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Evaluation of *In vitro* Adjunct Antimycotic Effects of Most-Preferred Chewing Sticks on Human Oral *Candida* Species

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ABSTRACT

Aqueous and ethanolic extracts of 12 most-preferred Nigerian indigenous chewing-sticks were assayed for in vitro antimycotic potentials against 86 human oral Candida species isolated from tongues, teeth and saliva. The oral Candida strains were not species-specific as regards their sources of isolation but recovery rates were C. albicans (43), C. glabrata (5), C. pseudotropicalis (10) and C. tropicalis (28). In vitro susceptibility rates of [C. albicans (8.3-58.3%), C. glabrata (8.3-33.3%), C. pseudotropicalis (8.3-25.0%), C. tropicalis (8.3-50.0%)] and [C. albicans (8.3-83.3%), C. glabrata (16.7-66.7%), C. pseudotropicalis (8.3-75.0%), C. tropicalis (8.3-66.7%)] were recorded respectively among the oral Candida strains towards aqueous and ethanolic extracts of the chewing-sticks. In vitro inhibitory activities of each aqueous chewing-stick extract were, Massularia acuminata (0.0-20.0%), Fagara xanthoxyloides / Zanthoxylum xanthoxyloides (0.0-23.3%), Pseudocedrela kotschyi (10.0-23.3%), Parquetina nigrescen (20.0-25.0%), Distemonanthus benthamianus (0.0-27.9%), Garcinia cola (0.0-28.6%), meyinro (16.3-28.6%), Terminalia avicenniordes (0.0-30.0%), Vernonia amygdalina (20.0-37.2%), Terminalia glaucescens (18.6-40.0%) and Periscopsis laxiflora / Prosopis africana, Olax subscorpioide (20.0-40.0%). Relatively higher susceptibility rates were exhibited by ethanolic extracts of Fagara xanthoxyloides / Zanthoxylum xanthoxyloides (0.0-23.3%), Pseudocedrela kotschyi (0.0-25.0%), Olax subscorpioide (20.0-34.9%), Parquetina nigrescen (0.0-37.2%), Periscopsis laxiflora / Prosopis africana (14.3-40.0%), Massularia acuminata (0.0-53.6%), Garcinia cola (17.9-60.0%), Distemonanthus benthamianus, Vernonia amygdalina (20.0-60.0%), Terminalia glaucescens (30.0-60.0%) and Terminalia avicenniordes (27.9-70.0%) chewing sticks. The findings of this study indicated the phenotypic potentials of Nigerian chewing sticks as adjunct cleansing agents in oral hygiene, which is of clinical relevance in dentistry.

Keywords: *Candida*, chewing sticks, dental caries, natural plant products, oral hygiene, oral trush, periodontal diseases

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INTRODUCTION

Oral candidiasis affects many sectors of the population, including the very young, the elderly and severely immuno-deficient people [1], while *Candida* species are the aetiological agents of oral candidasis, which usually reside as commensals and part of normal oral microflora. They are frequently cultured from oral and oesophageal surfaces

and have been known to reach the oesophagus in oral secretions [2-4] but determining exactly how transformation of *Candida* species from being commensals to pathogens takes place and how it can be prevented is a continuous clinical challenge, although candidal adherence to mucosal surfaces is considered as a critical initial step in the pathogenesis of oral candidiasis [2, 5-7].

Symptoms of oral candidiasis include burning mouth syndrome; white lesions of the oral mucosa are increased in frequency [8], while treatment for oral candidiasis includes antifungal therapy if candidiasis is diagnosed. The purpose of oral hygiene through regular removal of dental plaque and food deposits is an essential factor in the prevention of dental caries and periodontal diseases, although methods for oral hygiene vary among countries and cultures. It has been shown that *Candida* species can survive in the biofilm of the mouth [9], so, there is the need for adjunct mouth-cleansing agents for oral hygiene, with regards to oral fungi.

For centuries, chewing sticks have been used as a tooth-cleaning device; some clinical epidemiological studies are in support of beneficial activities of chewing sticks in oral cases, while many laboratory investigations have also suggested the presence of heterogeneous antimicrobial components, which are extractable with the use of different chemical procedures. Today, chewing sticks are still used in many developing countries based on tradition, religion or because of their ready availability, low cost and simplicity; even, the World Health Organisation also encouraged their use. The Year 2000 Consensus Report on Oral Hygiene stated that chewing sticks may have a role to play in the promotion of oral hygiene and that evaluation of their effectiveness warrants further research [10].

