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Qualitative and quantitative phytochemical profiling of crude fractions of *Pechuel-Loeschea leubnitziae* leaves

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Pechuel-Loeschea leubnitziae **is a predominant medicinal plant in Namibia. It is traditionally utilized in treatment of gastrointestinal issues, sexually transmitted infections and common cold. Despite its therapeutic potential, the phytochemicals profile and compounds compositions are yet to be scientifically validated. This study was conducted to determine the spectrum and total phytochemical constituents present in *P. leubnitziae* leaves extract using standard qualitative and quantitative methods. Fine powder of *P. leubnitziae* was extracted by cold maceration using a serial exhaustive approach in a 1:5 dilution in hexane, ethyl acetate, ethanol, methanol and water. After 72 h, obtained filtrate was concentrated using a rotary evaporator or lyophilizer. Crude fraction of each solvent obtained was screened for phytochemicals using qualitative and quantitative methods. The yield of the extracts post-extraction ranged from 5.361 to 23.93 g. The extracts contain alkaloids, phenolics, flavonoids, saponins, terpenoids and tannins. There were significant differences in the total phytochemical contents of each solvent. Total alkaloids contents (TAC) ranged from 29.12 to 106.44 µg, total flavonoids contents (TFC) ranged from 30.41 to 78.84 µg, total phenolic contents (TPC) ranged from 57.14 to 58.70 µg and total tannins contents (TTC) ranged from 13.06 to 30.85 µg. The leaves of *P. leubnitziae* contain phytochemicals including alkaloids, flavonoids, phenolic, terpenoids, tannins and saponins that are known to have pharmacological properties and they can be explored for biological potentials.**

Key words:*Pechuel-Loeschea leubnitziae* extracts, qualitative and quantitative analysis, phytochemicals content.

INTRODUCTION

Traditional medicines are defined by WHO as knowledge, skills, and practices based on beliefs, theoretical aspects and indigenous experiences which differ among different cultures in prevention or curing physical or mental illness (Mansoor and Sanmugarajah, 2018). Medicinal plants are rich in active secondary metabolites known as phytochemicals. The phytochemical compounds are non-dietary plants derivatives which are determined

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as secondary metabolites based on their structures and biological functions. The phytochemicals include alkaloids, phenols, terpeneoids, tannins, saponnis, flavanoids, steroids, and glycosides (Komolafe, 2014). In order to determine phytochemicals from medicinal plants, numerous processes should be considered such as extraction, isolation, purification, concentration, *in-vitro* activity and *in-vivo* efficacy. Qualitative phytochemical screening will assist in comprehension of phytochemical compounds present in the particular plant while quantification of those secondary metabolites will assist in extracting, purification and identification of the bioactive compounds for further convenient application. According to several studies conducted by World Health Organization, about 80% of the world's population relies on using traditional medicine (WHO, 2014). There are more than 350, 659 medicinal plants globally of which only 2% have been explored and scientifically validated based on their phytochemicals composition and effective bioactivities (De Ghosh, 2014).

Uses of some medicinal plants in Namibia are well documented and are highly in demand due to their long traditional uses. Although there is sufficient information on their traditional use, there is a huge gap on scientific evidence of their phytochemicals composition and biological efficacy (Chinsembu et al., 2015; Iikasha et al., 2017).

P. leubnitziae belongs to the family of Asteraceae (Mannheimer and Curtis, 2009; Ndongo, 2017). The shrub is bitter, smelly and commonly known as bitter bush in English while in Oshiwambo it is denoted by its local name as Oshizimba (Ndongo, 2017). It is a small shrub with silver-grey felt-like pubescence on stems and leaves, with leaves tapering petiole-like towards the base, lanceolate, entire and it can grow up to about 3 cm long (Von Koenen, 2001). The plant is predominant in Namibia excluding the far north-eastern part (Mannheimer and Curtis, 2009). It is used among the Ovahimba (indigenous people living in Kunene region, Namibia and Angola) for gastrointestinal issues, sexually transmitted diseases, skin cleansing as well as a cosmetic product (Curtis and Mannheimer, 2005; Ndongo, 2017). The Herero people of Namibia reportedly use *P. leubnitziae* to delay menstruation by steaming and baths with leaf decoctions of the plant, in such a manner that it cleans the abdomen (Von Koenen, 2001). *P. leubnitziae* leaves are reported to be an effective mosquito repellent. The Nama tribe of Namibia uses *P. leubnitziae* plant materials to alleviate sweaty feet by stuffing it in their shoes (Von Koenen, 2001). Boiled roots and leaves are used for enemas by placing crushed leaves into anus in cases of intestinal conditions, or as steam to relieve colds (Von Koenen, 2001; Ndongo, 2017). In addition to the aforementioned, Aawambo tribe prepare the plant material by heating and sniff the smoke to cure common cold (Von Koenen, 2001). Notwithstanding the largely traditional usage of this plant, no published phytochemicals composition using different solvents are accessible in literature. Consequently, this study was executed to determine constituents of the hexane, ethyl acetate, ethanol, methanol and water extract of *P. leubnitziae* leaves by profiling of present phytochemicals and their total contents with the aim to validate its traditional uses.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were purchased from Legacy Lab Africa Ltd., Kenya. All chemicals used were of analytical grade.

