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Effect of *Randia acuminate, Garcinia manii* and *Symphonia globulifera* on Oral Flora

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Abstract: *Microbial analysis of oral flora of individual from ten to sixty years was carried out alongside with sensitivity test, using routine microbiological methods. Plant extraction and phytochemical analysis was also carried out using standard chemical technique. Total Bacterial Count (TBC) obtained from male subject before mouth brushing with R.acuminata, ranged from $4.4x10^4$cfu/ml to $8.1x10^4$cfu/ml and $2.1x10^3$cfu/m1 to $3.6x10^3$cfu/ml after brushing. TBC obtained from female subject before brushing ranged from $4.4x10^4$cfu/ml to $8.1x10^4$cfu/ml and 1.7 $x10^3$ cfu/ml to 3.6 $x10^3$ cfu/ml after brushing. TBC obtained from male subject before brushing with Garcinia manii ranged from $3.9x10^4$ cfu/ml to $6.9x10^4$ cfu/ml and 2.0 $x10^3$ cfu/ml to 3.3 $x10^3$ cfu/ml after brushing. TBC obtained from female subjects ranges from 6.1 $x10^4$ cfu/ml to 8.1 $x10^4$cfu/ml and $4.2x10^3$cfu/ml to $6.1x10^3$cfu/ml after brushing. TBC obtained from male subject before brushing with Symphonia globulifera ranged from $4.1x10^4$cfu/ml to $7.1x10^4$cfu/ml and 1.2 $x10^3$cfu/ml to 4.1 $x10^3$cfu/ml after brushing. TBC obtained from female subjects range from $6.2x10^4$ cfu/ml to 8.4x10 cfu/ml and 1.2 $x10^3$ to 4.1 $x10^3$ after brushing. The predominant isolates were lactobacillus sp 25.6%, Staphylococcus sp 16.8% Enterobacter sp and Campylobacter sp 10.2% and 10.1% respectively, Strptococcuss mutant 8.0%, Corynebacteria sp 7.1%, Veillonella sp and Streptococcus oralis 4.7% and 4.2% respectively. The least were Strptococcus virans 3.9%, Fusabacterium sp 3.8%, Klebsiella sp 3.6% and Atinobacillus sp 1.9%. The sensitivity test revealed the potency of the plants, among the organism tested, the highest average zone of inhibition at 300mg/ml was obtained from Randia acuminata 19.7mm followed by Garcinia manii 10.4mm while Symphonia globulifera had the list 7.8 mm. The potency observed by these plants extract may be as a result of alkaloid, flavonoids, phlobatannins, tannins, anthroquinones, terpenes and cardiac slycosides present in the plants.*

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I. Introduction

Organs and structures in the oval cavity are infected by bacteria. Mouth serves as portal of entry into the body, the mucous membrane of the mouth and pharynx are often sterile at birth but may be contaminated by passage of the birth canal (Jaluetz *et al*., 2004). Report by Menichetti 2005, revealed that mouth habour a wide range of microorganism both aerobic and anaerobic including *staphylococci*, gram negative *diplococci*, *Brahamela cartarrhalis*, *Diptheriods* and *lactobacilli* are added when teeth begins to emerge. The anaerobic spirochetes, provotella species especially (*P. milaninogenica*), *Fusobacterium* species, Rothia species and Capnocytophage spp establish themselves together with some anaerobic vibros. Actinomyces spp are usually found in Tonsillar tissue and on adult gingival. Various protozoa may be present (Menichetti 2005). *Streptococci mutant* are one of the major colonizer of the teeth surface (Chambers 2001). Types of microorganism found in the mouth varies and it is best on the sites. People that eat sucrose diet, the bacteria that break down the sugar produces substance that help them stick to the tooth surface. In this process lactic acid bacteria produced lowers the pH in the mouth enhancing the growth of lactobacillus (Chambers 2001). Report revealed that about 1000,000,000 bacteria contains in one milliliter of saliva and other digestive enzymes, including a number of other substance especially antimicrobial compound. These include secretary 1gA, lysozyme and lactoterrin, saliva thus help to prevent colonization with potentially pathogenic bacteria (Fauci 2005).

