

PHYTOCHEMICAL EVALUATION OF SELECTED PLANTS USED AS CHEWING STICKS IN OWERRI, IMO STATE, NIGERIA

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PHYTOCHEMICAL ANALYSIS AND DETERMINATION OF PHYTOCHEMICAL COMPOSITIONS OF VARIOUS EXTRACTS FROM THE TWIGS OF SOME SELECTED PLANTS USED AS CHEWING STICKS IN NIGERIA

Abstract

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Chewing sticks have been identified as potential tools for teeth cleaning and protection against dental conditions, including caries. This study determined the phytochemical composition of specific medicinal plants commonly utilized as chewing sticks by determining the qualitative and quantitative composition in various medicinal plants used as chewing sticks: bitter leaf, orange, neem, napoleanna, kolanut, and guava.

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Chewing sticks from bitter leaf, orange, neem, napoleanna, kolanut and guava plants have been used as tooth cleaner. Additionally, these chewing sticks protect against several dental conditions including caries. This study aimed to analyze the presence of various classes of phytochemicals and to determine their compositions in various extracts obtained from the twigs of these plants.

Soxhlet extraction of the plants were done using standard method and aqueous, methanolic and ethanolic extracts of the plants were used for their various phytochemicals. The investigation focused on alkaloids, flavonoids, tannins, saponins, steroids, and glycosides, examining their concentrations in aqueous, ethanolic, and methanolic extracts. Bitter leaf extracts exhibited substantial levels of alkaloids (methanolic: 47.44%, ethanolic: 43.73%, aqueous: 35.9%) and flavonoids (methanolic: 12.28%, ethanolic: 8.85%, aqueous: 5.05%). Tannin content was higher in aqueous extracts (4.92%) compared to ethanolic (4.59%) and methanolic (4.35%) extracts. Saponin levels were highest in aqueous extracts (18.83%), while steroids (0.86%) and glycosides (0.48%) were predominant in methanolic extracts. Quantitative analysis of orange extracts revealed elevated steroid and glycoside levels in methanolic extracts. Neem extracts consistently displayed high concentrations of steroids and glycosides across all solvents, with statistical analysis indicating no significant differences ($p > 0.05$). Napoleanna extracts showed variations in alkaloid (ethanolic: 0.59%), flavonoid (aqueous: 6.72%), tannin (methanolic: 0.37%), saponin (methanolic: 4.27%), steroid (aqueous: 0.22%), and glycoside (ethanolic: 0.37%) content. Kolanut extracts exhibited high alkaloid, tannin, and steroid levels across all extracts, with glycosides prominent in aqueous and ethanolic extracts. Guava extracts demonstrated varying concentrations of alkaloids (methanolic: 0.87%, aqueous: 0.18%), flavonoids (ethanolic: 1.60%, methanolic: 1.08%), tannins (methanolic: 1.09%, ethanolic: 0.83%), saponins (methanolic: 3.28%, ethanolic: 0.92%), steroids (methanolic: 1.13%, ethanolic: 0.43%), and glycosides (methanolic: 0.46%, ethanolic: 0.02%). Statistical analysis revealed no significant differences ($p > 0.05$) in the phytochemical amounts among the tested extracts for each plant.

Comment [D.P4]: Sentence not clear. Modify.

Comment [D.P5]: Sentence not clear

Comment [D.P6]: Please, omit unnecessary words

Comment [D.P7]: Format it into textual language.

Keywords: Aqueous, Extract, Ethanolic, Methnolic, Napoleanna, Phytochemical.

Comment [D.P8]: This part should be brief and concise

Comment [D.P9]: What is the conclusion based on the result?

Comment [D.P10]: Include a few plant names and methodology used

Comment [D.P11]: These are not proper key words

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Comment [D.P13]: Reference should be cited in numeric number in square bracket as [1]

1.0 INTRODUCTION

Chewing sticks are used widely in Africa and Asia as a means of maintaining good oral hygiene

(Adeoti, *et al.*, 2020). They are made from the roots, twigs or stem of a plant. The preferred part

or parts are cleaned with water to remove dirt, cut to a convenient length which varies from 15-

Follow this comments for the rest of the references.

30 cm long, and field into a bundle. The user holds one end directly in into the mouth and chews it into a fibrous brush-like fringe, which is used to scrub the surfaces of the teeth. A combination of vertical and horizontal strokes of the “brush” on tooth surfaces removes plaque.

