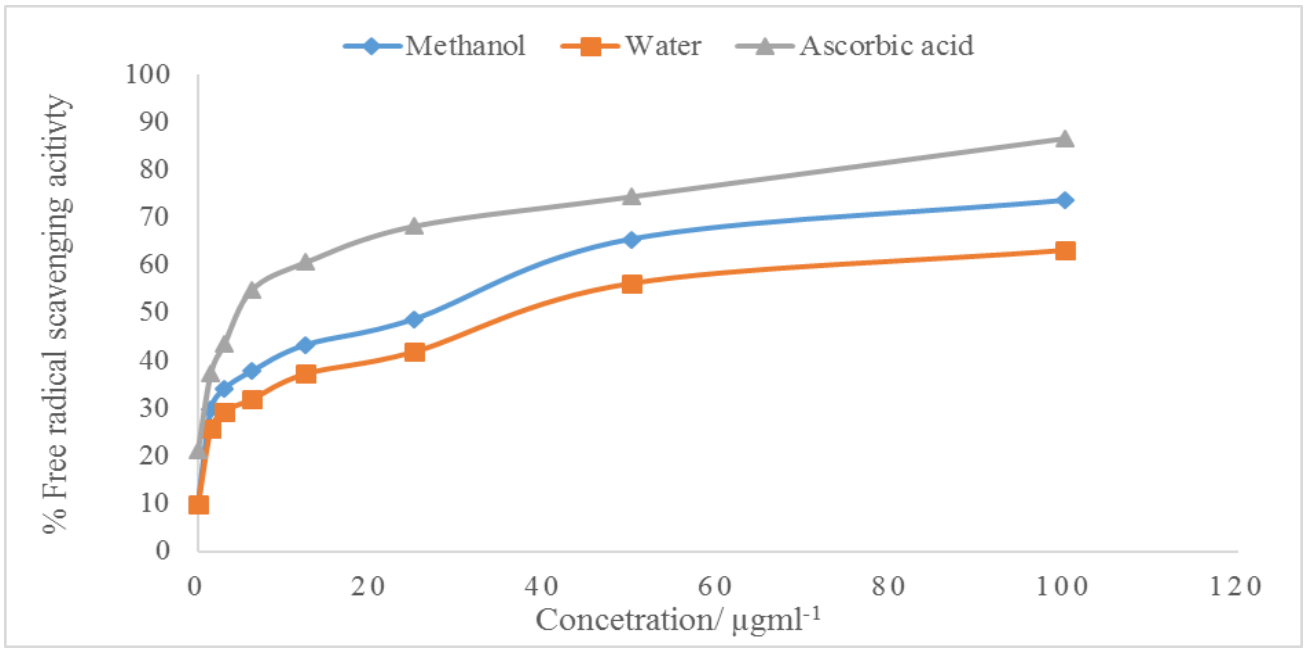

Fig 2: DPPH radical scavenging activity of methanol and water extracts of *G. Kola* and control (Ascorbic acid).

capacity as previously described (Mensah et al., 2015; Mensah et al., 2016). In all cases, Ascorbic acid was used as the standard free radical scavenger and radical scavenging activity was expressed as the inhibition percentage according to the formula shown in Eq. 1

$$\% \text{ radical scavenging activity} = (1 - A_s/A_c) \times 100 \quad (1)$$

Where A_s is the absorbance of sample and A_c is the absorbance of the control. The antioxidant activity for each extract was expressed as IC_{50}; the concentration (mg/mL) of sample required to scavenge DPPH radical formation by 50% and was computed from the dose response curve plotted between % inhibition and extract concentrations.