Oral cleansing is an age long practice among human beings, the use of medicinal sticks for oral cleansing is an ongoing practice in some rural part of the world particularly in Nigeria. The medicinal plant used for oral cleansing varies from place to place depending on their medicinal value and understanding.

Randia acuminata is a commonly used chewing stick for mouth cleansing. It is a small tree of about 5mm or a shrub size flowering plant with yellow white fruit with long stem (Iwu 2014). It is a tropical plant with medicinal values. The stem is used as chewing stick in southern Nigeria, pulped root are used for treatment of dysentery through anal route (as anema), incised juice from fruit are used for eye treatment as eye drop in

Sierra Leone. The Iwu 2014 reported that *R. acuminata* has significant antimicrobial effect against oral pathogens including bacterial species. *R. acuminata* are significantly and more frequently used as medicinal plant for oral hygiene (Ajibesin *et al*., 2008) Garcinia Manii has stem (Logs), a perennial tall evergreen forest tree of about 3m-9m from family Garciniaceae usually found in Cross River, Akwa Ibom and southern Cameroon forest ecosystem (Nucitoh *et al* 2010) *G. manii* has medicinal value and has been used for treatment of mouth odors and in production of tooth brushes for traditional uses by South Eastern Nigeria, West and central African countries (Nwatoh 2010 and 2011) *Symphonia globulifera* is a timber tree occurring naturally in rainforest of tropical Africa and America along river. It is both use as ornamental plant and as medicinal plant. It is used for treatment of gonorrhea and as a diuretic in some part of Nigeria, boiled bark and root are used for the treatment of itching. The bark is also used for treatment of cough in children (Nkengfack *et al.,* 2002; Ajibesin *et al.,* 2008).

The extract of *Symphonia globulifera* exhibited antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *E. coli* (Nkengfack *et al*., 2002). Bioflavonoid and xanthories extracted from the stem of symphonia globulifera revealed effective antimicrobial activity (Mkounga *et at.,* 2009).

II. Methodology

Ethical condition upon the entrance of a compound, the family head was approach and informed of the study. Following the informed consent members of the family were sampled with questionnaires which were filled. Persons who refused consent were excluded from the study.

Sample collection: Each registered subject served with structured questionnaire and oral gargle with sterile water was collected from each subject before and after brushing the mouth in the morning.

Isolation: Serial dilution was carried out with the sample using bactopeptone, the samples were serially diluted up to 1:1000 for samples before brushing and 1:100 for sample after brushing. I milliliter from each was introduced to sterile Petri dish in triplicate as inocullum, 20ml each of media was added hence pour plate technique was applied. The nutrient agar plates were for total viable counts while the MacConkey plates were for total coliform counts, (Cheesbrough 2003). The plates were incubated in the incubator at 37^0c for 24 to 48 hours. The emerging colonies were subcultured cheesbrough (2003). Colonies were stocked for characterization and further analysis.

Characterization: Pure isolates were achieved by continous sub-culturing, 18 hours old was subjected to the following test. Gram staining for gram's reaction and morphology, motility test to detect motile organisms, catalase test for catalase producing organisms, oxidase tests for oxidase positive, urease activity, citrate utilization, indole test, MR VP test, coagulase test and carbohydrate fermentation test for acid and gas production.

Extraction: The stems of each plant were cut into piece and dried at 39^oC to dryness. The dried piece were ground to powder, 200g each were macerated in a flask with one liter of 70% ethanol for seventy two hours (72 hours). The crude extracts were filtered and the marc were discarded, the filtrates were concentrated to dryness at 40^oC using digital regulated water bath Trease and Evans (2002).

Phytochemical Screening: Basic Phytochemical Screening consists of performing simple chemical test according to Soforowa 2006 to specifically elucidate the presence of alkaloids, tannins, saponins, flavonoids, antroquinones, phlobatanins and glycosides in the plants extract.

Preparation of Concentrations of the Extracts

Stock solution was prepared from each concentrated extracts by dissolving 1g of extract in 1mg of sterile distilled water. This gave a concentration of 1000mg/ml, other concentrations were prepared by using simple dilution ratio $C_1V_1 = C_2 V_2$ to obtain 100mg/ml, 200mg/ml and 300mg/ml.

