Qualitative and quantitative phytochemical profiling of ethnomedicinal folklore plant- *Globimetula oreophila.*

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Abstract

Globimetula oreophila is a hemiparasitic plant commonly called mistletoe, found growing on several dicotyledonous trees using them as a host for its root-like structure called haustoria. *Globimetula oreophila* has been used to cure a variety of ailments, including diarrhea, stomachache, headache, and fever. As a treatment for these ailments, the leaves and stem are boiled and the resulting decoction is drunk. The phytochemical compositions have not yet been scientifically confirmed despite their potential for medicinal use. The present study is aimed at investigating the leaves of *G. oreophila* fraction for phytochemicals, by qualitative and quantitative analysis. It was observed that from the qualitative screening, different fractions confirmed the presence of carbohydrates, saponins, cardiac glycoside, tannins, flavonoids, steroid/triterpene, and alkaloids. Anthraquinones were not observed in any of the fraction of *G. oreophila* plant. Further, the study of the quantitative findings revealed that the butanol fraction had more secondary metabolites when compared with other fractions and the crude extract. Higher flavonoid content was found in the butanol fraction as compared to crude ethanol extract (CEE) while phenols were found to be higher in crude ethanol extract as compared to the

butanol fraction. There were significant differences in the total phytochemical contents of each fraction. The results also show that the analyzed plant is a beneficial source of secondary metabolites and may have contributed to the therapeutic use of the plant in traditional medicine for the management of diseases.

Keywords: *Globimetula oreophila*, phytochemicals content, flavonoids, *Azadirachta indica*, qualitative and quantitative analysis.

Introduction

The relationship between mankind and nature has been long and very complicated. Since historical record, geography, time, and culture have not stopped mankind from continuously searching, inquiring and exploring the vast range of natural diversity. The earliest known records of mankind often reveal impressive and ingenious 'responses' to these natural diversities and to this day, mankind life is inseparable from the rich source nature offers mankind existence (Kong *et al.,* 2003). Historically speaking, mankind has explored nature to satisfy two major needs i.e. food and herbs for alleviating pain and suffering.

Traditional medicine refers to health practices, knowledge, and beliefs incorporating plants, animals, and mineral-based medicines, spiritual therapies, manual techniques, and exercise, applied singularly or in combination to treat, diagnose, and prevent illnesses or maintain well-being (Mansoor and Sanmugarajah, 2018; NNMDA, 2008; WHO 2005). Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain (Okigbo e*t al.,* 2008). The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). Medicinal plants are the "backbone" of traditional medicine, which means that about 3.3 billion people in less developed countries utilize medicinal plants on a regular basis. These medicinal plants are considered rich resources of active secondary metabolites known as phytochemicals such as alkaloids, flavonoids, saponins, terpenoids, tannins, phenols, steroids, and glycosides which can be used in drug development and synthesis (Davidson-Hunt, 2000).

Besides that, these plants play a critical role in the development of human cultures around the world. The qualitative phytochemical screening will help in understanding active secondary metabolites present in a particular plant while quantification of those active secondary metabolites will assist in extracting,

purification, and identification of the bioactive metabolites for further convenient application. At a global scale, there are more than 350, 659 medicinal plants of which only 2% have been explored and scientifically validated based on their phytochemical composition and effective bioactivities (De Ghosh, 2014).

Nigeria has rich vegetation and many of the plant species are used by some indigenous people for medicinal purposes (Adebayo and Krettli, 2011). *Globimetula oreophila* is a member of the Loranthaceae family, popularly known as mistletoe (Adesina *et al.,* 2013). *G. oreophila* is a parasitic plant with a known therapeutic value that is widely used in the treatment of various ailments including malaria (Dauda *et al.,* 2016), hypertension, cancer, diabetes, epilepsy, and as a diuretic agent (Ojewole and Adewole, 2007). For example, the tea made from the Loranthaceae family is believed to cure bone fracture and body pain (Ken'ichi, *et al*., 2006). The present study aims to investigate the fractions of the extract of the leaf of *G. oreophila* for phytochemical analysis, by qualitative and quantitative analysis which have bioactive and medicinal value.