RESULTS AND DISCUSSIONS

Table 3: Broth dilution for methanol extracts

Plant species	Concentration (mg/ml)							
	Organisms	**128**	**64**	**32**	**16**	**8**	**4**	**2**	**1**
A. kamerunensis	*E. coli*	-	-	-	-	+	+	+	+
	B. subtilis	-	-	-	+	+	+	+	+
	S. aureus	-	-	-	-	+	+	+	+
	S. pneumoniae	-	-	-	-	+	+	+	+
	C. albicans	-	-	-	-	+	+	+	+
	P. aeruginosa	-	-	-	+	+	+	+	+
	S. typhi	-	-	-	-	-	+	+	+
	E. faecalis	-	-	-	-	-	+	+	+
	K. pneumoniae	-	-	-	-	-	-	+	+
		128	**64**	**32**	**16**	**8**	**4**	**2**	**1**
G. kola	*E. coli*	-	-	-	+	+	+	+	+
	B. subtilis	-	-	-	+	+	+	+	+
	S. aureus	-	-	-	+	+	+	+	+
	S. pneumoniae	-	-	-	-	+	+	+	+
	C. albicans	-	-	-	-	-	-	+	+
	P. aeruginosa	-	-	-	-	-	-	+	+
	S. typhi	-	-	-	-	-	-	+	+
	E. faecalis	-	-	-	-	-	+	+	+
	K. pneumoniae	-	-	-	-	-	+	+	+
		128	**64**	**32**	**16**	**8**	**4**	**2**	**1**
G. epunctata	*E. coli*	-	-	-	+	+	+	+	+
	B. subtilis	-	-	-	-	-	+	+	+
	S. aureus	-	-	-	-	-	-	+	+
	S. pneumoniae	-	-	-	-	-	+	+	+
	C. albicans	-	-	-	-	-	-	+	+
	P. aeruginosa	-	-	+	+	+	+	+	+
	S. typhi	-	-	+	+	+	+	+	+
	E. faecalis	-	-	-	+	+	+	+	+
	K. pneumoniae	-	-	-	+	+	+	+	+

+ indicates microbial growth; - indicates no microbial growth

Table 4: MIC values of extracts of plant species.

Organisms	**Aqueous extracts**			**Methanol extracts**		
	G. kola	***G. epunctata***	***A. kamerunensis***	***G .kola***	***G. epunctata***	***A. kamerunensis***
E. coli	32	64	64	32	32	16
B. subtilis	32	16	64	32	8	32
S. aureus	4	4	64	32	4	16
S. pneumoniae	8	16	64	16	8	16
C. albicans	4	4	64	4	4	16
P. aeruginosa	4	64	128	4	64	32
S. typhi	4	64	128	4	64	8
E. faecalis	4	64	No inhibition	8	32	8
K. pneumoniae	32	64	No inhibition	8	32	4

Phytochemical screening and TLC

Phytochemical screening of *A. kamerunensis* chewing sponge revealed the presence of flavonoids and saponins while that of the chewing sticks of *G. kola* indicated the presence of alkaloids and saponins (Table 1). *G. epunctata* revealed the presence of saponins only (Table1). The presence of alkaloids and saponins in the chewing stems of Garcinia kola agrees with earlier finding by other researchers (Okwu and Ekeke, 2003). Observed phyto-constituents are notably anti-microbial in bioactivity and their presence in all three plant species lends credence to the endowment of potential antimicrobial activities to the constituents of the extracts (Cushie and Lamb, 2011)

With the exception of the water extracts of *G. kola* and *G. epunctata* that showed only one spot, TLC analysis revealed the presence of multiple compounds with different Rf values in all the other extracts (Table1). Reasons for the display of one TLC spot for the water extracts of *G. kola* and *G. epunctata* are multiple but may potentially be due to the