Traditional medical knowledge of medicinal plants and their use by indigenous cultures are not only useful for conservation of cultural traditions and biodiversity but also for community healthcare and drug development in the present and future [12][23]. Several chewing-sticks have been used for centuries as oral hygiene tools in many parts of the world, while many studies have demonstrated the anticaries, antiplaque, antiperiopathic and antibacterial effects of these sticks [13][24]. Aderinokun *et al*. [11] reported that slight improvements were detected in the gingival status of those using chewing-sticks relative to those in the group using toothbrush. Similarly, various researchers have also advocated the use of chewing sticks in community oral health programmes because they are readily available, cheaper than toothbrushes and pastes, and also in consideration of oral reactions by some users, principally due to adulteration of some toothpastes or due to allergic reactions.

A number of studies have been conducted on the efficacy of local chewing sticks on oral bacterial flora [11-15] but there is sparsity of data on the effect of chewing sticks on oral *Candida* spp., especially with regards to usage of chewing sticks in oral hygiene in cases of oral candidasis. Apart from intrinsic nature, it is well known that most of the characteristics of microorganisms in close associations with humans are affected by genetic compositions, geographical/ environmental factors, diets etc., This study will therefore, be one of the very few (if any) that determine *in vitro* antimycotic effects of the most-preferred Nigerian indigenous chewing sticks on *Candida* species of oral origin.

MATERIALS AND METHODS

Collection of oral specimens and Oral *Candida* isolates:
Candida strains [16-19] used in this study were obtained in form of three sets of early-morning oral swabs from oral cavities of 40 healthy volunteers, who were 19-28 years old students of various faculties of University of Ibadan, and who had not been on antifungal therapy at least six months prior to collection of specimens [20].

Chewing sticks:
Local Nigerian chewing sticks used in this study were [*orogbo* (*Garcinia cola*) common name (bitter kola), *idi pupa* (*Terminalia avicenniordes*), *ogbo* (*Parquetina nigrescen*), *ewuro* (*Vernonia amygdalina*) Del., common name (bitter leaf); *pako Ijebu* (*Massularia acuminata*), *idi funfun* (*Terminalia glaucescens*), *emi gbegiri* (*Pseudocedrela kotschyi*), *aayan* (*Periscopsis laxiflora* / *Prosopis africana*), common name (mesquite); *modunmoro* (*Distemonanthus benthamianus*), *meyinro*, *orin ata* (*Fagara zanthoxyloides*) Lam, common name (candle wood) / (*Zanthoxylum xanthoxyloides*) (Engl.). Zepernick & Timter and *ifon* (*Olax subscorpioide*)]. The chewing sticks were obtained from local Nigerian herbal markets, and tentatively identified with common names by traditional herbal plants sellers, while final identifications were done at the Herbarium, Department of Botany & Microbiology, University of Ibadan, Nigeria.

Antimycotic susceptibility test:

Determination of *in vitro* inhibitory activities of test chewing sticks on oral *Candida* strains using modified agar well-diffusion method:

Aqueous and ethanolic extracts of the local chewing sticks were used for the determination of *in vitro* inhibitory activities. Holes, measuring 6.0 mm in diameter were aseptically bored and removed from sterile SDA agar plates, followed by surface flaming of the agar plates. Each SDA agar plate was then seeded by streaking the entire surface of the culture plate with each oral *Candida* strain. By modification of the method of Tagg *et al*. [21], 500µl of each aqueous and ethanolic chewing stick extracts was dispensed into the agar wells in the seeded plates, followed by incubation at 30-35^{0}C for 24-48 hrs. The modification was by incorporating the chewing stick extracts into sterile semi-solid agar before dispensing into the wells to prevent spreading of the extracts on the agar surface. Inhibitory activities of the extracts depended on the release of diffusible inhibitory metabolites into the assay medium during incubation, so, inhibition zones surrounding the agar wells were noted and recorded in mm diameter, while holes without zones of inhibition or inhibition zones less than 10.0 mm were recorded as negative. Results were recorded in triplicates.

