Qualitative and quantitative phytochemical evaluation of *Quassia undulata* (Guill. & Perr.) D. Dietr. leaves using different solvent polarities

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This study investigated solvent effects on the phytochemical composition of *Quassia undulata* leaves a medicinal plant used in treating arrays of diseases including fever and cough. The leaves were collected, washed, air-dried, pulverized and evaluated for some inherent phytochemicals using four different solvent systems based on their polarities. The solvents are methanol, acetone, ethyl acetate and chloroform. The methanol extract was found to have the highest number of secondary metabolites (saponins, tannins, flavonoids, steroids, coumarins, anthraquinones, alkaloids and phenols). None of the extracts tested positive for the presence of phlobatannins, terpenoids and emodins. The methanol extract was further analyzed quantitatively for some of the determined phytochemicals. Tannins had a concentration of 3.131 mg of catechin equivalents per 100 mg sample (mg CE/100g), alkaloids - 5.200 %, total phenolics - 11.828 mg of gallic acid equivalents per gram of extract (mg GAE/g), flavonoids - 8.074 mg of quercetin equivalents per gram of extract (mg QE/g) while 0.673 % saponins were detected. The presence of these secondary metabolites might justify the ethnomedicinal uses of *Quassia undulata* leaves as their bioactivity has been found to be dependent on the solvent used for extraction.

Key words: *Quassia undulata*; secondary metabolite; medicinal plant; solvent system

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1. INTRODUCTION

Plants are known to produce a wide variety of chemical compounds which do not contribute to their growth and development directly. These chemicals are termed secondary metabolites (Asadi-Samani et al., 2016) or phytochemicals (Shakya, 2016) and have been channeled into curing a wide spectrum of diseases such as cancer, diabetes, neurological disorders, atherosclerosis, cardiovascular diseases and malaria to name a few (Asadi-Samani et al., 2016; Odubanjo et al., 2018a). The use of plants for medicinal and therapeutic purpose is referred to as herbal or phytomedicine and is presently practiced worldwide. WHO has recognized herbal medicine as an important component of primary health care (Shakya, 2016) and active ingredients of some drugs have been extracted from plants. *Quassia undulata* (Guill. & Perr.) D.Dietr. commonly called Oriji by the Yoruba ethnic group of Nigeria and Akan-asante hotoro by Ghanaians is a perennial shrub or a small to fairly large tree of the family Simaroubaceae (Adeniyi and Lawal, 2020; Odubanjo et al., 2018a). It grows in grasslands of subtropical and tropical Africa, America, Australia and Asia (Iko and Eze, 2012). In Africa, the decoctions of its bark or root are used in treating fever, stomach complaints, cough, leprosy, insanity or dementia. A decoction of its leaves is used in treating ankylosis, rickets and varicose veins (Adeniyi and Lawal, 2020; Gyakari and Cobbinah, 2008; Odubanjo et al., 2018b). The fruit is used against head lice, and the seed is used in treating fever in Nigeria although it is considered poisonous in other places. Quassinoids isolated from the plant have been found to have antitumour and antimalarial activities (Ajaiyeoba et al., 1999). Extracts of its leaves and stems possess antibacterial and antifungal activities (Gyakari and Cobbinah, 2008). Ajayeoba et al. (1995) also reported the antimicrobial activity of crenatine (an alkaloid isolated from *Q. undulata*) against strains of *Staphylococcus aureus* and *Bacillus cereus*. The vast array of therapeutic or medicinal properties of medicinal plants can be linked to the presence of phytochemicals.

This study aimed to determine the effect of different solvents extraction on the phytochemical constituents of the leaves of *Quassia undulata*. A good solvent gives optimal extraction and conserves the chemical structure of desired compounds

(Thouri et al., 2017). The type and polarity of different solvents usually determine the quantity, quality, toxicity, biological activities and biosafety of extracts (Wakeel et al., 2019). The solvents used in this study in the order of decreasing polarity are methanol > acetone> ethyl acetate > chloroform.

2. MATERIALS AND METHODS

2.1. Sample Collection

Fresh leaves of *Quassia undulata* were collected from the herbal garden of Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo state, Nigeria. Plant identification and authentication was carried out at the Forest Herbarium Ibadan with voucher number FHI-102099.

2.2. Preparation of extracts

The leaves were washed by placing them under running water, they were air dried at room temperature for about two weeks; the dried samples were pulverized and stored in an air tight container prior to extraction. Amount of 20 g of the sample was soaked in 100 mL of each of the solvents in the ratio 1:5 (Fayinminnu et al., 2017). The mixture was stirred at intervals and filtered after 72 hours (Osibemhe and Onoagbe, 2015); the solvents were removed by means of a rotary evaporator at 40 °C under reduced pressure (Adeniyi, 2019). The concentrated extracts were stored in airtight bottles at 4 °C until they were screened.