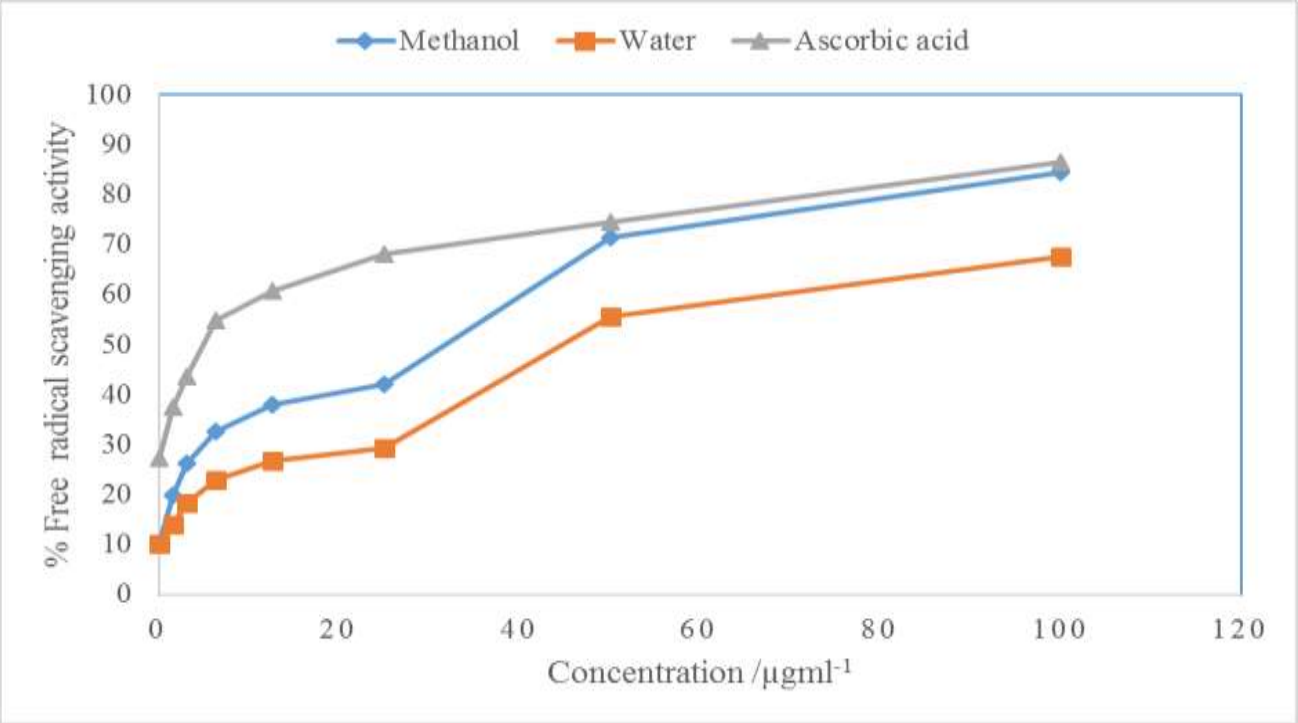

Fig 3: DPPH radical scavenging activity of methanol and water extracts of *G. epunctata* and control (Ascorbic acid).

inability of the mobile phase to resolve the constituents of the water extracts.

Broth dilution assay

Except for the water extract of *A. kamerunensis*, that failed to show activity against *E. feacalis* and *K. pneumoniae*, all examined extracts exhibited activity against all test organisms at concentrations that suggest potent bioactivity (Tables 2 and 3). The methanol extracts of *A. kamerunensis* and *G. epunctata* displayed higher antimicrobial activities as demonstrated by the relatively lower minimum inhibitory concentrations (MICs) (Table 4). No differences in antimicrobial potency were observed for either extract for *G. Kola* as both water and methanolic extracts of *G. kola* demonstrated similar inhibitory activities against all the test organisms (Table 4).

No discernable consistent pattern of growth inhibitory effect were observed with the bioactivities of the extracts. Different test microbes showed varied patterns of susceptibility to the growth inhibitory effect of different extracts as demonstrated by the varied MICs values. The water extract of *A. kamerunensis* showed the least inhibitory activity against *P. aeruginosa* and *S. typhi* at the highest extract concentration (128 mg/ml). On the other hand, the highest bioactivity of the methanol extract of *A. kamerunensis* was exhibited against *K. pneumoniae* at an MIC of 4.0 mg/ml. Both water and methanol extracts of *G. epunctata* showed highest inhibitory activities against *S. aureus* and *C. albicans* with MIC values of 4.0 mg/ml. The water extract of *G. kola* showed the highest inhibitory activity against four of the test organisms with MIC values of 4.0 mg/ml while the methanol extract of the same plant recorded the highest MIC value of 4.0 mg/ml against three of the test organisms (Table 4). For all test organisms, the standard drugs (antibacterial Ciprofloxacin and antifungal Fluconazole) and mouthwash (Chlorhexidine) used as positive controls demonstrated higher growth inhibition with lowest MICs that any of the extracts (Table 5).