RESULTS

In this study, 86 strains of oral *Candida* were phenotypically characterised as *C. albicans* 43 (50.0%), *C. glabrata* 5 (5.8%), *C. pseudotropicalis* 10 (11.6%) and *C. tropicalis* 28 (32.5%). The recovery patterns of the oral *Candida* species were tongue [*C. albicans* 22 (25.6%), *C. glabrata* 1 (1.2%), *C. pseudotropicalis* 3 (3.5%) and *C. tropicalis* 8 (9.3%)]; teeth [*C. albicans* 15 (17.4%), *C. glabrata* 1 (1.2%), *C. pseudotropicalis* 3 (3.5%) and *C. tropicalis* 11 (12.7%)] and saliva [*C. albicans* 8 (9.3%), *C. glabrata* 3 (3.5%), *C. pseudotropicalis* 4 (4.7%) and *C. tropicalis* 7 (8.1%). More of *C. albicans* strains were recovered from tongue, more of *C. glabrata* and *C. pseudotropicalis* strains were isolated from saliva, while more of the *C. tropicalis* strains were isolated from teeth (Fig. 1). *In vitro* susceptibility rates of 8.3-58.3% were exhibited by oral *C. albicans* strains towards the aqueous extracts of twelve local chewing sticks but the highest overall mean inhibitory activities of 32.6% and 37.2% were recorded in *ifon* (*Olax subscorpioide*) and *ewuro* (*Vernonia amygdalina*) respectively (Table 1).

Oral *C. glabrata* strains also exhibited susceptibility rates of 8.3-33.3% towards the aqueous extracts of the local chewing sticks but the maximum overall mean inhibitory activities by the aqueous extracts of the local chewing sticks was 40.0%. 20.0% were inhibited by *ogbo* (*Parquetina nigrescen*), *ewuro* (*Vernonia amygdalina*), *emi gbegiri* (*Pseudocedrela kotschyi*), *modunmoro* (*Distemonanthus benthamianus*), *meyinro* and *ifon* (*Olax subscorpioide*); 40.0% of the strains were inhibited by *idi funfun* (*Terminalia glaucescens*) and *aayan* (*Periscopsis laxiflora, Prosopis africana*), while none of the *C. glabrata* strains was inhibited by the remaining local chewing sticks.

Overall susceptibility rates of the 10 oral *C. pseudotropicalis* strains towards aqueous extracts of the chewing sticks were 8.3-25.0%; 20.0% of the *C. pseudotropicalis* strains were inhibited by six of the aqueous extracts of local chewing sticks- *ogbo* (*Parquetina nigrescen*), *ewuro* (*Vernonia amygdalina*), *pako Ijebu* (*Massularia acuminata*), *aayan* (*Periscopsis laxiflora, Prosopis africana*), *orin aata* (*Fagara xanthoxyloides / Zanthoxylum xanthoxyloides*) and *meyinro*. Ten percent of the strains were inhibited by *orogbo* (*Garcinia cola*) and *emi gbegiri* (*Pseudocedrela kotschyi*); 30.0% were susceptible to *idi pupa* (*Terminalia avicenniordes*) and *idi funfun* (*Terminalia glaucescens*); 40.0% were inhibited by *ifon* (*Olax subscorpioide*), while none of the strains was inhibited by *modunmoro* (*Distemonanthus benthamianus*) (Table 1).

Overall susceptibility rates of the 28 oral *C. tropicalis* strains towards aqueous extracts of the chewing sticks were 8.3-50.0%. The most inhibitory chewing sticks towards *C. tropicalis* strains were *ewuro* (*Vernonia amygdalina*) and *ifon* (*Olax subscorpioide*) (35.7%). 28.6% of the strains were inhibited by aqueous extracts of *orogbo* (*Garcinia cola*) and *meyinro*, 25.0% by *ogbo* (*Parquetina nigrescen*), 21.4% by *idi pupa* (*Terminalia avicenniordes*), *idi funfun* (*Terminalia glaucescens*), *aayan* (*Periscopsis laxiflora, Prosopis africana*) and *orin aata* (*Fagara xanthoxyloides/ Zanthoxylum xanthoxyloide*) but lower inhibitory rates were recorded in other chewing sticks (Table 1).

Susceptibility rates of 16.3-37.2% were recorded among *C. albicans* strains towards the ethanolic extracts of local chewing sticks. The relatively more inhibitory chewing sticks were *orogbo* (*Garcinia cola*) (25.6%), *idi pupa* (*Terminalia avicenniordes*) (27.9%), *ewuro* (*Vernonia amygdalina*) (30.2%), *pako Ijebu* (*Massularia acuminata*) (30.2%) and *meyinro* (30.2%), *aayan* (*Periscopsis laxiflora, Prosopis africana*) (32.6%), *idi funfun* (*Terminalia*

glaucescens) (34.9%), *modunmoro* (*Distemonanthus benthamianus*) (34.9%), *ifon* (*Olax subscorpioide*) (34.9%) and *ogbo* (*Parquetina nigrescen*) (37.2%), while lower susceptibility rates of 16.3% and 20.9% were recorded in *emi gbegiri* (*Pseudocedrela kotschyi*) and *orin aata* (*Fagara xanthoxyloides/ Zanthoxylum xanthoxyloides*) respectively (Table 2).