Sensitivity Test: Sterile plates of Muller Hinton agar 25ml each were prepared and allowed to set. A sterile cork borer 4mm diameter was used to burrow holes on each plate. Sterile micropipette was used to drop 5 microliter of the molten Muller Hinton agar as base for the holes. With the aid of sterile swap sticks, the test organisms were spread on the surface of the media. 0.1ml of each of the concentration 100mg/ml, 200mg/ml and 300mg/ml were introduced into the holes and incubated for 18 hours. The zone of inhibitions were read using vernier caliper. The NCCLS standard was used for interpretation of the reading.

III. Results/Discussion

It has long been noticed that mouth habours a wide range of bacteria both gram-positive and gram-negative bacteria. A total of six hundred humans were sampled in Uyo municipality before and after brushing, two hundred each with *R. acuminata, S. globulifera* and *G. manii*.

The total bacterial count obtained from male/female subjects before brushing with *Randia acuminata* ranged from 4.4 x 10^4cfu/ml to 9.5 x 10^4cfu/ml after brushing ranged from 1.7 x 10^3cfu/ml to 5.6 x 10^3cfu/ml. Total bacterial count obtained from male/female subject before brushing with *symphonia globulifera* ranged from 3.9 x 10^4cfu/ml to 9.0 x 10^4cfu/ml after brushing ranged from 2.0 x 10^3cfu/ml to 6.1 x 10^3cfu/ml while total count bacterial obtained from male/female subjects before brushing with *Garcinia manii* ranged from 3.8 x 10^4cfu/ml to 8.4 x 10^4cfu/ml after brushing 2.2 x 10^3cfu/ml to 6.9 x 10^3cfu/ml. There was no significant different at $p<0.05$ between male and female subject may be because of mode of sharing things or eating same nature of food within the municipality. The bacterial load agrees with Menichett 2005 that mouth harbor a wide range of microorganism.

In this study, a total of three hundred male subject and a total of three hundred female subject aged 10-60 residents in Uyo municipality participated, questionnaire analyses reveals common mouth and dental disorders among the studied subjects which were mouth odour, tooth decay, bleeding gum, toothache and uprooted tooth which may be due to dietary intakes or lack of proper oral hygiene. This conforms to the study conducted by Touger-Decker and Van (2003).

Much sugary food consumed by certain class of individual aid bacteria to produce acid which demineralize enamel dentin and cementum, thus may lead to the decay of gums, mouth odour and tooth removal. Left over particle in the mouth if not properly brush may also give the same effect. Some odour is as a result of decayed food particle deposited in root canal.

The investigation reveals the present of *streptococcus mutant* 8.0%, *lactobacillus* 25.6%, *Atinobacillus* 1.9%. This in line with Rogers (2008), most of the disorders and dental caries observed from individuals tooth may be as a result of habouring these organisms. *Staphylococcus* sp, *enterobacter* sp, *campylobacter* sp, *streptococcus virian, fusabacterium* sp, *veillonella* sp, *corynebacterium* sp, *streptococcus oralis and klebsiella* sp were isolated. Unhygienic condition and regular consumption of certain class of food support growth of these organisms. The attachment of these organisms to the tooth causes caries and also causes other mouth defect and diseases. From total bacterial count obtained in tables 1-6 reduction in bacterial load of the mouth was observed after brushing with each of the medicinal plant used. *Randia acuminata* gave the highest reduction followed by *Garcinia manii* and *symphonia globulifera* is the least. Local use of these medicinal plants is of great important.

The occurrence of isolates decrease with age; in table 9 more isolate were obtained from age 10-40, followed by 41-50. These age bracket is a picture of juvenile and active people who depend much on eateries and sweet pleasant foods. Table 7 shows their percentage.

Photochemical screening of the plants revealed the present of alkaloids, saponins, phlobatannins, anthroquinones, flavonoids, tannins, cardiac glycosides. These qualify the plants as medicinal plants and some of the metabolites obtained are of antimicrobial qualities. The sensitivity test of the plants with mouth isolate table II, revealed the broad spectrum effect of each of the plant. The efficacy of each of these plants on mouth isolates increases with the increase of the concentration. The most potent plant among them is *Randia acuminata*. Others also showed varied effect as reveal by their zone of inhibition.