DPPH radical scavenging activity

All examined extracts exhibited considerable DPPH radical scavenging activity (Figures 1-3). Scavenging activity of extracts increased with increase in extract concentration. For all extracts, scavenging activities of methanol extracts were marginal higher than that of their corresponding water extracts (Figures 1- 3). The higher scavenging activities of methanol extracts compared to water extracts could be attributed to the higher extraction efficiency of methanol that led to the extraction of a larger spectrum of antioxidant compounds than water did. In all cases, DPPH radical scavenging activities of extracts were significantly lower than that of the ascorbic acid control as demonstrated by the lowest IC_{50} value of ascorbic acid (Table 6).

Table 5: Broth dilution for Standard Drugs (Ciprofloxacin and Fluconazole) and standard mouthwash (Chlorhexidine)

Test organisms	**Concentration (mg/ml)**					
	0.01	**0.005**	**0.0025**	**0.00125**	**0.000625**	**0.0003125**
	Ciprofloxacin					
E. coli	-	-	-	-	-	+
B. subtilis	-	-	-	-	-	+
S. aureus	-	-	-	-	-	+
S. pneumoniae	-	-	-	-	-	+
K. pneumoniae	-	-	-	-	-	+
P. aeruginosa	-	-	-	-	-	+
S. typhi	-	-	-	-	-	+
E. faecalis	-	-	-	-	-	+
	0.01	0.005	0.0025	0.00125	0.000625	0.0003125
	Fluconazole					
C. albicans	-	-	-	-	-	+
	Concentration (% w/v)					
	0.08	0.04	0.02	0.01	0.005	0.0025
	Chlorhexidine					
E. coli	-	-	-	-	-	-
B. subtilis	-	-	-	-	-	-
S. aureus	-	-	-	-	-	-
S. pneumoniae	-	-	-	-	-	-
K. pneumoniae	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-
S. typhi	-	-	-	-	-	-
E. faecalis	-	-	-	-	-	-
C. albicans	-	-	-	-	-	-

+ indicates microbial growth; - indicates no microbial growth

Table 6: IC_{50} values for DPPH radical scavenging activities of aqueous and methanol extracts and control (ascorbic acid).

Plant species	*A. kamerunensis*		*G. kola*		*G. epunctata*		Ascorbic acid
Extracts	Methanol	Water	Methanol	Water	Methanol	Water	
IC_{50}	34.246	55.85	38.97	55.02	38.46	59.18	11.45

For each plant species, differences in DPPH Radical scavenging activities between the water and methanol extracts were marginal at the lowest extract concentration (0.78 µg/ml) but gradually increased to more substantial values at higher extract concentrations (25, 50 and 100 µg/ml) (Figures 1-3). As shown in Table 6 increasing scavenging effects of methanol extracts on DPPH radical were in the order: *A. Kamerunensis* > *G. Epunctata* > *G .kola* > while that of the water extracts is ordered: *G. kola*>*A. Kamerunensis* > *G. epunctata.* In view of present investigation, extracts have potential bioactive compounds and previious studies also support these findings (Adaramola et al., 2016; Asif, 2015a, b, c, d, e, f, 2016).

CONCLUSIONS

With the exception of the water extract of *G. epunctata* that showed the presence of only saponins, all other extract phyto-constituents comprised a mixture of saponins with either flavonoids (*A. kamerunensis*) or alkaloids (*G. kola*). While the water extract of *A. kamerunensis* failed to show activity against *E. Faecalis* and *K. pneumoniae*, all other extracts exhibited a dose-dependent inhibition of microbial growth of all test organisms. Levels of growth inhibitory activities were significantly lower for all extracts compared to that of the standard drug control (antibacterial Ciprofloxacin and antifungal Fluconazole) and mouthwash (Chlorhexidine). Extracts showed varied DPPH Radical Scavenging activities that increased with increasing extract concentration. Compared to ascorbic acid control, all extracts scavenging efficacy were relatively lower with demonstrably

higher IC_{50}s than that of the ascorbic acid. Phytochemical constituents of the aqueous extracts of *Garcinia kola* and *Garcinia epunctata* chewing sticks and *Acacia kamerunensis* chewing sponge likely inhibits microbial growth in the oral cavity and potentially confers anti-oxidant properties to oral tissues.

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