Materials and Methods

Collection, identification and preparation of plant materials

The plant sample of *Globimetula oreophila* parasite on *Azadirachta indica* comprising the leaves and fruit was collected from Sokoto metropolitan, Nigeria in July 2019.The plant sample was authenticated by a Taxonomist in the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University Zaria by comparing it with herbarium reference voucher specimen (Number ABU0886). After collection and authentication, the leaves were air dried, ground into powder, and weighed.

Extraction

The powdered plant material 1000 g was macerated using 70% ethanol for 3 days with occasional shaking and filtered through muslin cloth and then through filter paper. The filtrate was concentrated using rotary evaporator (Heidolph Laborota 4000, Schwabach, Germany) under reduced pressure at 40°C resulting in 74.55 g (7.46%) of dark green semisolid mass referred to as crude ethanol extract (CEE). The crude extract (74.55 g) was partitioned with n-hexane, chloroform, ethyl acetate and n-butanol to give the following yield; hexane (21.78 g), chloroform (9.83 g), ethyl acetate

(6.96 g), and butanol (17.19 g) fraction respectively.

Qualitative Phytochemical Screening of the Fractions of *G. oreophila* leaves

Different chemical tests were arried out on the hexane fraction (HF), chlororform fraction (CF), ethylacetate fraction (EF) and n-butanol fraction (BF) to identify the presence of various phytochemical constituents.

Test for Carbohydrates

Molisch test: To a small portion of the extract and fractions, distilled water was added and mixed with a few drops of Molisch reagent, and 1ml of concentrated H_2SO_4 was carefully added down the side of the inclined tube, to form a reddish-coloured ring at the interphase (Silva *et al*., 1998).

Test for Alkaloids

For each extract and fraction, 0.5 g was stirred with 5 ml of 1 % aqueous hydrochloric acid in a water bath and filtered. 3 ml of the filtrate was divided into three test tubes and treated as follows:

a. To the first portion, 1 ml of freshly prepared Dragendoff's reagent was added to the extract and fractions to form a reddish-brown precipitate.
b. To the second portion, 1 drop of Meyer's reagent was added to the extract and fractions to form a cream precipitate.
c. To the third portion, 1 ml of Wagner's reagent was added to the extract and fractions to form a reddish-brown precipitate (Evans, 2009).

Test for Flavonoids

a. Sodium hydroxide test

Some portions of the extract and fractions were separately dissolved in 10 % aqueous NaOH solution and dilute HCl was added to form yellow coloration (Silva *et al*., 1998).

b. Shinoda test

To a small portion of the extract dissolved in 1-2 ml of 50 % methanol plus heat, metallic magnesium chips and a few drops of concentrated HCl were added to form red colour (Evans, 2009)

Test for Anthraquinones

Bontrager test

The extract and fractions (0.5 g) were shaken with 5 ml carbon tetrachloride each, this was filtered and 10 % dilute ammonia solution was added. The mixture was shaken with visible change (Silva *et al*., 1998).

Test for Saponins

Frothing test

The extract (0.5 g) and fraction (0.5 g) were shaken with distilled water in a test tube each. It was allowed to stand for 10 minutes. It

forms honey comb froth that persists 10-15 (Silva e*t al*., 1998).

Test for Steroids and Triterpenes

a. Liebermann-Burchard's test: A small portion of the extract and fractions was dissolved in chloroform. Equal volumes of acetic anhydride and concentrated H_2SO_4 were added down the test tube. It formed a red color (Silva *et al.,* 1998).
b. Salkowski test: A small quantity of the extract and fractions was dissolved in 1 ml chloroform and concentrated H_2SO_4 (1 ml) was added down the test tube. It formed a reddish ring at the interphase (Sofowora, 1993).

Test for Tannins

Lead sub-acetate test

To a small portion of the extract, distilled water was added. 3-5 drops of lead acetate solution was added. Formation of a cream-colored precipitate (Silva *et al.,* 1998).

Test for Cardiac Glycosides

Keller-kiliani Test

A small portion of the extract was dissolved in 1ml glacial acetic acid containing traces of ferric chloride solution. The solution was then transferred into a dry test tube to which an equal volume of sulphuric acid was added, a brown ring obtained at the interface will indicate the presence of a deoxy sugar. (Evans, 2009).