IV. Conclusion

Having observed the present of these organisms from mouth of occupant of Uyo municipality, it is obvious that proper mouth cleaning should be emphasized. These medicinal plants especially *Randia acuminata*, which is commonly available in the area should be used for mouth cleaning because they have broad spectrum effect on the organism and will be very useful for mouth diseases and other mouth defect. Proper health education should be done concerning consumption of some food (e.g. much sugary foods both raw and prepared). Healthy mouth equals health life, Health officers should educate the public on these exposed danger so as to curb feature problem of mouth outbrake diseases. Especially those living within the municipality.

Table 1: Total bacterial count obtained from female subject before and after mouth brushing with Randia acuminuta

Ages (years)	Number involved	Average total bacterial count (cfu/ml)	
	n = 100	Before brushing	After brushing
10 - 20	34	7.8 X 10^4	2.2 X 10^3
21 - 30	26	9.5 X 10^4	5.6 X 10^3
31 - 40	16	9.1 X 10^4	3.7 X 10^3
41 - 50	15	8.7 X10^4	4.4 X 10^3
51 - 60	9	8.9 X 10^4	3.6 X 10^3

Table 2: Total bacterial count obtained from Male subject before and after mouth brushing with *Randia acuminuta*

Ages (years)	Number involved	Average total bacterial count (cfu/ml	
	n = 100	Before brushing	After brushing
10 - 20	36	6.7×10^4	2.7×10^3
21 - 30	24	8.1×10^4	3.1×10^3
31 - 40	22	7.3×10^4	3.6×10^3
41 - 50	14	4.4×10^4	2.1×10^3
51 - 60	14	6.9×10^4	1.7×10^3

Table 3: Total bacterial count obtained from female subject before and after mouth brushing with *Gacinia manii*

Ages (years)	Number involved	Average total bacterial count (cfu/ml)	
	n = 100	Before mouth brushing	After mouth Brushing
10 - 20	30	6.1×10^4	4.2×10^3
21 - 30	21	9.0×10^4	6.1×10^3
31 - 40	30	8.1×10^4	5.8×10^3
41 - 50	9	7.4×10^4	5.0×10^3
51 - 60	10	7.9×10^4	4.9×10^3

Table 4: Total bacterial count obtained from Male subject before and after mouth brushing with *Gacinia manii*

Ages (years)	Number involved	Average total bacterial count (cfu/ml	
	n = 100	Before mouth brushing	After mouth brushing
10 - 20	22	5.7×10^4	2.7×10^3
21 - 30	34	5.8×10^4	2.2×10^3
31 - 40	23	6.9×10^4	3.3×10^3
41 - 50	14	5.1×10^4	2.9×10^3
51 - 60	7	3.9×10^4	2.0×10^3

Table 5: The bacterial count obtained from Female subject before and after mouth brushing with *Symphonia globulifera*

Ages (years)	Female Subject n = 100	Before mouth brushing	After mouth brushing
10 - 20	27	6.8×10^4	3.2×10^3
21 - 30	30	7.3×10^4	3.6×10^3
31 - 40	22	8.4×10^4	6.9×10^3
41 - 50	4	7.3×10^4	4.7×10^3
51 - 60	7	6.2×10^4	3.1×10^3

Table 6: Total bacterial count obtained from male subject before and after mouth brushing with *Symphonia globulifera*

Ages (years)	Male Subject n = 100	Before mouth brushing	After mouth brushing
10 - 20	35	5.2×10^4	3.7×10^3
21 - 30	26	7.1×10^4	3.1×10^3
31 - 40	18	6.0×10^4	4.1×10^3
41 - 50	13	4.1×10^4	2.4×10^3
51 - 60	8	3.8×10^4	1.2×10^3

Table 7: Total Male and Female Subject Involved in the Study

Ages	*Garcinia manni*		*Randia acuminata*		*Symphonia globulifera*			
	Male n = 100	Female n = 100	Male n =100	Female n =100	Male n =100	Female n =100	Total	Percentage
10-20	35	27	36	34	22	30	184	30.7
21-30	26	30	24	26	34	21	161	26.8
31-40	18	22	23	16	24	30	133	22.2
41-50	13	14	13	15	12	9	76	12.7
51-60	8	7	4	9	8	10	46	7.6
Total							600	100