Plant material collection

P. leubnitziae leaves were collected from Onayena, Oshikoto region in Namibia in May 2020 and transferred to Pan African University of Basic Science, Technology and Innovation, Kenya. A permit for importing the plant materials was obtained from Kenya Plant Health Inspectorate Services (KEPHIS, Kenya) and for exporting was obtained from Ministry of Agriculture, Water and Forestry (MoAWF, Namibia). The plant leaves material was identified and authenticated by Mr. John Kamau Muchuku, Department of Botany, Jomo Kenyatta University of Agriculture and Technology.

Sample preparation and extraction

The leaves materials were washed under running tap water, air dried in the shade at room temperature and pulverized into fine powder using an electric blender. About 360 g of the fine powdered leaves extract was extracted subsequently with 1800 ml of hexane, ethyl acetate, ethanol, methanol and water in order of polarity and incubated on an orbital shaker incubator at 25°C for 72 h. The resulting mixture was filtered through double layers of muslin cloth, then finally filtered by gravity using Whatman number 1 filter papers. The organic filtrate was concentrated using a rotatory evaporator, while aqueous extract was lyophilized (Ushie et al., 2014; Fisher and Shepherd, 2016). The crude mass obtained was weighed in grams and percentage yield was calculated as follows:

Percentage Yield (%) = Dry weight of extract/Dry weight of plant material × 100

The extracts were then be kept at 4°C for further analysis.

Phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out to detect the presence or absence of secondary metabolites in hexane, ethyl acetate, ethanol, methanol and aqueous leaves extract using standard analytical procedure as described by Iikasha et al. (2017) and Mendhulkar and Kharat (2017). An amount of 0.5 g of hexane, ethyl acetate, ethanol, methanol and aqueous plant extracts was dissolved in 100 ml of each solvent, respectively to make a stock solution concentration of 5 mg/ml for qualitative phytochemical analysis.

Test for alkaloids: Wagner's test

About 2 ml of each solvent extract was transferred in a test tube, respectively. An amount of 2 ml of diluted 1% HCL was added followed by 10 drops of Wagner's reagent and incubated for 15 min. Formation of reddish brown precipitate indicates presence of alkaloids. A stock solution of Wagner's reagent was prepared by dissolving iodine (1.3 g) with potassium iodide (2.0 g) into 100 ml of distilled water. The mixture transferred and stored in an amber bottle.

Test for tannins: Lead acetate test

To 5ml of each solvent extract in a test tube, few drops of freshly prepared 10% lead acetate were added and dissolved. A yellow precipitate indicates presence of tannins. The 10% of lead acetate was prepared by weighing 10 mg into 100 ml of distilled water.

Test for saponins: Froth test

An amount of 1ml of each solvent extract was dissolved in 10 ml of distilled water in the test tubes, stoppered and shaken vigorously for 30 s and incubated for 30 min. Saponins presence indicated by a honeycomb froth above the surface after 30 min.

Test for flavonoids: Alkaline reagent test

To 3ml of each solvent extract in test tubes, 2 ml of dilute NaOH was added. Formation of intense yellow color indicates presence of flavonoids. The dilute NaOH was prepared by dissolving 4.25 g of NaOH in 50 ml of distilled water.

