IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 13, Issue 9 Ser. I (September. 2020), PP 34-41
www.iosrjournals.org

Phytochemical profile of *Plecranthusesculentus* N.E.Br obtained from Zimbabwe authenticates its medicinal uses

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Abstract:
The purpose of this study was to assess the ethnobotanical uses of Plecranthusesculentus N.E.Br in Makoni district, Zimbabwe as well as to evaluate the phytochemical profile of aqueous acetonic extract of the plant tubers using UV-Visible spectrophotometry, Fourier transform- Infrared (FTIR) analysis and high performance liquid chromatography (HPLC).Face to face interviews were conducted among residents of Makoni district in order to gather information about the ethnobotanical uses of Plecranthusesculentus. The phytochemical profile of Plecranthusesculentus tubers was determined using UV-Visible spectrophotometry and FTIR analysis after solvent extraction using conventional phytochemical extraction protocols. High performance liquid chromatography (HPLC) analysis was used to confirm the presence of two selected phenolic compounds.Data from interviews showed that Plecranthusesculentus is widely used as food and folklore medicine in Makoni district, Zimbabwe. The UV-Visible and FTIR analysis of the tuber extract showed the presence of phytochemical compounds with organic chromophores including phenyl, organic hydroxyl, amines, carboxyl, carbonyl, acyl, alkyl and aromatic compounds. HPLC analysis confirmed the presence kaempferol and vanillic acid in the tuber extract. The study establishes the common ethnobotanical uses of Plecranthusesculentus and avails data on the phytochemical profile of Plecranthusesculentus tubers found in Zimbabwe. It also promotes the use, further study and conservation of the plant basing on its potential phyto-pharmaceutical importance.

Key Words*:Ethnobotany, Medicinal plants, Plecranthusesculentus, Phytochemicals, Folklore medicine*

Date of Submission: 12-09-2020 Date of Acceptance: 29-09-2020

I. Introduction

Plants are commonly known to be important sources of thousands of natural medicinal compounds [1]. Phytochemicals are secondary metabolites from plants that have been documented to perform several functions ranging from pathogen defense, protein synthesis, protection against bad weather, nutrient uptake and enzyme activity [2, 3]. Phenolic acids and flavonoids are essential polyphenolic secondary metabolites found in plants [4]. Plant polyphenols have antioxidant function and are also useful in helping to wash free radicals and avoid reactive oxygen-related diseases [5-7]. Plant polyphenols are known to prevent and reduce diseases such as diabetes, cancer, cardiovascular diseases, cataracts, liver toxicity as well as stroke and ageing related disorders [8].

Medicinal plants are of great value to individuals and communities due to their health benefits. The medicinal value of these plants lies in their active chemical constituents, which have definite physiological behavior on human body systems. Flavonoids, phenolic acids, alkaloids, and tannins are the most important of these chemically active plant constituents [9]. A number of phytochemicals have been isolated from plants including phenolic acids, flavonoids, alkaloids, saponins, glycosides, phytols and essential oils [10]. Exploitation of biological properties of medicinal plants has been driven by their natural origin, cost effectiveness and less side effects [11]. Medicinal plants have been described as an expensive gift from nature to man [9].

The present work focused on phytochemical evaluation of tuber extracts of *Plecranthusesculentus* N.E.Br obtained from Zimbabwe. *Plecranthusesculentus* is a medicinal plant native to Africa. It is a perennial herb that belongs to the genus *Plecranthus* and the *Lamiaceae* family. *Plecranthusesculentus* is an erect, annual herbaceous plant growing to about 1 meter tall. It is native to Africa, and is both grown and found wild [12, 13]. *Plecranthusesculentus* usually grows in the wild, in woodlands, forest margins and rocky slopes of mountains. In Zimbabwe, the plant is usually found in the wild as well as in cultivated lands [14]. It is widely produced by farmers in the Eastern districts of Zimbabwe which include Rusape, Nyanga, Makoni, and Mutasa [15].

Plecranthusesculentus is widely distributed in African countries which include Nigeria, Central African Republic, Democratic Republic of Congo, Angola, Botswana, Swaziland, Tanzania, Zimbabwe, South Africa and Mozambique [16].