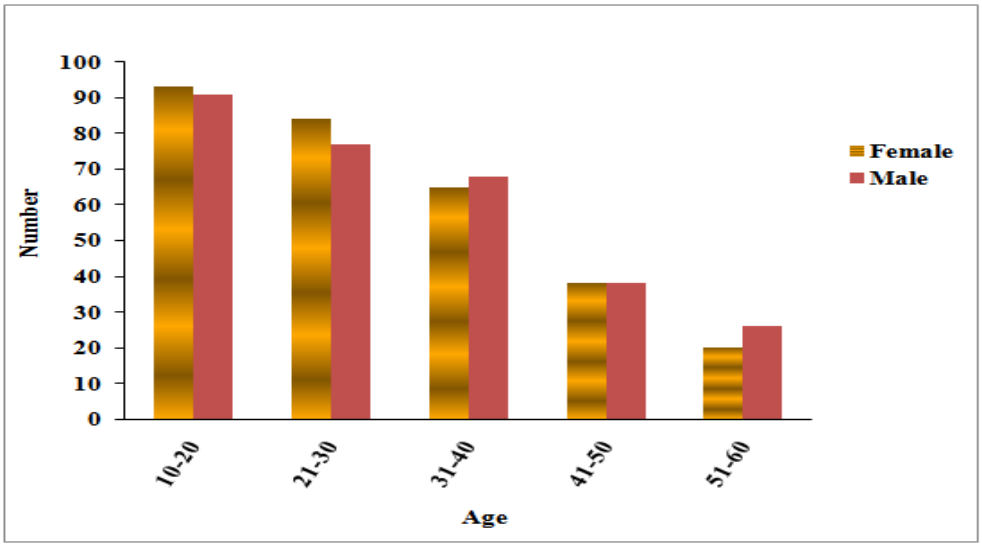

Fig. 1: Male and Female Subjects

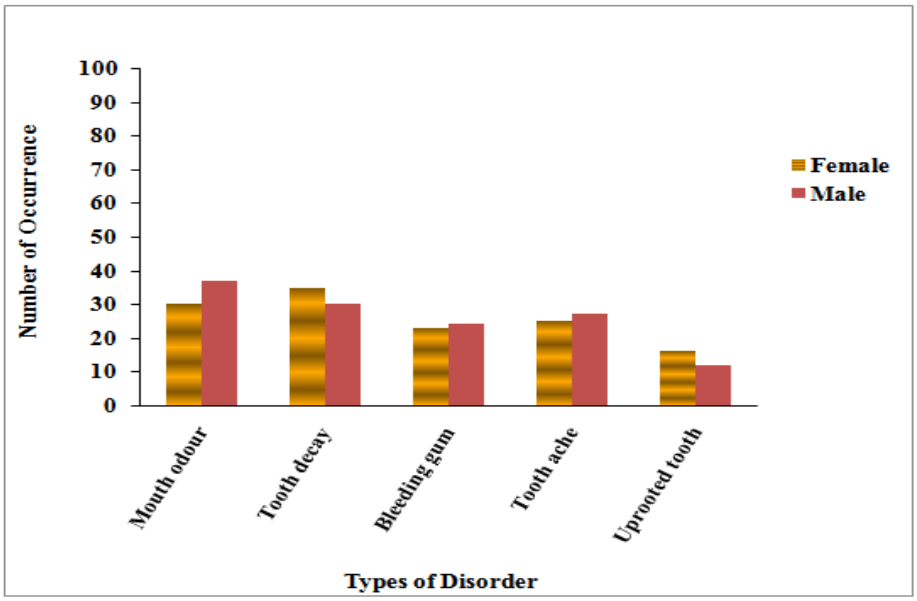

Fig. 2: Common mouth disorder associated with studied subject

Table 8: Phytochemical screening of stem bark of *R. acuminata, G. manii* and *S. globulifera*