Test for phenolics: Ferric chloride test

About 10% Ferric chloride and 10% Ferro cyanide were mixed, 3 drops of this mixture were added to 3 ml of each solvent extract. Formation of orange brown precipitate indicates presence of phenolics. To prepare a working solution, 10 mg of both ferric chloride and Ferro-cyanide was dissolved into 100 ml of distilled water.

Test for terpenoids: Salkowski's test

About 5 ml of each solvent extract was added in test tubes. An amount of 2 ml of chloroform and 1 ml of H_2SO_4 was added. Formation of reddish brown ring indicates presence of terpenoids.

Quantitative phytochemical constituents' analysis

The total phenolics, tannins, alkaloid and flavonoid content were determined according to (Madhu et al., 2016; Selvakumar et al., 2019).

Estimation of total content of alkaloids

About 1 ml of 1 mg/ml of sample was transferred to a separating funnel whereby there was an addition of 5 ml of bromocresol green solution and 5 ml of 4.7 pH phosphate buffer. The mixture was shaken with 1, 2, 3 and 4 ml of chloroform by vigorous shaking, collected in a 10 ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of Atropine (20, 40, 60, 80 and 100 µg/ml) was prepared in the same manner as described already. The absorbance for standard solutions and test solutions was determined on the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total content of alkaloids was expressed as Atropine equivalents (µg AE/mg of dried extract). Reagent blank was prepared in the same manner but without extracts.

Estimation of total content of phenolics

The phenolic compounds concentration in extracts was estimated using Folin-Ciocalteu method with slight modification. Briefly, 1 ml (1 mg/ml) of the sample extracts was mixed with 2 ml of (10%) Folin-Ciocalteu reagent in 10 ml volumetric flask. After 5 min, 3 ml of 7.5% sodium carbonate was added and made up to the volume with distilled water. A set of Gallic acid standard solutions (20, 40, 60, 80 and 100 µg/ml) will be prepared in the same manner and the tubes were incubated for 90 min at room temperature in dark place. The absorbance was measured at 760 nm. The results of total content of phenols were expressed as gallic acid equivalents (µg GA/mg of dried extract). Reagent blank was prepared in same manner but without extracts.

Estimation of total content of flavonoids

Colorimetric assay was used to determine the total content of flavonoid using aluminium chloride. For the reaction, 1 ml (1 mg/ml) of crude extract followed by 4 ml of distilled water were added to a 10 ml volumetric flask plus 0.30 ml of 5% sodium nitrite. After 5 min, 0.30 ml of 10% aluminium chloride was added. Moreover, after 6 min of incubation, 2 ml of 1M sodium hydroxide was added to the reaction mixture and immediately final volume was diluted to 10 ml with distilled water. A set of standard solutions of Rutin (20, 40, 60, 80 and 100 µg/ml) was prepared in same manner mentioned earlier. The absorbance for the test and standard solutions was measured using reagent blank at 510 nm wavelength by UV-Visible spectrophotometer. The total content of flavonoid was expressed as Rutin equivalents (µg RE /mg dried extract). Reagent blank was prepared in same manner but without extracts.

Estimation of total content of tannins

The tannins were determined by Folin and Ciocalteu method with slightly modification. About 0.5 ml (0.5 mg/ml) of sample extract was mixed with 3.75 ml of distilled water in a 10 ml volumetric flask. About 0.25 ml of Folin Phenol reagent and 0.5 ml of 35% sodium carbonate solution were added and diluted to 10 ml using distilled water. A set of tannic acid solutions as standard dilutions (10, 20, 30, 40 and 50 µg/ml) was prepared in same manner as mentioned earlier. The reagent mixture was well shaken and incubated at 30°C temperature for 30 min. The absorbance of standard and test solutions was analyzed with blank at 725 nm wavelength using UV-Visible spectrophotometer. The total content of tannins expressed as Tannic acid equivalents (µg TA/mg dried extract). Reagent blank was prepared in same manner but without extracts.

Statistical analysis

Experimental values are expressed as Mean±standard deviation (SD). Comparison of mean values between various groups was performed by independent t-test and one way-analysis of variance (one way-ANOVA) followed by post hoc test (multiple comparison)

Table 1. Crude extracts from leaves of *Pechuel-Loeschea leubnitziae*.