Susceptibility rates of 16.7-66.7% were exhibited by the oral *C. glabrata* strains towards ethanolic extracts of local chewing sticks. Out of the five *C. glabrata* strains, none was susceptible to the ethanolic extracts of *pako Ijebu* (*Massularia acuminata*), *emi gbegiri* (*Pseudocedrela kotschyi*) and *orin aata* (*Fagara xanthoxyloides/ Zanthoxylum xanthoxyloides*); 20.0% were susceptible to *idi pupa* (*Terminalia avicenniordes*), *ogbo* (*Parquetina nigrescen*) and *ifon* (*Olax subscorpioide*); 40.0% were susceptible to *ogbo* (*Parquetina nigrescen*) and *aayan* (*Periscopsis laxiflora, Prosopis africana*), while 60.0% were susceptible to *orogbo* (*Garcinia cola*), *ewuro* (*Vernonia amygdalina*), *idi funfun* (*Terminalia glaucescens*), *modunmoro* (*Distemonanthus benthamianus*) and *meyinro* (Table 2).

Overall, 8.3-75.0% of the oral *C. pseudotropicalis* strains were susceptible to ethanolic chewing sticks extracts *in vitro*. None of the strains was inhibited by *ogbo* (*Parquetina nigrescen*); 10.0% of the strains were inhibited by *pako Ijebu* (*Massularia acuminata*) and *emi gbegiri* (*Pseudocedrela kotschyi*); 20.0% of the strains were inhibited by *ewuro* (*Vernonia amygdalina*), *modunmoro* (*Distemonanthus benthamianus*), *meyinro* and *orin aata* (*Fagara xanthoxyloides / Zanthoxylum xanthoxyloides*); 30.0% were inhibited by *idi funfun* (*Terminalia glaucescens*), *aayan* (*Periscopsis laxiflora, Prosopis africana*) and *ifon* (*Olax subscorpioide*); 40.0% were inhibited by *orogbo* (*Garcinia cola*), while 70.0% were inhibited by *idi pupa* (*Terminalia avicenniordes*).

In vitro, 8.3-66.7% of the oral *C. tropicalis* strains were susceptible to the ethanolic extracts of local chewing sticks. The most inhibitory chewing sticks were *ogbo* (*Parquetina nigrescen*), *idi pupa* (*Terminalia avicenniordes*) and *pako Ijebu* (*Massularia acuminata*) with inhibitory rates of 35.7%, 46.2% and 53.6% respectively. The inhibitory rates by *idi funfun* (*Terminalia glaucescens*), *modunmoro* (*Distemonanthus benthamianus*) and *meyinro* were 32.1%; 25.0% inhibitory rates were recorded in *ewuro* (*Vernonia amygdalina*), *emi gbegiri* (*Pseudocedrela kotschyi*) and *ifon* (*Olax subscorpioide*), while 17.9%, 14.3% and 14.3% inhibitory rates were recorded in *orogbo* (*Garcinia cola*), *aayan* (*Periscopsis laxiflora, Prosopis africana*) and *orin aata* (*Fagara xanthoxyloides/ Zanthoxylum xanthoxyloides*) respectively (Table 2).

DISCUSSION

The diagnosis of oral candidiasis can be based on the clinical recognition of a particular form of *Candida*, while fungal opportunistic infections, and in particular those caused by various *Candida* species have gained considerable significance as cause of morbidity and often, mortality [22, 23]. Several clinical forms of oropharyngeal candidiasis (OPC) exist but the most common and widely recognised is acute pseudomembranous candidiasis, which is commonly referred to as thrush [24], including oral thrush. Oral candidiasis, primarily caused by *C. albicans*, is an opportunistic infection [25]; however, species of non-*albicans Candida* such as *C. glabrata*, *C. krusei* and *C. parapsilosis*, have also been implicated more frequently. This was corroborated by the types of *Candida* species (*C. albicans*, *Candida glabrata*, *C. pseudotropicalis* and *C. tropicalis*) also isolated from oral specimens (teeth, toungue and saliva) in this current study, although the *Candida* species were not species-specific as regards their sources of isolation.