2.3. Qualitative phytochemical analysis

This was carried out using the following standard procedures:

2.3.1. Test for saponins

Amount of 5 mL of each extract was added to 5 mL of distilled water and shaken vigorously. It was warmed in a water bath and the formation of a stable persistent froth indicates the presence of saponins (Rohit, 2015).

2.3.2. Test for tannins

This was carried out using Braymer's test. Amount of 2 mL of each extract was dissolved in 5 mL of distilled water, it was filtered and to the filtrate, 2-3 drops of 5 % $FeCl_3$ was added. The presence of green precipitate indicates the presence of tannins (Mir et al., 2016; Yadav et al., 2014).

2.3.3. Test for flavonoids

This was carried out using lead acetate test as described by Rohit (2015). Amount of 1 mL of each extract was added to 1 mL of 10 % lead acetate solution. Formation of a yellow precipitate indicates the presence of flavonoids.

2.3.4. Test for steroid

Salkowski test was used to determine the presence of steroids. Amount of 2 mL of each extract was added to 2 mL of chloroform and 2 mL of conc. H_2SO_4.Production of a reddish brown ring at the junction shows the presence of steroids (Yadav et al., 2014).

2.3.5. Test for phlobatannin

Amount of 2 mL of 1 % HCl was mixed with 2 mL of each extract; it was boiled in a water bath for about 5 minutes. Presence of red precipitate shows positive result for presence of phlobatannins (Osibemhe and Onoagbe, 2015; Yadav et al., 2014).

2.3.6. Test for terpenoids

Amount of 2 mL of chloroform was added to 5 mL of each extract and 3 mL conc. H_2SO_4 was also added carefully. Reddish brown color at the interface shows the presence of terpenoids (Mir et al., 2016).

2.3.7. Test for coumarins

To 2 mL of each extract, 3 mL of 10 % NaOH was added. Yellow coloration indicates the presence of coumarins (Yadav et al., 2014).

2.3.8. Test for emodins

Amount of 2 mL of each extract was mixed with 2 mL NH_4OH and 3 mL benzene, red coloration shows the presence of emodins (Yadav et al., 2014).

2.3.9. Test for anthraquinone

Borntrager's test as described by Yadav et al. (2014) was used to check for the presence of anthraquinones. Amount of 3 mL of each extract was added to 3 mL of benzene and 5 mL of 10 % ammonia. A pink color in the ammonical layer indicates the presence of anthraquinones.

2.3.10. Test for anthocyanins

Amount of 2 mL of each extract was added to 2 mL of 2N HCl and ammonia. A pinkish red coloration shows the presence of anthocyanins (Yadav et al., 2014).

2.3.11. Test for alkaloids

Amount of 2 mL of each extract was added to few drops of Hager's reagent, a yellow precipitate shows positive result for presence of alkaloids (Yadav et al., 2014).

2.3.12. Test for cardiac glycosides

Legal's test was used to test for glycosides; 2 mL of extract was mixed with 3 mL chloroform and 10 % NH_3 solution. Pink coloration shows a positive result (Osibemhe and Onoagbe, 2015).

2.3.13. Test for phenols

Amount of 2 mL of each extract was added to 2 mL of 5 % aqueous ferric chloride. Appearance of blue color indicates the presence of phenols (Prabhavathi et al., 2016).

2.4. Quantitative phytochemical screening

Based on the result of the preliminary phytochemical analysis, the content of secondary metabolites (phenols, tannins, saponins, flavonoids and alkaloids) present in the methanol extract of *Q. undulata* leaves was determined.

2.4.1. Determination of total phenolics

The total phenolic content was determined using the method of Singleton et al. (1999) as described by Stanković (2011). Amount of 0.5 mL of methanolic solution of the extract was mixed with 2.5 mL of 10 % Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5 % $NaHCO_3$. The blank was made up of 0.5 mL methanol, 2.5 mL of 10 % Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5 % $NaHCO_3$. They were incubated at 45 °C for 45 minutes and absorbance was read on a spectrophotometer at 765 nm. A similar procedure was carried out for standard solutions of gallic acid and calibration curve was constructed. The concentration of phenolics was read from the calibration curve and total phenolics present in the extract were expressed in terms of mg of gallic acid equivalent per gram of extract (mg GAE/g) Stanković (2011).