Solvent	Initial weight (g)	Extract yield (g)	Percentage yield	Extract colour	Extract texture
Hexane	360	5.361	1.49	Dark green	Sticky mash
Ethyl acetate	360	23.93	6.65	Greenish	Dry mash
Ethanol	360	13.775	3.83	Greenish	Sticky mash
Methanol	360	13.977	3.88	Greenish	Sticky mash
Water	360	13.667	3.80	Brownish	Dry powder

Table 2. Qualitative profile of phytochemicals in leaves extract of *Pechuel-Loeschea leubnitziae.*

Solvent	Alkaloids	Flavonoids	Phenolics	Saponins	Tannins	Terpenoids
Hexane	+	-	-	+	+	+
Ethyl acetate	+	-	-	-	+	+
Ethanol	+	+	-	+	+	+
Methanol	+	+	+	-	+	+
Water	+	+	+	-	-	+
Type of test	Wagner's reagent	Alkaline reagent	Ferric chloride	Froth test	Lead acetate test	Salkowski's test

+ = Present, -= absent.

Table 3. Total alkaloids content of *Pechuel-Loeschea leubnitziae* leaves extracts

Solvent extract	Total alkaloids content (µgTAE/mg extract)±SD
Hexane	64.53±7.44[a]
Ethyl acetate	106.44±6.54[a,b]
Ethanol	53.28±1.71[b,c]
Methanol	51.63±4.56[b,d]
Water	29.12±5.53[a,b,c,d]

[a]Statistical significance of Hexane, [b]Statistical significance of ethyl acetate, [c]Statistical significance of ethanol, [d]Statistical significance of methanol.

at significant level of P<0.05.

RESULTS

Qualitative phytochemical analysis

The yield crude extracts and percentage yield obtained from the serial exhaustive extraction of leaves of *P. leubnitziae* using hexane, ethyl acetate, ethanol, methanol and water in the order of increasing polarity are summarized in Table 1. The yield of the extracts post-extraction ranged from 5.361 to 23.93 g, with hexane being the least and ethyl acetate being the highest. The percentage yield of crude extracts ranged from 1.49 to 6.65%, whereby hexane was the minimal and ethyl acetate was maximal. Varying pigmentations were observed across the extracts with ethyl acetate, ethanol and methanol with similar color (greenish). The hexane and water extracts exhibited color variation ranging from dark green and brown, respectively. In addition, the crude extracts were either sticky or dry mash (Table 1).

As presented in Table 2, all the extracts contained alkaloids and terpenoids. Also all the extracts except the aqueous indicated the presence of tannins. The presence of flavonoids, phenolics and saponins varied among the extracts. Only the polar extracts (ethanol, methanol and water) showed ability to extract flavonoids. Ethanol and methanol showed the highest extraction potential for phytochemicals. Phenolics and saponins were only present in two extracts each.

Quantitative phytochemical analysis of total alkaloids content (TAC)

The standard calibration curve of atropine showed a linear regression of: $y = 0.007x + 0.0144$, $R^2 = 0.9917$. The total content of alkaloids was expressed as atropine equivalent (µg AE/mg of dried extract). In Table 3, the results obtained were expressed as Mean ± SD of

Table 4. Results of one way ANOVA of hexane, ethyl acetate, ethanol, methanol and water total alkaloids content.

Source of variation	Sum of squares	df	Mean square	F	Sig.
Between groups	9724.062	4	2431.015	83.109	0.000
Within groups	292.508	10	29.251	-	-
Total	10016.570	14	-	-	-

Table 5. Total flavonoids content of *Pechuel-Loeschea leubnitziae* leaves extracts.

Solvent extract	Total alkaloids content (µg TFE/mg extract)±SD
Ethanol	47.82±0.29[e]
Methanol	78.84±0.47[e,f]
Water	30.41±0.12[e,f]

[e]Statistical significance of ethanol, [f]Statistical significance of methanol.

Table 6. Results of one way ANOVA of hexane, ethyl acetate, ethanol, methanol and water total alkaloids content.

Source of variation	Sum of squares	df	Mean square	F	Sig.
Between groups	3611.13	2	1805	16922.597	0.000
Within groups	0.640	6	0.107		
Total	3611.753	8			

Table 7. Total phenolic content of *Pechuel-Loeschea leubnitziae* leaves extracts.