Quantitative Determination of Secondary Metabolites of the Fractions of *G. oreophila* leaves using Spectrophotometric Method.

Total Steroids Content (TSTC): The Liebermann-Burchard reaction method was used to detect sterols and terpenoids that give dark pink to green colour, due to the hydroxyl group reacting with acetic anhydride and H_2SO_4. Varying concentrations of cholesterol (10-100 µg/ml) was used for standard calibration curve, which was read spectrophotometrically at 640 nm. The concentration of steroids was expressed in milligrams/gram of the crude extract (Harborne, 1973).

Total Terpenoid Content (TTPC): A solution of 1.0 mL of the extract was mixed with vanillin-glacial acetic acid solution 1.5 mL, 5% w/v) and per chloric acid solution (5.00 mL). The sample solution was heated for 45 min at 60◦C and then cooled in an ice-water bath to the ambient temperature. After the addition of glacial acetic acid (2.25 mL), the absorbance was measured at 548 nm, using a UV-visible-light spectrophotometer. Ursolic acid/Cholesterol (20–100 ug/mL in methanol) was used as a standard. Results were expressed as milligram

ursolic/cholesterol/linalool acid equivalents (mg ursolic acid/cholesterol/g extract) (Harborne, 1973).

Determination of total phenolic content (TPC): Estimation of total phenol content was measured spectrophotometrically by the Folin–Ciocalteu colorimetric method, using Gallic acid as the standard and expressing results as Gallic acid equivalent (GAE) per gram of sample. Different concentrations (0.01-0.1 mg/ml) of Gallic acid were prepared in methanol. Aliquots of 0.5 ml of the test sample and each sample of the standard solution will be taken, mixed with 2 ml of Folin–Ciocalteu reagent (1:10 in deionized water) and 4 ml of saturated solution of sodium carbonate (7.5% w/v). The tubes were covered with silver foils and incubated at room temperature for 30 minutes with intermittent shaking. The absorbance was taken at 765 nm using methanol as a blank. All the samples were analyzed in three replications. The total phenol was determined with the help of a standard cure prepared from pure phenolic standard (Gallic acid) (Ainsworth and Gillespie, 2007; Alhakmani *et al*., 2013).

Determination of total flavonoid content (TFC): The total flavonoid content (TFC) was determined by an aluminum chloride colorimetric assay (Zhishen *et al*., 1999). Briefly, 0.5 ml aliquots of the samples (Methanol extract and fractions) and standard solution (0.01-1.0 mg/ml) of quercetin were added with 2 ml of distilled water and subsequently with 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v) solution will be added. After 6 minutes, 0.15 ml of (10% $AlCl_3$, w/v) solution was added. The solutions were all allowed to stand for 6 min and after that 2 ml of sodium hydroxide (4% NaOH, w/v) solution was added to the mixture. The final volume was adjusted to 5 ml with immediate addition of distilled water, mixed thoroughly and, allowed to stand for another 15 min. The absorbance of each mixture was determined at 510 nm against the same mixture. TFC was determined as mg of quercetin equivalent per gram of sample and all determinations were performed in triplicate (Zhishen *et al.,* 1999).

Determination of total alkaloid content (TAC): Total alkaloid content (TAC) was quantified by the spectrophotometric method. This method is based on the reaction between alkaloid and bromocresol green (BCG). The plant extract and fractions (1 mg/ml) were dissolved in 2 N HCl and then filtered. The pH of the phosphate buffer solution will be adjusted to neutral with 0.1 N NaOH. About 1 ml of this solution was transferred to a

separating funnel, and then 5 ml of BCG solution along with 5 ml of phosphate buffer was added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extract was collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform will be measured at 470 nm. TAC was determined as mg atropine equivalent per gram of sample. The whole experiment was conducted in three replicates (Shamsa *et al*., 2008; Sharief *et al*., 2014).

Determination of tannin Content (TTC): The tannins were determined by the Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin –Ciocalteu phenol reagent, 1 ml of 35 % Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Gallic acid (20, 40, 60, 80, and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions was measured against the blank at 725 nm with a UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract (Marinova *et al*., 2005).