Plecranthusesculentus tubers have been reported to contain active phytochemicals [17]. The presence of phytochemicals which includes alkaloids, tannins, steroids, saponins and flavonoids in *Plecranthusesculentus* tubers was verified by a preliminary phytochemical screening study [15]. Despite its wide medicinal uses, *Plecranthusesculentus* is counted amongst the poorly studied and under-utilized food crop species in Africa [18]. It has been noted that the abandoning of traditional foods in favor of exotic food crops in Africa is likely to result in extinction of tuberous food plants such as *Plecranthusesculentus* [13]. The need for investigations on non-food uses and potential applications of *Plecranthusesculentus* in the pharmaceutical industry was recommended by other researchers [14]. The present work seeks to find out the ethnobotanical uses of *Plecranthusesculentus* as well as to evaluate the phytochemical profile of *Plecranthusesculentus* tubers grown in Zimbabwe.

Several techniques have been developed for qualitative and quantitative analysis of compounds of plant origin [19]. Among the techniques for the identification of phytochemicals are thin layer chromatography (TLC), capillary electrophoresis (CE), UV-Vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR), high performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS) and nuclear magnetic resonance (NMR) [20]. The choice of a suitable analytical technique depends on several factors that include extraction method used, storage conditions, storage time, particle size, interfering substances and impurities [21]. Availability of the desired analytical instrument and cost effectiveness of the technique are other factors which largely affects the choice of an analytical technique. In the present work, UV-Visible spectrometry and FTIR have been used to qualitatively identify phytochemicals in *Plecranthusesculentus* tuber extracts. The HPLC technique was employed to identify and quantify selected phenolic compounds in *Plecranthusesculentus* tuber extract.

II. Materials and Methods

2.1 Study Area

The study was carried out in Makoni district communal area (18°12′30″S to 32°48′00″E). Makoni district is located in Manicaland, the eastern province of Zimbabwe. The area receives an average annual rainfall of 750-1000 mm [22]. The district is largely populated with peasant farmers. The major cash crops grown in the area are tobacco and maize. *Plecranthusesculentus* is widely grown in the area as a supplementary food crop. The area was chosen on the notion that it is one of the major producers of *Plecranthusesculentus* tubers in Zimbabwe.

2.2 Data Collection and Analysis

Face to face interviews were conducted among the residents of Makoni district, Zimbabwe to determine the common uses of *Plecranthusesculentus* in the area. Prior informed consent was obtained from all interview respondents. Respondents were purposively sampled among the residents of the study area. The frequency of quote of each use was calculated from the collected data. Only those uses with a frequency of quote equal to or above 50.00 % were taken into consideration.

2.3 Materials

All chemicals and reagents used in the experiments were purchased from reputable suppliers. HPLC grade organic solvents were procured from Skylabs, South Africa. Analytical reagent grade kaempferol and vanillic acid standards were procured from Sigma Aldrich, Germany. *Plecranthusesculentus* tubers (2.50 Kg) were obtained in July 2019 from farmers residing in Makoni district, Zimbabwe.

2.4 Sample Pretreatment

The species of the plant was firstly identified from a sample plant by a botanist at Harare Polytechnic. The herbarium specimen (voucher number SCI44) was deposited in the horticultural section at Harare Polytechnic. To remove foreign contaminants, the tubers were thoroughly cleaned with running tap water and further washed with sterilized distilled water. Prior to further use, the tubers were stored in a refrigerator at 5 °C.

2.5 Extraction of Phytochemicals

Fresh *Plecranthusesculentus* tubers were chopped with a sterile knife and pounded using a mortar and pestle into a homogeneous mixture. The extraction of phytochemicals was then carried out using the common solvent extraction technique, as demonstrated in previous works [9, 23]. A separate 150.00 g crushed tuber sample was extracted using 250.0 mL aqueous acetone (60.00 %) as the extraction solvent on a laboratory shaker for a period of 24 hours. The residue plant material was separated from the extract by filtering through a whatman filter paper. The extract was subjected to fractionation using hexane and chloroform to eliminate non-

polar components and pigments. The fractionated extract was then concentrated to dryness using rotary vacuum evaporation at 50 °C, accompanied by air drying as suggested by other researchers [24, 25]. The resultant solid extract was weighed and stored for further experiments. The phytochemical extraction yield (percentage) from the tuber extract was determined according to Equation 1 [26].