Solvent extract	Total phenolic content (µg GAE/mg extract)±SD
Methanol	58.70±0.21[g]
Water	57.14±0.05[g]

[g]Significance difference between methanol and water.

triplicates. Accordingly, ethyl acetate extract had higher total alkaloids content (106.44±6.54 µg AE/mg) as compared to other solvents extract while aqueous had least total alkaloids content (29.12±5.53 µg AE/mg). According to Table 4 there was statistical significant difference between groups as demonstrated by one-way ANOVA ($F(4, 10) = 83.109$, $p = 0.000$. A Tukey post hoc test denoted with superscript letters in Table 3 showed that the TAC of ethyl acetate and water extracts was statistically significant with other four solvents. Furthermore, there was no statistical difference between hexane TAC and Ethanol TAC, between hexane TAC and methanol TAC and between ethanol TAC and methanol TAC. The mean difference is significant at the 0.05 level.

Total flavonoids content (TFC)

The standard calibration curve of Rutin showed a linear regression of: $y = 0.0085x + 0.0419$ $R^2 = 0.9859$. The total content of flavonoids was expressed as rutin equivalent (µg RE/mg of dried extract). As stated in Table 5, the results obtained were expressed as Mean ± SD of triplicates. Methanol extract had higher total flavonoids content (78.84±0.47 µg RE/mg) as compared to others while water had least total flavonoids (30.41±0.12 µg RE/mg). Table 6 showed there was a statistically significant difference between groups as demonstrated by one-way ANOVA ($F(2,6) = 16922.597$, $p = 0.000$. A Tukey post hoc test results denoted with superscript letters in Table 5 showed that the TFC of ethanol, methanol and water was statistically significant among each. The mean difference is significant at the 0.05 level.

The standard calibration curve of Gallic acid showed a linear regression of: $y = 0.0124x + 0.3267$, $R^2 = 0.9908$. The total content of phenolic was expressed as Gallic equivalent (µg GAE/mg of dried extract). According to Table 7, the results obtained were expressed as

Table 8. Independent sample *t*-test results of total phenolics content between methanol and water.

Means difference	1.546
Std. error difference	0.125
df	4
t-cal.	12.358
Sig.	0.005
t-tab.	2.776

The mean difference is significant at the 0.05 level.

Table 9. Total tannins contents of *Pechuel-Loeschea leubnitziae* leaves extracts

Solvent extract	Total tannins content (µg TTE/mg extract)±SD
Hexane	30.85±4.92[g]
Ethyl acetate	13.06±0.54[g,i]
Ethanol	27.70±0.09[i]
Methanol	28.68±0.63[i]

[g]Statistical significance of hexane; [i]Statistical significance of ethyl acetate.

Table 10. Results of one way ANOVA of hexane, ethyl acetate, ethanol and methanol total tannins content means.

Source of variation	Sum of squares	df	Mean square	F	P-value
Between groups	592.821	3	197.607	31.794	0.000
Within groups	49.721	8	6.215		
Total	642.542	11			

Mean ± SD of triplicates. The methanol extract had maximal total phenolic content (58.69±0.21 µg GAE/mg) while water extract had minimal total phenolic content (57.14±0.05 µg GAE/mg). Table 8 shows independent sample t-test results that determine the statistical significance between methanol total phenolic content and water total phenolic content means. There was significance difference between TPC of methanol extract (58.70±0.21 µg/ml) and TPC of water extract (57.14±0.05 µg/ml); $t(4) = 12.358$, $p = 0.005$.

Total tannins content (TTC)

The standard calibration curve of tannic acid showed a linear regression of: $y = 0.0064x + 0.1052$ $R^2 = 0.9899$. The total content of tannins was expressed as tannic equivalent (µg TAE/mg of dried extract). As illustrated in Table 9, the results acquired were expressed as Mean ± SD of triplicates. The hexane extract had higher total tannins content (30.85±4.92 µg TAE/mg) as compared to other solvents extracts while ethyl acetate extract had least total tannins content (13.06±0.54 µg TAE/mg). As shown in Table 10, there was a statistically significant difference between groups as demonstrated by one-way ANOVA ($F(3, 8) = 31.794$, $p = 0.000$. A Tukey post hoc test results denoted with superscript letters in Table 9 illustrated that TTC of ethyl acetate extract was statistically significant to other extracts TTC. There was no statistical difference between hexane TTC and ethanol TTC, between hexane TTC and methanol TTC, and between ethanol TTC and methanol TTC. The mean difference is significant at the 0.05 level.