In numerous African households, a traditional method of teeth cleaning involves chewing on the roots, twigs, or slender stems of specific plants until they form brush-like ends (Muhammad and Shinkafi, 2007). These fibrous ends are then utilized in a combination of vertical and horizontal strokes on tooth surfaces, offering a thorough method of teeth cleaning. Oral diseases stand as a significant global health concern, impacting approximately 3.9 billion people worldwide (FDI, 2015). Among these conditions, untreated tooth decay, commonly known as dental caries, emerges as the most prevalent oral health issue affecting humans. The Global Burden of Disease Study revealed that dental caries affects nearly half of the world's population (44%), making it the most widespread condition among the 354 studied from 1990 to 2017 (James *et al.*, 2018). Given the expensive nature of treatments for oral health conditions and the high prevalence and recurrent nature of these diseases, the importance of good oral hygiene becomes paramount importance.

Comment [D.P14]: Full stop is required

According to the report of Alaribeet *al.* (2022), the utilization of chewing sticks has received endorsement from the World Health Organization. This traditional oral hygiene practice has been associated with lower prevalence and incidence of dental caries in specific regions of Nigeria where its usage is prevalent. Notably, Nigeria boasts a diversity of chewing sticks derived from various plants, with reports identifying 24 different tree species employed in their production.

Comment [D.P15]: Not clear

Plants for thousands of years have been used to enhance health and for medicinal purposes. *Psidiumguajava* has an enormous wealth of medicinal value. It for long has been known for its anti-inflammatory, antimicrobial, antioxidant, antidiarrheal, and antimutagenic

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properties. Despite its widespread biologic uses, there is a dearth of information on its therapeutic effect in the treatment of periodontal disease (Rishika and Sharma, 2012). According to Joseph et al. (2016), a phytochemical investigation revealed the presence of glycosides, flavonoids, alkaloids, saponins, vitamin, tannins, carbohydrate, amino acids, and steroids in the extracts, which showed phytoconstituents in various proportions in aqueous and alcoholic extracts of seeds, fruit pulp and leaves of *Psidium guajava*. Leaf extract showed the presence of maximum number of phytoconstituents and alcoholic fruit pulp extract showed minimum constituents.

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Kola nut is the nut of kola tree (*Kola acuminata*), a genus of tree native to the tropical rainforest of Africa. Classified in the family of Malvaceae, subfamily of Stercuhoideae (or treated in the separate family Sterculiaceae) (Dark and Reed 2009). Phytochemical analysis of extracts from root, stem and seed of *Garcinia kola* and other members of the genus show that they contain reasonable amounts of phenolic compounds including biflavonoids (GB-1, GB-2), xanthenes and benzophenones (Kanomaet al., 2014). Kanomaet al., (2014) acknowledged the presence of various secondary metabolites like tannins, flavonoids, saponins, glycoside, steroids, saponins, alkaloids, cardiac glycoside, and volatile oil in the kola nut.

Comment [D.P21]: This is not a sentence

Comment [D.P22]: reported

Neem tree (*Azadirachta indica*) is a very common tree and belongs to the family Meliaceae. It is a tall evergreen tree with clear foliage originally native of India. *Azadirachta indica* is one of the most widespread introduced tree species in Nigeria. *A. indica* is extensively naturalized in drier parts of Nigeria and the most successful shade and fuel plantation tree. Analysis reveals the following phytochemicals: Alkaloid and glycoside were found to be moderately present while flavonoid and steroid were found to be present whereas saponin was not present in the qualitative analysis of neem (*Azadirachta indica*) leaf sample (Uwagwe, 2019). In the quantitative analysis,

Comment [D.P23]: *A. indica*

Comment [D.P24]: revealed the presence of

Comment [D.P25]: *A. indica*

Neem(*Azadirachta indica*) was found to contain 4.00% of saponin, flavonoid was found to contain 2.10% alkaloid was found to contain 14.5%, glycoside was found to contain 0.27%, and steroid was found to contain 0.03% (Uwague, 2019).

Comment [D.P26]: *A. indica*

Napoleonaea imperialis P. Beauv is a medicinal plant commonly found and known in South-Eastern Nigeria and local people called it as 'mkpodu'. It is a small tropical plant which belongs to the family Lecythidaceae (popularly known as 'The Brazil Nut Family') (Lawalet *al.*, 2010). Aqueous *Napoleonaea imperialis* stem bark extract had yield of 76.9g (7.69%) while methanol stem bark extract also yielded 58.5g (5.85%) when 1000g pulverized was used for each (Odeyemiet *al.*, 2023). Qualitative phytochemical analysis of this extract exhibited moderate quantities of alkaloid, steroid and terpenoid in aqueous extract while methanol extract had flavonoid, saponin and tannin also in moderate quantities. Scanty quantities of flavonoid, saponin, cardiac glycoside and tannin were seen in aqueous extract so also, scanty quantities of alkaloid, steroid, terpenoid and cardiac glycoside were seen in methanol extract (Odeyemiet *al.*, 2023).