Adhesion of *Candida* cells to oral surfaces is an initial event in the development of oral candidiasis [26], and there is the possibility that the strong synergistic interactions among oral microbial pathogens can influence their adhesion to oral surfaces. This can be easily explained by the fact that the oral microbial pathogens usually form a layer (biofilm) over the teeth enamel [9]. It has also been reported that whole saliva was shown to promote the attachment of *Candida* yeast cells to hard surfaces [27, 28]. The study of Khan *et al*. [29] demonstrated that most bark extracts possessed antimicrobial activity and therefore, chewing sticks can serve as adjunct cleansing agents, especially as good abrasive teeth/mouth-cleansing agents in interproximal health [30].

Although minimal to moderate *in vitro* inhibitory activities in the aqueous extracts and relatively higher inhibitory activities in the ethanolic extracts of some Nigerian chewing sticks were recorded in this study. However, in cases of oral hygiene, observed *in vitro* results of the teeth cleansing agents are not exclusively the same results obtained *in vivo*. Thus, the acclaimed five most-popular local chewing sticks, especially among the south-western Nigerian (Yoruba tribe) habitual chewing-stick users, *orin aata* (*Fagara xanthoxyloides/ Zanthoxylum xanthoxyloides*), *idi*

pupa (*Terminalia avicenniordes*), *idi funfun* (*Terminalia glaucescens*), *pako Ijebu* (*Massularia acuminata*) and *ewuro* (*Vernonia amygdalina*) was based mostly on the reported significant natural tasty/foaming properties due to the barks, as well as the mouth-feel after their usage as mouth cleansing agents.

In addition to teeth cleansing effects, the subjects used in the study of Ogunshe and Odumesi [15] also supported usage of local chewing sticks as natural means of aiding mastication, being the only oral hygiene agent that can be daily chewed for some time. It was also advocated that chewing sticks can aid in inducing salivation and thereby, can also be responsible for cleansing of the salivary organs, since the main purpose of mouth-cleansing is oral hygiene and dental health. It has been recommended that chewing-sticks will be a great help in developing countries with financial constraints and limited oral health-care facilities for their populations [15, 25, 31] but much more importantly, *Candida* species are members of mixed biofilms (including fungal-bacterial interactions) *in vivo*, and subject to various antagonistic and synergistic interactions; therefore, as claimed by habitual chewing stick users, the abrasive importance of chewing sticks cannot be replaced by other mouth / teeth-cleansing method. The study of Khan *et al*. [29] advised the use of unpeeled rather than peeled chewing sticks for tooth-cleaning in order to fully exploit the antimicrobial effects of chewing sticks; therefore, such study is recommended for further consideration.

Table 1: *In vitro* mean percentage susceptibility rates of oral *Candida* species towards aqueous extracts of twelve Nigerian indigenous chewing sticks

Lab codes of chewing sticks	*In vitro* mean percentage susceptibility rates of *Candida* species			
	C. albicans (43)	*C. glabrata* (5)	*C. tropicalis* (28)	*C. pseudotropicalis* (10)
	[8.3-58.3]	[8.3-33.3]	[8.3-50.0]	[8.3-25.0]
ORO	23.3	0.0	28.6	10.0
IDP**	20.9	0.0	21.4	30.0
OGB	23.3	20.0	25.0	20.0
EW*	37.2	20.0	35.7	20.0
PKIJ**	14.0	0.0	17.9	20.0
IDF**	18.6	40.0	21.4	30.0
EMI	23.3	20.0	14.3	10.0
AAY	27.9	40.0	21.4	20.0
MOD	27.9	20.0	17.9	0.0
MEY	16.3	20.0	28.6	20.0
AAT***	23.3	0.0	21.4	20.0
IF	32.6	20.0	35.7	40.0

Keys: *1. ORO = Orogbo (Garcinia cola), 2. IDP = Idi pupa (Terminalia avicenniordes), 3. OGB = Ogbo (Parquetina nigrescen), 4. EW = Ewuro (Vernonia amygdalina), 5. PKIJ = Pako Ijebu (Massularia acuminata), 6. IDF = Idi funfun (Terminalia glaucescens), 7. EMI = Emi gbegiri (Pseudocedrela kotschyi), 8. AAY = Aayan (Periscopsis laxiflora, Prosopis africana), 9. MOD = Modunmoro (Distemonanthus benthamianus), 10. MEY = Meyinro, 11. AAT = Orin aata (Fagara xanthoxyloides/ Zanthoxylum xanthoxyloides), 12. IF = Ifon (Olax subscorpioide).*