Determination of Saponins Content (TSC): Total saponins were determined according to the method described by Makkar *et al.* (2007). A known quantity of dried extract will be dissolved in aqueous 50% methanol and a suitable aliquot (5 mg/ml) was taken. Vanillin reagent (0.25 ml; 8%) was added followed by sulphuric acid (2.5 ml; 72% v/v). The reaction mixtures were mixed well and incubated at 60°C in a water bath for 10 min. After incubation, the reaction mixtures were then cooled on ice and absorbance at 544 nm (UV visible spectrophotometer) was read against a blank that does not contain extract. The standard calibration curve was obtained from suitable aliquots of diosgenin (0.5 mg/ml in 50% aqueous methanol). The total saponins concentration was expressed as mg diosgenin equivalents (DE) per g dry weight (DW).

Results and Discussion

Table 1. Results of % Yield of Partitioned Fractions from 100g Crude Ethanol Extract

Solvent	Fraction yield (g)	Percentage yield	Fraction colour	Fraction texture
Hexane	21.78	29.22	Oily green	Sticky
Chloroform	9.83	13.19	Bright green	Dry
Ethylacetate	6.96	9.34	Light brownish	sticky
Butanol	17.19	23.08	Greenish brown	Sticky
Crude ethanol extract	74.55	-	Dark greenish	Sticky

Qualitative phytochemical analysis

Table 2. Qualitative Profile of Phytochemicals in Leaves extract of *Globimetula oreophila*

Phytochemical Constituent	Hexane	Chloroform	Ethylacetate	Butanol	Type of Test
Carbohydrate	-	+	+	+	Molish test
Saponins Glycoside	-	-	+	+	Frothing test
Cardiac Glycoside	-	-	+	+	Keller-killiani test
Tannins	-	-	+	+	Lead acetate
Flavonoids	-	-	+	+	Sodium Hydroxide & Shinoda test `
Steroid/Triterpenes	+	+	+	+	Libermann Buchard & Salkowski test
Alkaloid	-	+	+	+	Dragendoff's test, Meyer's test & Wagner's test
Anthraquinones	-	-	-	-	Bontrager Test

+=Present, -=absent

Phytochemical compounds are non-dietary plant derivatives that are determined as secondary metabolites based on their structures and biological functions. From the qualitative finding on the various fractions of *G. oreophila* plant presented in table 2, it is observed that different fraction confirmed the presence of carbohydrate, saponins, cardiac glycoside, tannins, flavonoids, steroid/triterpene and alkaloid. Surprisingly, anthraquinone was not observed in any of the fraction of *G. oreophila* plant growing on *Azadirachta indica.* Oluwole *et al.* (2013) reported the presence of combined anthraquinones, however, the host of the plant was not identify. Though, steroid and triterpenes were found in all the fractions. Carbohydrate and alkaloid components are present in three fractions of chloroform, ethylacetate and butanol screened except hexane. Fractions such as ethylacetate and butanol which are polar showed the ability to extract flavonoids. Tannins, saponins glycoside and cardiac glycoside were only present in two fraction (ethylacetate and butanol). Hexane have the least phytochemical constituents when compared to other fractions screened of *G. oreophila* plant. *G. oreophila* is known as a "health protective agent" because of the phytochemicals it contains, which have varied degrees of disease-preventive antimalarial, antioxidant, anti-inflammatory, and antibacterial properties. In addition to their role in plant protection, flavonoids are the best antioxidants that shield cells from damage caused by free radicals (Omoregie and Osagie, 2012). For instance, polyphenols like flavonoids have been shown to reduce the risk of cancer by preventing the growth of tumors (Devasagayam *et al.,* 2004); they also stimulate hormones and have antibacterial, anti-aging, and anti-diarrheal properties (Mathew *et al.,* 2012; Saxena *et al.,* 2013); they also have anti-diarrheal properties. The mode of action of these phytochemicals were found to increase colonic water and electrolyte reabsorption (Enzo, 2007). Because of their pharmacological qualities as antibiotics, the alkaloid families of phytochemicals have been researched in contemporary medicine to support their traditional use (Kubmarawa *et al.,* 2011, Mensah *et al.,* 2008), antiviral, anticancer, antifungal, and antimalarial (Thawabteh *et al.,* 2019; Casciaro *et al.,* 2020). Many studies have reported the antibacterial properties of tannins (Banso and Adeyemo, 2007). Terpenes are known to facilitate membrane disruption using lipophilic compounds and they possess some medical properties such as analgesic agents (Singh, 2006), anti-