2.4.2. Determination of tannins

The tannin content was determined according to the modified vanillin-HCl method described by Lawal et al. (2015) and Omoruyi et al. (2012). The vanillin-HCl reagent was prepared just before use by mixing equal volumes of 8 % HCl and 1 % vanillin in methanol. Amount of 10 mL of 1 % concentrated HCl in methanol was added to about 0.2 g of ground sample in a conical flask. The flask was stoppered and continuously shaken for 20 minutes; the content was further centrifuged at

2500 rpm for 5 min. About 1.0 mL of the supernatant was transferred into a test tube containing 5 mL of vanillin-HCl reagent. It was incubated at 30 °C for 20 minutes and absorbance was read at 450 nm. The tannin content was expressed as mg of catechin equivalents per 100 mg of sample.

2.4.3. Determination of saponins

This was conducted using the method of Obadoni and Ochuko (2002) as described by Biradar and Rachetti (2013) with slight modifications. Amount of 50 mL of 20 % aqueous methanol was added to 10 g of sample in a conical flask. This was placed on hot water bath (about 55 °C) for 4 h with continuous stirring. It was then filtered and the residue re-extracted using 100 mL of 20 % aqueous methanol. The extracts were combined and reduced to 40 mL on water bath at 90 °C. The concentrate was poured into a separating funnel and 10 mL diethyl ether was added to it and shaken vigorously. The ether layer was discarded while the aqueous layer was recovered and purification process was repeated. Amount of 30 mL of *n*-butanol was added and the *n*-butanol extracts were washed twice with 10 mL of 5 % aqueous NaCl. The remaining solution was heated to evaporation on water bath, the samples were then dried in the oven to a constant weight and saponin content was calculated as a percentage (Biradar and Rachetti, 2013).

2.4.4. Determination of flavonoids

This was measured using aluminium chloride colorimetric assay. Amount of 1 mL of extract and 4 mL of distilled water were added to a volumetric flask; 0.3 mL of 5 % $NaNO_2$ was added and after 5 minute, 0.3 mL of 10 % $AlCl_3$ was also added. Amount of 2 mL of 1 M NaOH was added after 5 minutes and the content of the flask was made up to the 10 mL mark with distilled water. Standard solutions of quercetin at 20, 40, 60, 80 and 100 µg/mL were prepared in the same manner, absorbance for test and standard solutions were read against the blank at 510 nm on a spectrophotometer. Total flavonoid content was expressed as mg of quercetin equivalents per gram of extract (Mythili et al., 2014).

2.4.5. Determination of alkaloids

This was conducted using the method of Harborne (1984) as described by Biradar and Rachetti (2013) with slight modifications. To 5 g of sample in a 250 mL beaker, 200 mL of 10 % acetic acid in methanol was added; it was covered and allowed to stand. After 4 h, it was filtered and the filtrate was concentrated on a water bath to about one-quarter of its original volume. Conc. NH_4OH was added to the extract dropwisely until precipitation stopped. The solution was allowed to settle, precipitate was collected and washed with dilute NH_4OH and then filtered. The residue was dried and weighed as the percentage of alkaloids (Biradar and Rachetti, 2013).

3. RESULTS

3.1. Qualitative analysis

The result of the qualitative phytochemical constituent of the four different leaf extracts of *Quassia undulata* is presented in Table 1. It shows the presence of numerous secondary metabolites which are of great medicinal importance. Steroids, coumarins, anthraquinones, and alkaloids were present in all the four extracts, while phlobatannins, terpenoids and emodins were absent in all the extracts. The methanolic extract contained the highest number of phytochemicals, followed by the acetone extract, while the ethyl acetate and chloroform extracts had similar composition. Extraction of the phytochemicals with solvents of different polarity resulted in the identification of diverse chemical constituents in each of the extracts.

Generally, polar solvents provide optimum extraction of polyphenols when compared to non-polar solvents (Abbas et al., 2017; Abdel-Shafy and Mansour, 2017). This could be due to the interaction of the hydrogen bonds between their polar sites and those of the polyphenols (Thouri et al., 2017). This explains the extraction of these metabolites by the polar solvents (methanol and acetone) used in this study. Ethyl acetate, a semipolar solvent can dissolve sterols, alkaloids and glycosides (Widyawati et al., 2015).