Comment [D.P27]: *N. imperialis*

Comment [D.P28]: Introduction about *Citrus sinensis* (orange), and *Vernonia amygdalina* (bitter leaf) are not given.

Introduction on these plants are required.

The investigation sheds light on the phytochemical composition of selected plants used as chewing sticks, providing insights into the potential therapeutic effects on dental health. Understanding these traditional practices may inform oral care strategies and contribute to the preservation of cultural dental hygiene practices.

Comment [D.P29]: Please include the aim of this study here.

Comment [D.P30]: The introduction part should be written two or maximum of three paragraphs.

The introduction part is somewhat vague; remove unnecessary words and/or sentences.

2.0 MATERIALS AND METHOD

2.1 Plant Collection and Identification

Fresh plant samples of *Psidium guajava* (guava), *Cola acuminata* (Kola nut), *Azadirachta indica* (Dongoyaro), *Napoleonaea*, *Citrus sinensis* (orange), and *Vernonia amygdalina*

Comment [D.P31]: *Citrus* (*C* should be in upper case)

(bitter leaf) twigs were obtained from herbal sellers from Akabor in Mbaise and identified by a Botanist at the Federal University of Technology, Owerri.

Comment [D.P32]: Identification is not necessary for commercially available products.

2.2 Extraction and Sterilization of Aqueous Extracts of the Plants

Preparation of extracts was done according to the method reported by (Ojiuko *et al.*, 2021), with a few modifications as follows: fresh twigs from the plants were crushed using a wooden mortar and pestle and allowed to dry under shade for 3 weeks, and sterile manual grinder used to crush the twigs into powder.

2.3 Aqueous Extraction of Plant Twigs

Aqueous extracts of the plants twigs were prepared by adding 10 gm of each plant twig powder to 90 ml of deionized distilled water. The extraction process was allowed to boil for 30 minutes.

Comment [D.P33]: twigs

Comment [D.P34]: please specify the temperature

Occasional shaking of extraction flasks is done to facilitate the process. The different extractions were filtered to get an extract of 10% concentration of each type of plant twig. Finally, the filtered extracts were sterilized by passing each through a bacterial membrane filter (0.45 µm pores) under positive pressure. The filtrates were then labeled and stored under refrigeration awaiting antibacterial tests.

Comment [D.P35]: not clear; modify

Comment [D.P36]: sentence not clear

Comment [D.P37]: twigs

Comment [D.P38]: what does it mean?

2.4 Soxhlet Extraction

This was done using 95% ethanol in a Soxhlet apparatus for the three plants (Obomanu *et al.*, 2017).

Comment [D.P39]: Incomplete procedure; How much plant material? How much solvent? Reflux time? Amount of extract obtained etc...? Specify various solvents...

2.5 Phytochemical Analysis of the Plants

The aqueous and soxhlet extracts obtained from the twig of the plant were subjected to phytochemical test using standard methods (Obomanu *et al.*, 2017).

2.5.1 Test for Saponins: (Frothing Test): Some 3 ml of each extract was shaken vigorously for about 5 min; allowed to stand for 30 seconds and observed for frothing which is indicative of the presence of saponins.

Comment [D.P40]: mL: change it throughout

2.5.2 Test for Tannins: (Ferric Chloride Test): Two drops of 5% FeCl_3 were added to 1 ml of the extract. A greenish precipitate indicates the presence of tannin in the extracts.

2.5.3 Test for Glycosides: Ten milliliters (10 ml) of 50% H_2SO_4 was added to 10 ml of each extract in a test tube. The mixture was heated in boiling water for 15 minutes. Fehling's solution (10 ml) was added and the mixture boiled. A brick red precipitate if observed in all the samples, showed the presence of glycosides.

3.5.4 Test for Alkaloids: Two Drops of Mayer's reagent were added to 1 ml of each extract in a test tube and observed for a creamy precipitate indicative of the presence of alkaloid.

3.5.5 Test for Steroids (Salkowski's Test): Five (5) drops of concentrated H_2SO_4 were added to 1 ml of each extract. A red colouration observed for each extract indicated the presence of steroids.