Metabolites	Randia acuminata	Garcinia Manii	Symphonia globulifera
Saponins	+++	-	+++
Alkaloids	++	+++	++
Flavonoids	+++	+++	+++
Tannins	++	++	-
Phlobataninins	-	-	-
Anthroquinones	-	-	+++
Carcliac glycoside	++	++	-
Lieberman's test	-	-	-
Keller – killiani's test	++	+	++

+++ = High concentration , ++ = Moderate concentration
+ = Low concentration , -- = Absent

Table 9: Organisms isolated from mouth with different groups from mouth with different age group

Ages	Organism	Number Occurrence
10-20	**Staphylococcus* sp	40
	Enterobacter sp	44
	Campylobacter sp	15
	Streptococcus mutant	17
	Corynebacterium sp	21
	Veillonella sp	10
	Lactobacillus sp	61
	Streptococcus virans	4
21-30	**Streptococcus virans*	6
	Lactobacillus sp	67
	Fusabacterium sp	8
	Veillonella sp	4
	Streptococcus oralis	9
	Atinobacillus sp	6
	Campylobacter sp	17
	Enterobacter sp	21
	Staphylococcus sp	36
31-40	**Lactobacillus* sp	30
	Streptococcus oralis	10
	Fusabacterium sp	12
	Klebsiella sp	10
	Campylobacter sp	31
	Staphylococcus sp	18
	Enterobacter sp	15
	Corynebacterium sp	17
41-50	**Streptococcus virans*	15
	Klebsiella sp	13
	Lactobacillus sp	3
	Streptococcus mutant	18
	Staphylococcus sp	6
	Enterobacter sp	5
	Streptococcus mutant	7
	Corynebaderium	9
	Veilconella sp	6
51-60	**Lactobacillus* sp	2
	Fusabacterium	4
	Veillonella sp	10
	Staphylococcus sp	6
	Streptococcus mutant	16
	Campylobacter sp	1
	Corynebacterium sp	4
	Streptococcus oralis	8

Table 10 Bacteria isolated from mouth and their percentage of occurrence

Organism	Number of occurrence	Percentage of occurrence
Staphylococcus sp	107	16.8
Enterobacter sp	65	10.2
Campylobacter sp	64	10.1
Streptococcus mutant	51	8.0
Lactobacillus sp	103	25.6
Streptococcus virans	25	3.9
Fusabacterium sp	24	3.8
Veillonella sp	30	4.7
Corynebacteria sp	45	7.1
Streptococcus oralis	27	4.2
Atinobacillus sp	12	1.9
Klebsiella sp	23	3.6

Please remove this table and replace table11 below here. Table 11 is the correct table that reflect in the abstract and in discussion

Table 11: The Potency of *R. acuminata, G. manii* and *S.* globulifera on mouth isolates and their Zones of Inhibition

Isolate Organism	No Tested	Randia acuminata					Ciarcinia manil					Symphonia globulifera				
		Concentration used					**Concentration used**					**Concentration used**				
		100	**Zn**	**200**	**Zn**	**300**	**100**	**Zn**	**200**	**Zn**	**300**	**100**	**Zn**	**200**	**Zn**	**300**
Staphylococcus sp	20	18		25		30	12		18		25	0		0		10
Campylobacter sp	20	15		18		20	0		0		15	0		15		18
Corynebacterium sp	20	0		0		15	0		0		0	15		18		18
Veillonella sp	20	0		18		25	0		0		15	0		0		0
Atinobacillus sp	10	0		0		15	0		0		0	0		0		0
Enterobacter sp	20	0		0		15	0		0		10	0		0		0
Fusabacterium sp	20	0		0		0	0		0		0	0		0		0
Strept. mutant	20	0		0		20	0		10		15	0		0		0
Streptococcus oralis	10	0		0		20	0		0		15	0		0		10
Streptococcus virans	10	0		0		18	0		0		10	0		0		0
Klebsiella sp	20	14		24		30	0		0		0	0		15		22
Lactobacillus sp	20	15		22		28	15		16		20	10		15		15
Average zone of inhibition		5.2mm		8.9mm		19.7mm	2.3mm		3.7mm		10.4mm	2.1mm		5.3mm		7.8mm

Legend:Zn= zone of inhibition

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