carcinogenic, antihypertensive, insecticidal, antiviral, and anti-ulcer and antimalarial which could validate the use of the plant as a potential anti-malarial drug (Ndongo, 2017, Dauda *et al.,* 2016; Kabera *et al.,* 2014). The presence of carbohydrates accounts for the nutritional value of the leaves of *Globimetula oreophila* (Dauda *et al.,* 2016). Cardiac glycosides are the earliest therapies discovered for congestive heart failure and arrhythmia (Ehle *et al.,* 2011). The saponins class of phytochemicals possess numerous pharmacological activities such as insecticidal, anthelmintic, molluscidal and pesticidal, antifungal, anticancer, antiviral, and antibacterial activity because of their amphiphilic properties (Dorota *et al.,* 2017; Marrelli *et al.,* 2016; Kezwon and Wojciechowki, 2014). Tannins are involved in the formation of complex molecules within bacteria cell walls and prevent the formation of biofilms (Mapiye, 2019, Trentin *et al.,* 2013; Prashant *et al.,* 2011). Tannin possesses pharmacological activities such as antioxidant, anticancer, anti-inflammatory, antifungal, and antibacterial (Mapiye, 2019; Mboweni, 2018; Ramachandran *et al.,* 2014).

Quantitative phytochemical analysis

Table 3. Total alkaloid content of *G. oreophila* leaves fractions

Solvent Fraction/Extract	Alkaloid (mg/g)±SEM
Hexane	0[a]
Chloroform	23.39±0.001[a,c,d,]
Ethylacetate	27.81±0.001[a,b,d,]
Butanol	19.27±0.001[a,b,c,]
Crude ethanol extract	45.02±0.005[,a,b,c,d]

[a]Statistical significance of Hexane, [b]Statistical significance of chloroform, [c]Statistical significance of ethyl acetate, [d]Statistical significance of butanol,

Table 4. Total flavonoids content of *Globimetula oreophila* leaves fractions

Solvent Fraction/Extract	Flavonoids (mg/g)±SEM
Hexane	0^{e}
Chloroform	$29.64\pm0.002^{e,g,h}$
Ethylacetate	$38.07\pm0.003^{e,f,h}$
Butanol	$128.75\pm0.003^{e,f,g}$
Crude ethanol extract	$98.66\pm0.008^{e,f,g,h}$

[e]statistical significance of hexane, [f]statistical significance of chloroform, [g]ethylacetate, [h]Statistical significance of butanol.

Table 5. Total phenols content of *Globimetula oreophila* fractions

Solvent Fraction/Extract	Phenols (mg/g)±SEM
Hexane	0^{i}
Chloroform	$27.91\pm0.003^{i,k,l}$
Ethylacetate	$35.74\pm0.003^{i,j,l}$
Butanol	$114.16\pm0.003^{i,j,k}$
Crude ethanol extract	$103.83\pm0.004^{i,j,k,l}$

[i]statistical significance of hexane, [j]statistical significance of chloroform, [k]ethylacetate, [l]Statistical significance of butanol

Table 6. Total tannins content of *Globimetula oreophila* leaves fractions

Solvent Fraction/Extract	Tannins (mg/g)±SEM
Hexane	0^{m}
Chloroform	$18.79\pm0.001^{m,o,p}$
Ethylacetate	$21.46\pm0.001^{m,n,p}$
Butanol	$46.23\pm0.000^{m,n,o}$
Crude ethanol extract	$86.46\pm0.191^{m,n,p}$

[m]statistical significance of hexane, [n]statistical significance of chloroform, [o]ethylacetate, [p]Statistical significance of butanol

Table 7. Total saponins content of *Globimetula oreophila* leaves fractions

Solvent Fraction/Extract	Saponins (mg/g)±SEM
Hexane	0^{q}
Chloroform	$14.93\pm0.001^{q,s,t,}$
Ethylacetate	$23.04\pm0.003^{q,r,t,}$
Butanol	$62.20\pm0.001^{q,r,s,}$
Crude ethanol extract	$31.60\pm0.003^{q,r,s,t}$

[q]statistical significance of hexane, [r]statistical significance of chloroform, [s]ethylacetate, [t]Statistical significance of butanol

Table 8. Total steroids content of *Globimetula oreophila* leave fractions.