3.5.6 Test for Flavonoids: One milliliter (1 ml) of 10% NaOH was added to 3 ml of the extracts. A yellow colouration indicated the presence of Flavonoids in each extract.

Comment [D.P41]: Experimental details for Quantitative determination of phytochemicals are missing. This is required.

3.0 Results and Discussion

Results

3.1: Qualitative Phytochemical Properties of Some Selected Plants Used as Chewing Sticks.

Results show (Table 1) that bitter leaf had alkaloid, flavonoid, and tannin in high amounts for both ethanolic and methanolic extracts respectively. On the other hand, saponin, steroid, and

Parameter	Bitter leaf	Orange	Neem	Napoleanna	Kola nut	Guava
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glycoside were in large amounts in aqueous and methanolic extracts respectively. Methanolic extracts of orange recorded high steroid and glycoside while alkaloids and glycoside were both high in aqueous and ethanolic extracts respectively. Tannin was detected in moderate quantity in aqueous and methanolic extracts but not detected in ethanolic extract. Neem had steroid and glycoside in high amount in all the extracts. Alkaloid, tannin and saponin were high in napoleanna while glycoside was not detected in aqueous and methanolic extracts of same. Alkaloid, tannin and steroid were present in high amounts in all the tested extracts of kolanut with high amount of glycoside present in aqueous and ethanolic extracts. Alkaloid was not detected in methanolic extracts of guava but was present in moderate amount in aqueous and ethanolic extracts.

Table 1 Qualitative Phytochemical Properties of Some Selected Plants Used as Chewing Sticks.

	A	E	M	A	E	M	A	E	M	A	E	M	A	E	M	A	E	M		
Alkaloid	+	+	+	+						+	+	+	+	+	+		+	+	-	
Flavonoid		+	+							+									+	+
Tannin	+			+	-	+	+	+			+	+	+	+	+	+	+	+		+
Saponin	+			+			+	+	+	+				+	+	+	+	+		+
Steroid	+	+		+			+	+	+	+				+	+	+		+	+	+
Glycoside	+				+	+	+	+	+					+	+					+
e	+				+	+	+	+	+					+	+					+

Key: + present in Moderate amount, ++ present in High amount., -Not detected

A – Aqueous extract

E – Ethanol extract

M – Methanol extract

Comment [D.P42]: ..

3.2 Quantitative Properties of phytochemicals of Bitter leaf extracts

Comment [D.P43]: properties

The quantitative phytochemicals present in bitter leaf are shown in Table 2. Methanolic extracts of alkaloids were highest with a value of 47.44%, this was followed by ethanolic extract and aqueous extract with values of 43.73% and 35.9 % respectively. A similar trend was observed for flavonoids with methanolic greater than ethanolic and aqueous extracts with values of 12.28%, 8.85%, and 5.05% for methanolic, ethanolic and aqueous extracts respectively. Values of tannin in aqueous extracts was greater in ethanolic and methanolic extracts with values of 4.92%,

	4.59% and
% concentration	4.35% for
	aqueous,
	ethanolic
	and
	methanoli

c extracts respectively. A similar trend was observed for saponin greater in aqueous extracts than in ethanolic and methanolic extracts (18.83%, 14.80% and 16.60% respectively). Methanolic extract of steroid (0.86%) was higher than ethanolic (0.68%) and aqueous (0.46%). Glycoside had values of 0.48%, 0.37%, and 0.18% for aqueous, ethanolic, and methanolic extracts respectively.

Statistical analysis showed no significant difference ($p > 0.05$) in the amount of phytochemicals produced by the various extracts.

3.3 Quantitative Values of Phytochemicals of Orange Extracts

Table 3 shows that the methanolic extract of alkaloid was higher than ethanolic extracts and aqueous extracts with values of 38.76% while 34.64% and 37.33% were recorded for ethanolic and aqueous extracts respectively. Flavonoid content was 14.73%, 16.5%, and 18.56% for aqueous, ethanolic and methanolic extract respectively. Tannin had values of 1.61%, 1.47%, and 1.03% for methanolic, aqueous, and ethanolic extracts respectively. Ethanolic extract of glycoside was 0.54% while 0.38% and 0.45% were recorded for aqueous and methanolic extracts. Statistical analysis showed no significant difference ($p > 0.05$) in the amount of phytochemicals produced by the tested extracts.