Saponins which were detected only in the methanol extract have been found to have antidiabetic, antiatherosclerotic, anti-HIV and gastroprotective effects. They also help in liver function and reduction of blood cholesterol (Chukwuebuka and Chinenye, 2015). Tannins, present in the methanol and acetone extracts inhibit HIV replication; have antibacterial and antiparasitic effects (Chukwuebuka and Chinenye, 2015; Lü et al., 2004). They have also been found to possess antioxidant and antiinflammatory effects (Osibemhe and Onoagbe, 2015). Flavonoids present only in the methanol extract have anticarcinogenic and antioxidant effects (Chukwuebuka and Chinenye, 2015; Yadav et al., 2014). Steroids which exhibit analgesic properties, and also influence activities of the central nervous system (Mir et al., 2016) were present in all the four extracts. Coumarins which have a wide array of medicinal properties ranging from antimicrobial, antidiabetic, antioxidant to antiinflammatory effects were found in all the extracts. They also have inhibitory action on enzymes and vitamin K (Poumale et al., 2013). Anthraquinones and alkaloids were present in all the extracts. The former have laxative effects which gives constipation relief (Bolen, 2020), while the latter protect against chronic diseases (Mir et al., 2016), malaria (Nafiu et al., 2013) and inflammation (Kumar et al., 2008). Anthocyanins are antioxidants that have been found to reduce the risk of heart disease and fight obesity (He and Giusti, 2010). Cardiac glycosides are used in treating congestive heart failure and cardiac arrhythmia. Phenols present in acetone and methanolic extracts have been found to have antioxidant, antiviral, hypotensive and antimicrobial properties (Chukwuebuka and Chinenye, 2015). Absence of phlobatannins, terpenoids and emodins in all the extracts suggests the absence of their therapeutic effects in the plant studied. Most of the phytochemicals detected have antinutritive properties such as enzyme inhibition, nausea, vomiting, diarrhea, paralysis, toxicity and death when taken in excess. Hence, they should be administered with caution and at reduced doses.

3.2. Quantitative analysis

The result of the quantitative analysis carried out on the methanol extract for the content of some secondary metabolites is shown in Table 2 below. The extract was found to contain total phenolics – 11.828 mg GAE/g, tannins – 3.131 mg CE/100 g, flavonoids – 8.074 mg QE/g, saponins – 0.673 % and alkaloids – 5.200 %.

The total phenolics content detected in this study - 11.828±0.269 mg GAE/g is slightly lower than 12.51±0.32 mg GAE/g detected by Odubanjo et al. (2018b). They also detected a total flavonoid of 7.62±0.12 mg QE/g in the aqueous leaf extract of *Quassia undulata*. Anusha and Sudha (2017) who analyzed the phytochemical profile and antimicrobial potential of methanol and aqueous extracts of bark and leaf of *Quassia indica* (Gaertn.) Nooteb reported that the methanol leaf extract of *Quassia indica* contained 1.08 mg/g flavonoid, 10.44 mg/g phenol, and 1.53 mg/g tannin.

Table 1. Results of qualitative phytochemical analysis of leaf extracts of *Quassia undulata*

Phytochemicals	Extraction solvent[a]			
	Chloroform	Ethyl acetate	Methanol	Acetone
Saponins (Froth's test)	-	-	+	-
Tannins (Braymer's test)	-	-	+	+
Flavonoids (Lead acetate test)	-	-	+	-
Steroids (Salkowaski's test)	+	+	+	+
Phlobatannins (Precipitate test)	-	-	-	-
Terpenoids (Salkowaski's test)	-	-	-	-
Coumarins (Reaction with 10 % NaOH)	+	+	+	+
Emodins (Reaction with ammonium hydroxide and benzene)	-	-	-	-
Anthraquinones (Borntrager's test)	+	+	+	+
Anthocyanins (Reaction with acid and ammonia)	+	+	-	-
Alkaloids (Hager's test)	+	+	+	+
CardiacGlycosides (Legal's test)	+	+	-	+
Phenols (Ferric Chloride's test)	-	-	+	+

[a] Signs plus (+) and minus (-) denote presence or absence of phytochemicals, respectively.

Table 2. Concentration of some phytochemicals present in the methanolic leaf extract of *Quassia undulata*

Phytochemicals[a]	Concentration[b]
Total phenolics [mg GAE/g]	11.828±0.269
Tannins [mg CE/100g]	3.131±0.000
Saponins [%]	0.673±0.003
Flavonoids [mg QE/g]	8.074±0.016
Alkaloids [%]	5.200±0.000

[a] Abbreviations: mg GAE/g - mg of gallic acid equivalent per gram of extract; mg CE/100g - mg of catechin equivalents per 100 mg sample; mg QE/g - mg of quercetin equivalents per gram of extract

[b] Data are presented as mean ± standard deviation of three replicates

CONCLUSION

Generally, the present study shows a range of phytochemicals that can be obtained from different extracts of *Q. undulata* leaves; they can be explored for the treatment of various diseases. This supports the claim that the amount of secondary metabolites extracted from plants is dependent on the polarity of solvents used. Solvent effects revealed that methanol extracted the highest number of phytochemicals, while the non polar solvents (ethyl acetate and chloroform) extracted the least. As a result of this, methanol can be said to be the most effective solvent compared to acetone, ethyl acetate and chloroform. The medicinal properties of *Quassia undulata* leaf extracts may be due to the presence of the above mentioned metabolites. Further research on the potentials of *Q. undulata* leaves is continuous.

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