Solvent Fraction/Extract	Steroids (mg/g)±SEM
Hexane	44.11 ± 0.003^{u}
Chloroform	$20.71\pm0.002^{u,w}$
Ethylacetate	$25.94\pm0.001^{u,vx}$
Butanol	$21.24\pm0.001^{u,w}$
Crude ethanol extract	$20.39\pm0.001^{u,w}$

[u]statistical significance of hexane, [v]statistical significance of chloroform, [w]ethylacetate, [x]Statistical significance of butanol

Table 9. Total terpenoids contents in *Globimetula oreophila* leaves fractions

Solvent Fraction/Extract	Terpenoids (mg/g)±SEM
Hexane	$35.44\pm0.003^{*}$
Chloroform	$27.65\pm0.002^{*,z,a'}$
Ethylacetate	$21.23\pm0.001^{*,y,a'}$
Butanol	$22.94\pm0.001^{*,y,z}$
Crude ethanol extract	$30.69\pm0.002^{*,y,z,\ a'}$

[*]statistical significance of hexane, [y]statistical significance of chloroform, [z]ethylacetate, [a']Statistical significance of butanol

Total alkaloids content (TAC)

In Table 3, the results obtained were expressed as Mean ± SEM of triplicates. Accordingly, crude ethanol extract had higher total alkaloids content (45.02±0.005mg/g) as compared to other solvents extract while in the hexane fraction, total alkaloids content was not found. A Tukey post hoc test denoted with superscript letters in Table 3 showed that the TAC of hexane, chloroform, ethyl acetate, butanol, and crude ethanol extracts was statistically significant with each other. The mean difference is significant at the 0.05 level.

Total flavonoids content (TFC)

As stated in Table 4, the results obtained were expressed as Mean ± SEM of triplicates. Butanol fraction had higher total flavonoids content (128.75±0.0.003 mg QE/g) as compared to others while hexane had no total flavonoids (0 mg/g). A Tukey post hoc test denoted with superscript letters in Table 4 showed that the TFC of chloroform, ethyl acetate, butanol, and crude ethanol extract was statistically significant among each. The mean difference is significant at the 0.05 level.

Total phenols content (TPC)

According to Table 5, the results obtained were expressed as Mean ± SEM of triplicates. The butanol fraction had the maximal total phenolic content (114.16±0.003 mg GAE/g) while the hexane fraction had minimal total phenolic content (0 mg GAE/g). A Tukey post hoc test denoted with superscript letters in Table 5 showed that the TFC of chloroform, ethyl acetate, butanol, and crude ethanol extract was statistically significant among each. The mean difference is significant at the 0.05 level.

Total tannins content (TTC)

As illustrated in Table 6, the results acquired were expressed as Mean ± SEM of triplicates. The crude ethanol extract had higher total tannins content (86.46±4.92 mg TAE/g) as compared to other solvents extracts while the hexane fraction had no total tannins content (0 mg TAE/g). A Tukey post hoc test results denoted with superscript letters in Table 6 illustrated that the TTC of hexane, chloroform, and butanol fraction was statistically significant to other extracts TTC. There was no statistical difference between ethyl acetate fraction TTC and crude ethanol extract TTC. The mean difference is significant at the 0.05 level

Total saponins content (TSC)

As illustrated in Table 7, the results acquired were expressed as Mean ± SEM of triplicates.

The butanol fraction had higher total saponins content (62.20±0.001 mg DE/g) followed closely by crude ethanol extract (31.60±0.003) as compared to other solvents extracts while hexane fraction had no total saponins content (0 mg DE/g). A Tukey post hoc test results denoted with superscript letters in Table 7 illustrated that the TSC of hexane, chloroform, ethyl acetate, butanol, and crude ethanol extract was statistically significant among each. The mean difference is significant at the 0.05 level.