Comment [D.P44]: space required

Table 2: Quantitative Values of Phytochemicals of Bitter Leaf extracts

	Aqueous	Ethanollic	Methanolic
Alkaloid	35.29±0.61 ^a	43.73±0.59 ^a	47.44±0.39 ^a
Flavonoid	5.05±0.02 ^a	8.85±0.12 ^a	12.28±0.43 ^a
Tannin	4.92±0.43 ^a	4.59±0.51 ^a	4.35±0.81 ^a
Saponin	18.83±0.06 ^a	14.80±0.11 ^a	16.60±0.07 ^a
Steroid	0.46±0.005 ^a	0.68±0.006 ^a	0.86±0.01 ^a
Glycoside	0.48±0.01 ^a	0.37±0.006 ^a	0.18±0.015 ^a

Comment [D.P45]: How these values are obtained? What is the procedure? What is the control used to get this values?
This comments are applicable to all other values.

Values are presented as mean ± standard deviation (n = 3), values with similar alphabet superscripts are not statistically (p > 0.05) different from each other across the column.

Table 3: Quantitative Evaluation of Phytochemicals of Orange Extracts

Parameter	% concentration		
	Aqueous	Ethanollic	Methanolic
Alkaloid	33.33±0.57 ^a	34.64±0.55 ^a	38.76±0.33 ^a
Flavonoid	14.73±0.37 ^a	16.5±0.41 ^a	18.56±0.37 ^a
Tannin	1.47 ±0.02 ^a	1.03 ±0.01 ^a	1.61 ±0.05 ^a
Saponin	0.87±0.02 ^a	1.39±0.05 ^a	2.64±0.02 ^a
Steroid	0.36±0.01 ^a	0.25±0.01 ^a	0.361±0.01 ^a
Glycoside	0.38±0.01 ^a	0.54±0.01 ^a	0.45±0.01 ^a

Comment [D.P46]: How these values are obtained? What is the procedure? What is the control used to get this values?
This comments are applicable to all other values.

Values are presented as mean ± standard deviation (n = 3), values with similar alphabet superscripts are not statistically (p > 0.05) different from each other across the column

3.4 Quantitative Values of Phytochemicals of Neem

The quantitative phytochemicals of Neem as presented in Table 4 show that alkaloid in ethanolic extract was lowest (0.69%) while 0.79% and 0.87% were recorded in aqueous and methanolic

extracts respectively. Flavonoid content was 1.00%, 1.41%, and 1.45% for aqueous, ethanolic and methanolic extracts respectively. Tannin had values of 2.23%, 2.45%, and 2.51% for aqueous, ethanolic, and methanolic extracts respectively.

High value of Saponin was recorded in aqueous extracts (8.59%) while methanolic and ethanolic extracts had values of 7.80% and 6.38% respectively. Steroids in Methanolic extracts was highest (0.33%), while aqueous and ethanolic extracts had the same value (0.28%).

Statistical analysis showed no significant difference in the amount of phytochemical produced by the extracts.

Table 4: Quantitative Values of Phytochemicals of Neem

Parameter	% concentration		
	Aqueous	Ethanolic	Methanolic
Alkaloid	0.79±0.08 ^a	0.69±0.01 ^a	0.87±0.04 ^a
Flavonoid	1.0±0.02 ^a	1.41±0.06 ^a	1.45±0.45 ^a
Tannin	2.23±0.46 ^a	2.45±0.45 ^a	2.51±0.34 ^a
Saponin	8.59±0.04 ^a	7.80±0.07 ^a	6.28±0.02 ^a
Steroid	0.28±0.01 ^a	0.28±0.02 ^a	0.33±0.20 ^a
Glycoside	0.77±0.21 ^a	0.65±0.02 ^a	0.33±0.29 ^a

Comment [D.P47]: How these values are obtained? What is the procedure? What is the control used to get this values? This comments are applicable to all other values.

Values are presented as mean ± standard deviation (n = 3), values with similar alphabet superscripts are not statistically (p > 0.05) different from each other across the column

3.5 Quantitative Values of Phytochemicals of Napoleanna

In Napoleannaethanolic extract, alkaloid was 0.59% while 0.46% and 0.45% were recorded in Aqueous and Methanolic extracts (Table 5). Flavonoid content was 6.72%, 3.53% and 2.79% for

Aqueous; Ethanolic and Methanolic extracts respectively. Tannin values were 0.37%, 0.37% and 0.43% for Methanolic, Ethanolic and Aqueous extracts respectively. Saponin values was highest (4.27%) in methanolic extract followed by Ethanolic (3.66%) while Aqueous had the lowest (3.55%). Steroid values were 0.22%, 0.23% and 0.24% for Aqueous, Ethanolic and Methanolic extracts respectively. Glycoside was only detected in Ethanolic (0.37%). Statistical analysis showed no significant difference in the amount of phytochemicals produced by the extracts.