As indicated in Figure 1, the histogram represents comparison of total phytochemical contents in each solvent fraction and their abundance.

DISCUSSION

Phytochemical compounds are non-dietary plant derivatives that are determined as secondary metabolites based on their structures and biological functions. They are the main compounds that pose various properties including antimicrobial, antioxidants, antiviral and many more. They act against broad pathogens (Dushimemaria et al., 2012; Iikasha et al., 2017). Phytochemical screening process involves qualitative and quantitative profiling of the chemical compounds classes of ethno

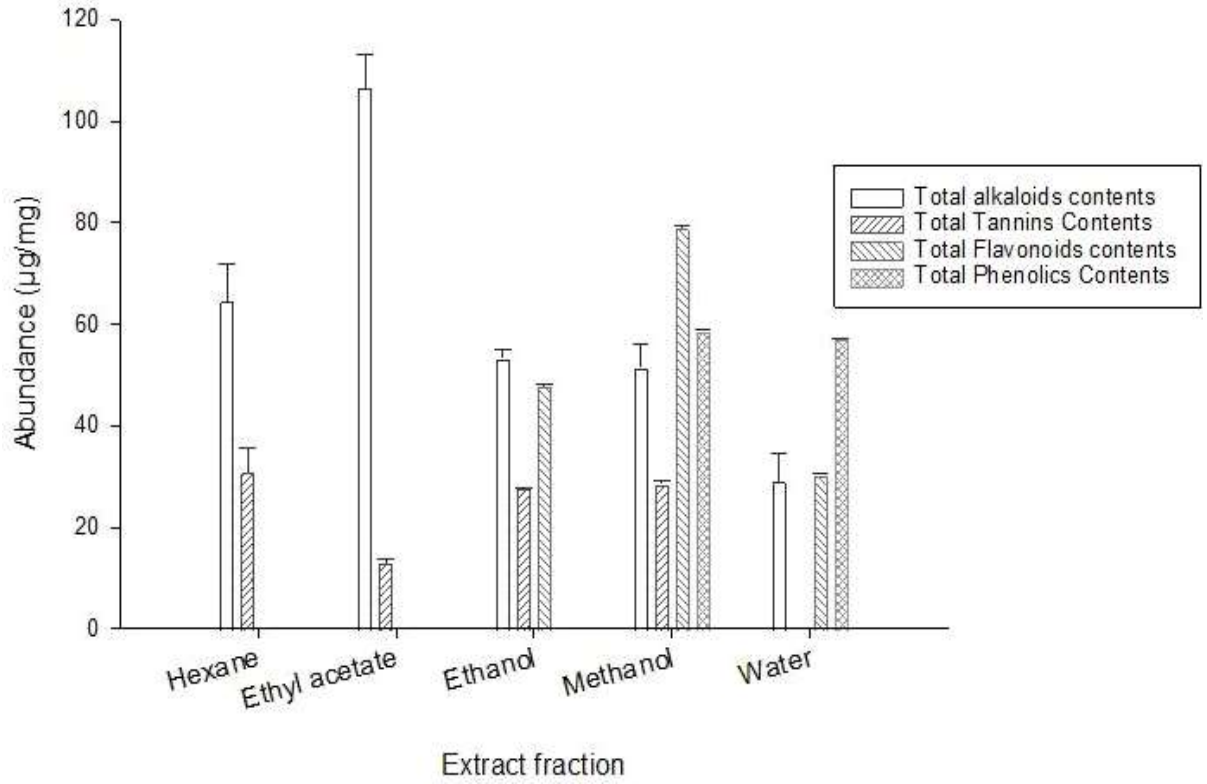

Figure 1. Evaluated total phytochemical contents of various fraction of *Pechuel-Loeschea leubnitziae* leaves. Error bars represent standard deviation (SD) of the data.