Total steroids content (TSTC)

As illustrated in Table 8, the results acquired were expressed as Mean ± SEM of triplicates. The hexane fraction had higher total steroids content (4.11±0.003 mg CE/g) as compared to other solvents extracts while the crude ethanol fraction had the least total steroids content (20.39±0.001 mg CE/g). A Tukey post hoc test denoted with superscript letters in Table 8 showed that the TSTC of hexane and ethyl acetate was statistically significant with other four fractions. Also, there was no statistical difference between chloroform TSTC and butanol TSTC, between chloroform TSTC and crude ethanol extract and butanol TSTC and crude ethanol extract TSTC.

Total terpenoids content (TTPC)

As illustrated in Table 9, the results acquired were expressed as Mean ± SEM of triplicates. The hexane fraction had higher total terpenoid content (35.44±0.003 mg UE/g) as compared to other solvent extracts while the ethylacetate fraction had the least total terpenoids content (21.23±0.001 mg UE/g). The result from Table 15 showed that the TTPC of hexane, chloroform, ethyl acetate, butanol and crude ethanol extract was statistically significant among each fraction. The mean difference is significant at the $p< 0.05$ level.

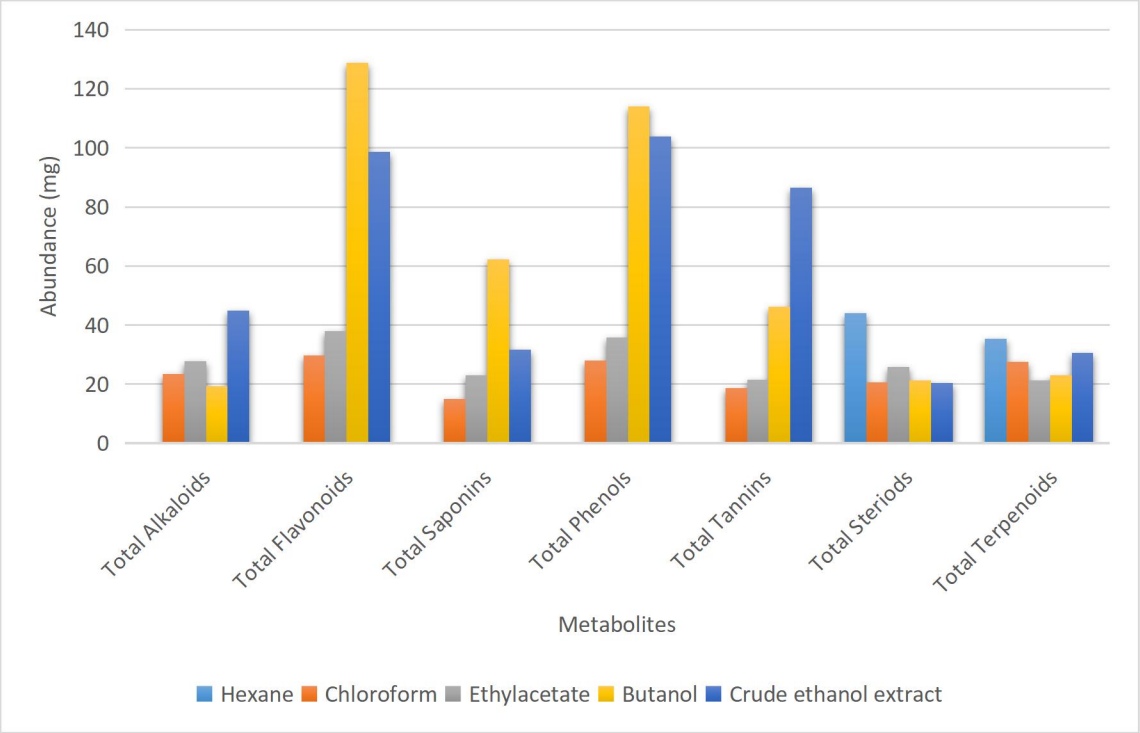


Figure 1. Quantitative phytochemical screening of *Globimetula oreophila* leaves of various fractions.

Conclusion: The results also show that the analyzed plant is a beneficial source of secondary metabolites and may have contributed the therapeutic use of the plant in traditional medicine for the management of diseases.

Conflict of interest

There is no conflict of interest. No external financial support was obtained for this study

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