Table 5: Quantitative Values of Phytochemicals of Napoleanna

Parameter	% concentration		
	Aqueous	Ethanolic	Methanolic
Alkaloid	0.46±0.03 ^a	0.59±0.06 ^a	0.45±0.02 ^a
Flavonoid	6.72±0.11 ^a	3.53±0.66 ^a	2.79±0.09 ^a
Tannin	0.43±0.01 ^a	0.37±0.02 ^a	0.37±0.02 ^a
Saponin	3.55±0.04 ^a	3.66±0.41 ^a	4.27±0.05 ^a
Steroid	0.22±0.01 ^a	0.23±0.02 ^a	0.24±0.01 ^a
Glycoside	-	0.37±0.02 ^a	-

Comment [D.P48]: How these values are obtained? What is the procedure? What is the control used to get this values? This comments are applicable to all other values.

Values are presented as mean ± standard deviation (n = 3), values with similar alphabet superscripts are not statistically (p > 0.05) different from each other across the column

3.6 Quantitative Values of Phytochemicals of Kola Nut

As shown in Table 6, ethanolic extract recorded highest value of Alkaloid (4.6%) followed by methanolic (4.34%) while Aqueous was lowest (0.80%). Flavonoid values were 2.30%, 1.81%, and 3.45% for Aqueous, Ethanol and methanol respectively. Tannin had values of 0.46%, 0.44%, and 0.36% for Methanolic, Ethanolic, and Aqueous extracts respectively. The saponin value of ethanolic extracts was 0.63% while 2.34% and 3.12% for methanolic and aqueous extracts

respectively. Steroids had values of 3.41%, 2.70%, and 1.87% for methanolic, ethanolic, and aqueous extracts respectively. The ethanolic extract value of glycoside was 0.23% while 2.46% and 1.24% were recorded for methanolic and aqueous extracts respectively. Statistical analysis showed no significant difference in the amount of phytochemicals produced by the extracts.

Table 6: Quantitative Values of Phytochemicals of Kola Nut

Parameter	% concentration		
	Aqueous	Ethanolic	Methanolic
Alkaloid	0.80 ± 0.00 ^a	4.6±0.00 ^a	4.34 ± 0.43 ^a
Flavonoid	2.30 ± 0.41 ^a	1.81±0.00 ^a	3.45±1.67 ^a
Tannin	0.36±0.02 ^a	0.44±0.00 ^a	0.46±0.01 ^a
Saponin	3.12±1.34 ^a	0.63±0.01 ^a	2.34±1.56 ^a
Steroid	1.87±0.45 ^a	2.70±0.11 ^a	3.41±0.68 ^a
Glycoside	1.24±1.43 ^a	0.23±0.01 ^a	2.46±0.35 ^a

Comment [D.P49]: How these values are obtained? What is the procedure? What is the control used to get this values? This comments are applicable to all other values.

Values are presented as mean ± standard deviation (n = 3), values with similar alphabet superscripts are not statistically ($p > 0.05$) different from each other across the column

3.7 Quantitative Values of Phytochemicals of Guava Extracts

Table 7 shows the quantitative phytochemical present in Guava extract. Aqueous extract of alkaloid (0.18%) was the lowest while methanolic extract (0.87%) was the highest. Flavonoid extracts had values of 1.25%, 1.60%, and 1.08% for aqueous, ethanolic and methanolic extracts respectively. Tannin had values of 0.54%, 0.83%, and 1.09% for aqueous, ethanolic and methanolic extracts respectively. Values of 0.92%, 3.28%, and 0.18% were recorded in methanolic, ethanolic, and aqueous extracts respectively for Saponin. Steroid values were 0.28%,

0.43%, and 1.13% in aqueous, ethanolic and methanolic extracts respectively. Glycosides had values of 0.19%, 0.02%, and 0.46% for aqueous, ethanolic, and methanolic extracts respectively.

Statistical analysis showed no significant difference in the amount of phytochemicals produced by the extracts.