*Values in parenthesis are the overall susceptibility rates of the Candida species towards the aqueous chewing sticks' extracts. * -*** = popular - most popular local chewing-sticks.*

Table 2: *In vitro* mean percentage susceptibility rates of oral *Candida* species towards ethanolic extracts of twelve Nigerian indigenous chewing sticks

Lab codes of chewing sticks	*In vitro* mean percentage susceptibility rates of *Candida* species			
	C. albicans (43)	*C. glabrata* (5)	*C. tropicalis* (28)	*C. pseudotropicalis* (10)
	[8.3-83.3]	[16.7-66.7]	[8.3-66.7]	[8.3-75.0]
ORO	25.6	60.0	17.9	40.0
IDP**	27.9	20.0	46.2	70.0
OGB	37.2	20.0	35.7	0.0
EW*	30.2	60.0	25.0	20.0
PKIJ**	30.2	0.0	53.6	10.0
IDF**	34.9	60.0	32.1	30.0
EMI	16.3	0.0	25.0	10.0
AAY	32.6	40.0	14.3	30.0
MOD	34.9	60.0	32.1	20.0
MEY	30.2	60.0	32.1	20.0
AAT***	23.3	0.0	14.3	20.0
IF	34.9	20.0	25.0	30.0

Keys: *1. ORO = Orogbo (Garcinia cola), 2. IDP = Idi pupa (Terminalia avicenniordes), 3. OGB = Ogbo (Parquetina nigrescen), 4. EW = Ewuro (Vernonia amygdalina), 5. PKIJ = Pako Ijebu (Massularia acuminata), 6. IDF = Idi funfun (Terminalia glaucescens), 7. EMI = Emi gbegiri (Pseudocedrela kotschyi), 8. AAY = Aayan (Periscopsis laxiflora, Prosopis africana), 9. MOD = Modunmoro (Distemonanthus benthamianus), 10. MEY = Meyinro, 11. AAT = Orin aata (Fagara xanthoxyloides/ Zanthoxylum xanthoxyloides), 12. IF = Ifon (Olax subscorpioide).*

*Values in parenthesis are the overall susceptibility rates of the Candida species towards the ethanolic chewing sticks' extracts * -*** = popular - most popular local chewing-sticks.*

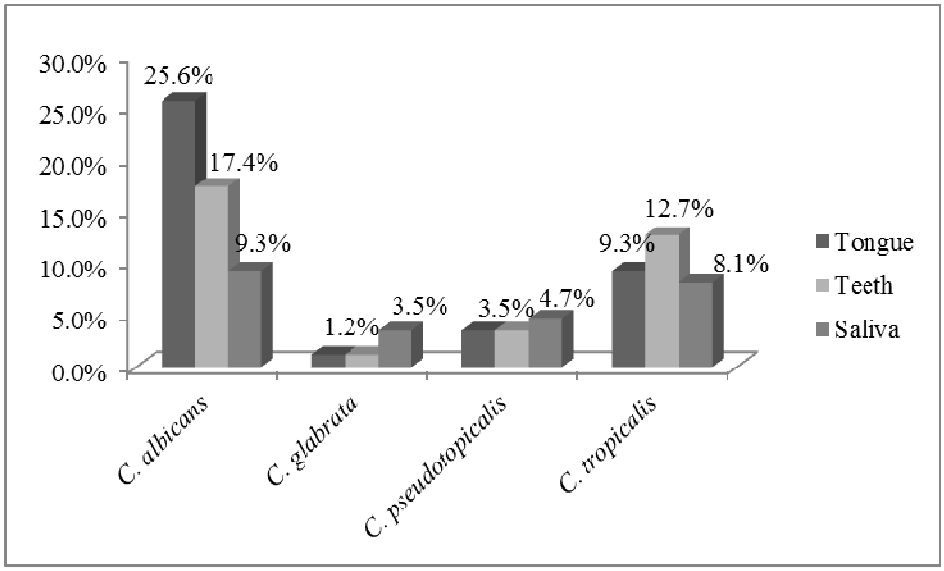

Fig. 1: Percentage recovery rates of oral *Candida* species from oral specimens

CONCLUSION

On the basis of the presented results and within the limitations of this study, we concluded significant *in vitro* inhibitory potentials of some Nigerian indigenous chewing sticks on oral *Candida* species, and that the abrasive importance of chewing sticks can aid in inhibition of oral candidal pathogens, and if incorporated into toothpastes can additionally influence the quality of oral microflora; thereby, leading to more efficacious oral hygiene.

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