medicinal plants. Generally, preparation of plant extracts is mostly based on traditional methods, however during research investigation other methods of extraction were applied in order to establish the most effective method that can lead to bioactive compounds isolation and purification (Prashant et al., 2011; Iikasha et al., 2017). In order to extract desired phytochemical compounds from plant materials, using various solvents based on polarity is very crucial (Pradeepa et al., 2016). Consequently, to determine the plant composition, we employed polar and non-polar solvents in profiling of phytochemicals. *P. leubnitziae* extracts from roots and stems are reported having pharmacological activities and are of significance in the medical field (Ndongo, 2017). Phenolics, alkaloids, terpenoids, saponins, flavonoids and tannins were detected in *P. leubnitziae* leaves extract. These results are in agreement with previous study done by Ndongo (2017) except for the presence of terpenoids and alkaloids. The quantitative determination is a major way of context of the standard of a crude drug while effectiveness of a solvent in extracting phytochemicals gives a preliminary fact on the quality of a drug. The percentage yield of crude extract was reported higher in ethyl acetate (6.65%) and lower in hexane (1.49%). When a solvent is a weak solubilizer, there is a reduced dissolution which leads to higher mass; this could be attributable to ethyl acetate. Hexane deals with fats and oils, which could be indicative of why it holds a low yield. Percentage yield in ethanol, methanol and water are cross related, this is because the polarity variance between ethanol, methanol and water is minimal.

Alkaloids had been studied in modern medicine to validate their traditional use due to their pharmacological properties mainly for antibacterial activities but also for antiviral, anticancer, antifungal and antimalarial (Thawabteh et al., 2019; Casciaro et al., 2020). Thus, higher level of alkaloids in this plant's leaves which ranged between 29.12 and 106.44 mg/ml across solvents fraction indicates its significance and application in treatment of broad infectious diseases associated with multidrug-resistant (MDR) cases (Ghirga et al., 2016; Casciaro et al., 2020). Terpenoids are known to facilitate membrane disruption using lipophilic compounds and they possess a quite number of medicinal properties such as anti-carcinogenic, antimalarial, antihypertensive, insecticidal, antiviral and anti-ulcer which could validates the use of this plant as a potential antimicrobial drug (Saxena et al., 2013; Kabera et al., 2014; Ndongo, 2017). Saponins exhibit numerous biological activities such as insecticidal activity, anthelmintic activity, molluscicidal and piscidal activity, antifungal, anticancer, antiviral, and antibacterial activity because of their amphiphilic properties (Kezwon and

Wojciechowski, 2014; Marrelli et al., 2016; Dorota et al., 2017). Tannis are reported to be involved in formation of complexes molecules within bacteria cell well and prevent formation of biofilms (Prashant et al., 2011; Trentin et al., 2013; Mapiye, 2019). Their pharmacological activities include antioxidant, anticancer, anti-inflammatory, antifungal and antibacterial (Ramachandran et al., 2014; Mboweni, 2018; Mapiye, 2019). The total tannins content in this plant varies with a range of 13.06 to 30.85 mg/ml. Phenolics are diverse compounds that cut across most of medicinal plants and they are reported to have antioxidants and antibacteria properties to fight against cardiovascular diseases (Saxena et al., 2013; Kabera et al., 2014; Ndongo, 2017). Phenolics total content present in this plant are average with range of 57.14 and 58.70 mg/ml. Flavonoids play a big role in protecting plants against abiotic factors and their biological properties including antimicrobial, anti-inflammatory, antitumor, anti-allergenic, anti-carcinogenic, anti-aging and antiviral activities (Saxena et al., 2013; Ndongo, 2017). The flavonoids total contents in this plant ranged between 30.41 and 78.84 mg/ml. The results of total phytochemical contents exhibited in this plant are in agreement with the study done by Ndongo (2017) but with a very higher concentration of total contents compared to Ndongo's findings and this may be attributable to use of several solvents based on polarity without combining them. Although traditional uses of the selected plant are documented, its phytochemicals quality and quantity is yet to scientifically validated. In addition, the findings of this study served as a justification to validate the *P. leubnitziae* as a medicinal plant.

Conclusion

The results from this study demonstrate that *P. leubnitziae* leaves have abundant secondary metabolites which are largely utilized in traditional medicine to combat and cure numerous infections. Qualitative and quantitative analysis indicates that the leaves of *P. leubnitziae* are potential source of phytochemicals that could be used in drug discovery and development. Ethanol and methanol solvent extraction yields maximum phytochemicals compared to the other solvents and therefore recommended for the extraction of this plant materials.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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