Table 7: Quantitative Values of Phytochemicals of Guava Extracts

Parameter	% concentration		
	Aqueous	Ethanolic	Methanolic
Alkaloid	0.18±0.45 ^a	0.21±0.23 ^a	0.87±0.34 ^d
Flavonoid	1.25±0.23 ^a	1.60±0.00 ^a	1.08±0.65 ^a
Tannin	0.54±0.42 ^a	0.83±0.00 ^a	1.09±0.36 ^a
Saponin	0.18±0.71 ^a	3.28±0.12 ^a	0.92±1.76 ^a
Steroid	0.28±0.45 ^a	0.43±0.01 ^a	1.13±0.45 ^a
Glycoside	0.19±0.78 ^a	0.02±0.01 ^a	0.46±0.36 ^a

Comment [D.P50]: How these values are obtained? What is the procedure? What is the control used to get this values? This comments are applicable to all other values.

Values are presented as mean ± standard deviation (n = 3), values with similar alphabet superscripts are not statistically ($p > 0.05$) different from each other across the column

Discussion

Qualitative Properties of Selected Plants used as Chewing Stick

Phytochemicals are non-nutritive plant chemicals that have protective or disease-preventive properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect against diseases (Gajalaksmiet *al.*,2012).

Comment [D.P51]: modify the sentence

The preliminary phytochemical screening revealed the presence of alkaloids, tannins, steroids, flavonoids, saponins, and alkaloids. Flavonoids and tannins have been reported to be antioxidants used to neutralize highly unstable and extremely reactive molecules like free radicals that attack the cells of the human body (Karthishwaran *et al.*, 2010). *V. amygdalina* contains a wide variety of secondary metabolites or compounds such as tannins terpenoids, alkaloids, and flavonoids; that dictate the therapeutic potency of the plants (Suleet *et al.*, 2010). The use of *V. amygdalina* stem for chewing helps inhibit mouth microbes; this may be due to the presence of these phytochemicals (Onwuliri, 2004). The presence of these phytochemicals in neem is in line with the reports of various authors (Barua *et al.*, 2017; Nagini *et al.*, 2021; Singhet *et al.*, 2020).

Comment [D.P52]: modify the sentence

Phytochemicals are biologically active plant constituents that are naturally present in plants. It is believed that phytochemicals take part in resisting disease formation in plants (Hanyet *et al.*, 2015). The phytochemical found in an orange extract from this study is not synonymous with the findings of Abdallah (2020) who revealed the presence of alkaloids, flavonoids, and steroids but synonymous with the findings of Nata'ala *et al.* (2018), who reported the absence of tannins. The presence of these compounds gives an insight into the medicinal importance of the stems of the tree, as flavonoids have been reported to have antibacterial and antimicrobial properties (Abdallah, 2020).

Comment [D.P53]: Coherency is required in dealing with same concept. The first three sentences of the above paragraph convey the same message.

Qualitative analysis carried out on the extracts of *N. imperialis* showed the presence of important phytochemical constituents. Tannins and saponins were the major phytochemical constituents present in the plant in a relatively high amount while glycoside was absent in aqueous and methanolic extracts. This finding is in line with the report of Etimet *et al.* (2017).

Comment [D.P54]: What do you mean by important phytochemicals?

Quantitative Properties of Selected Plants used as Chewing Stick

Alkaloid, flavonoid, saponin, tannin, steroid and glycoside were detected in the bitter leaf plant

sample in the three solvents used. The values obtained in this research are slightly higher than those reported by Aliet *al.*(2020) for leaves of *V. amygdalina* purchased from Sabon-Gari Market in Kano. The amount of alkaloid in all the extracts is not significant, though it is highest in methanolic extract. Alkaloids are present not only in human daily life in food and drinks but also as stimulant drugs. They have shown anti-inflammatory, anticancer, analgesics, local anesthetic and pain relief, neuropharmacology, antimicrobial, antifungal, and many other activities.

The present study shows that saponin with aqueous extracts was greater than ethanolic and methanolic extracts with values of 18.83%, 14.80%, and 16.60% for aqueous, ethanolic, and methanolic extracts respectively. Methanolic extract of steroid (0.86%) was higher than ethanol (0.68%) and aqueous (0.46%). This finding is in line with the report of Anibijuwon *et al.*(2012) who had the aqueous extract highest for saponin. Glycoside had values of 0.48%, 0.37%, and 0.18% for aqueous, ethanolic, and methanolic extracts respectively.

According to Sujata, (2018), the highest concentration of alkaloids %, flavonoids %, terpenoids %, and saponins % was seen in the leaves of *A. indica* than in the Stem-bark and root. Biuet *al.*(2009) reported that the aqueous leaf extract of *A. indica* possesses a higher amount of saponins and a low quantity of alkaloids which corroborates with this finding. The phytoconstituents alkaloids, glycosides, flavonoids, and saponins are antibiotic principles of plants. These antibiotic principles are actually the defensive mechanism of the plants against different pathogens. The result was also supported by Sujata (2018).

The variation in phytochemical content observed across different solvent extracts of *Napoleanna* underscores the significance of the extraction method in studying the chemical composition of medicinal plants. This diversity in chemical constituents can be attributed to the

Comment [D.P55]: Combine all paragraphs in this title into two paragraphs and precisely summarise the concepts.

varying solubility of compounds in different solvents, reflecting the selectivity of each solvent for specific classes of phytochemicals. For instance, the higher alkaloid content in the Ethanolic extract suggests that ethanol is more effective in extracting alkaloids from *Napoleanna* compared to the Aqueous and Methanolic solvents. Similarly, the Aqueous extract exhibited the highest flavonoid content, indicating its efficacy in extracting these compounds from the plant material. These findings emphasize the importance of carefully selecting the extraction solvent based on the targeted phytochemicals in a study.

In an earlier study, it had been confirmed that the tested chewing stick contained antibacterial compounds and that the exhibited bioactivities of these chewing sticks were consequent upon the phytochemicals that they contained (Ogundiya *et al.*, 2006). Results from the present study seem to suggest the phytochemical that is most possibly contributing to the exhibited antibacterial activities. The presence of tannin in chewing sticks has been suggested to be conferring antimicrobial activities to the chewing stick that contains it (Ogundiya *et al.*, 2006).

The importance of solvent selection in phytochemical studies is underscored by the findings of the quantitative phytochemical evaluation of Kola nut. The varying affinities of different solvents for specific classes of compounds, such as alkaloids, flavonoids, tannins, saponins, steroids, and glycosides, highlight the need for careful consideration when choosing extraction methods. In the present study, phytochemical screening showed that methanolic extracts of kola nut contain most of the phytochemicals like alkaloids, tannins, saponins, and glycosides. Several investigators are unanimous in observing that the medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body (Akinpelu *et al.*, 2008; Miret *et al.*, 2016). Phytochemical tests showed that aqueous, ethanolic, and methanolic extracts of kola nut contained alkaloids, phenols, tannins, flavonoids, glycosides, and saponins which could

be regarded as the active principles that confer antimicrobial properties to this plant. Possession of alkaloids by some plants for instance, has been shown to give distinct fungicidal activities and most likely to give the plants a specific advantage against fungal attacks in the humid rain forest where such plants exist (Omwirhirenet *al.*,2016). Also, Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include the formation of foams in aqueous solutions, hemolytic activity, cholesterol-binding properties (Eleazu *et al.* 2012; Mir *et al.*, 2016), and bitterness (Omwirhirenet *al.*, 2016).

The quantitative phytochemical components of extracts of *Guava* in this study revealed various amounts of phytochemicals. Studies have shown that the results of phytochemical analysis may differ because of various factors such as biochemical reactions within species, plant genotypes, developmental stages, and geographical locations. Furthermore, variations in extraction methods are usually found in the length of the extraction period, pH, temperature, particle size, and solvent-to-sample ratio (Taura *et al.*, 2014; Oncho *et al.*, 2021).

Conclusion

The identification of various phytochemicals in the tested plant extracts, such as flavonoids, tannins, saponins, and steroids, suggests that these plants may have health-promoting properties. These compounds have been associated with potential antioxidant, anti-inflammatory, and antimicrobial effects. The detection of phytochemicals in these plants supports their traditional medicinal use. It provides a scientific basis for the therapeutic effects attributed to these plants in folk medicine and traditional healing practices. This can lead to the development of new herbal remedies or pharmaceutical drugs. These findings may help preserve and validate traditional knowledge about the medicinal properties of these plants, bridges the gap between traditional and modern medicine.

Comment [D.P56]: May have... what does it mean?
Please provide your concrete statement based on your research and previous reports

Comment [D.P57]: Not clear.

Comment [D.P58]: The conclusion part should be modified.

In the title, you have cited that these plants are used as chewing sticks.

Justify that these chewing sticks are having tooth cleaning property based on your research and previous reports.

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Comment [D.P59]: Year should be placed after the journal name.

Example:
ClinExp Allergy. 2002;32(4):489-98.

Comment [D.P60]: Abbreviations are required. Full journal name is not required.

Also note that italics not necessary.

Example:
ClinExp Allergy. 2002;32(4):489-98.

Follow these comments for all references.

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