Extraction yield (%) = (Final dry weight of extract)/ (Initial weight of fresh plant material) ×100 (1)

2.6 UV-Visible Spectrophotometric Analysis

The tuber extract was subjected to UV-Visible spectrophotometric scanning on a UV-Visible spectrophotometer (*Merck Spectroquant, Pharo 300*) as described [27, 28], with a few modifications. Dried tuber extract sample (1.00 g) was firstly dissolved in methanol. Thereafter, the sample solution was diluted and subjected to UV-Visible scanning over a wavelength range of 200 nm-800 nm. The absorption profile and the wavelength of maximum absorbance for the sample were recorded. The UV-Visible spectrum for the sample was captured.

2.7 Fourier Transform Infra-Red (FTIR) Analysis

The dried tuber extract was subjected to FTIR analysis on a Fourier transform infra-red spectrophotometer (Infra 3000A) as described by other scholars [28-30]. Briefly, a 0.20 g portion of the dried tuber extract was thoroughly mixed with 10.00 g KBr salt using a mortar and pestle. A portion of the mixture was used to make 0.5 mm thick pellets. The pellets were then subjected to FTIR analysis in the wavenumber range 3800 to 400 cm-1. The FTIR spectra were captured and recorded.

2.8 High Performance Liquid Chromatography (HPLC) Analysis

2.8.1 HPLC method validation

The HPLC method used for identification of selected phenolic compounds was validated as suggested in past works [1, 31]. Linearity, limits of detection, limits of quantification and precision were considered in the validation process. To ascertain the linearity, five different standards in the concentration range 0.05-0.40 µg/mL were run on the HPLC system. Calibration curves were plotted from peak areas versus concentration for each standard. The measure of linearity was indicated by the correlation coefficient (R^2) value. Limit of detection for each standard was determined using the formula in Equation 2.

$$LOD = (3.3\times\delta)/S \quad (2)$$

Where, δ = standard deviation of response (peak area) and S = slope of the calibration curve.

Limit of quantification for each standard was calculated using the formula in Equation 3.

$$LOQ = (10\times\delta)/S \quad (3)$$

Where, δ = standard deviation of response (peak area) and S = slope of the calibration curve.

Precision was determined from standard deviations obtained by analysis of a series of standards. Precision as relative standard deviation (% RSD) was calculated from the measured standard deviations (SD) and mean values using Equation 4.

$$\% RSD = SD/Mean \times 100 \quad (4)$$

2.8.2 Column Purification

Column purification of the crude tuber extract was carried out as per the method outlined by [32], with minor modifications was conducted prior to HPLC analysis. A column was loaded with a paste of silica gel prepared in hexane: ethyl acetate solvent mixture of ratio 1:1. The column packing was allowed to settle. Solvents of varying polarity were used to fractionate the crude tuber extract in the order petroleum ether, acetone, ethyl acetate, methanol and water. The different solvent fractions were collected and preserved for further use.

2.8.3 Preparation of HPLC Standards

Standards for HPLC analysis were prepared as mentioned [31]. Stock solutions of concentration 1.00 mg/mL were prepared by dissolving 0.01 g standards (kaempferol and vanillic acid) in 10.0 mL HPLC grade methanol. Working standards in the 0.05-0.40 µg/mL concentration range were obtained by suitable dilutions from stock solutions.

2.8.4 Conditions for HPLC Analysis

The identification of two selected phenolic compounds (kaempferol and vanillic acid) in the tuber extract was done using the HPLC (Agilent, 1100 Series) system equipped with a binary pump (G1312A), degasser (G1379A) and a photodiode array detector (PDA G1315A) as described in previous works [33, 34].

The conditions used for HPLC analysis were as follows: HPLC grade methanol solvent, injection volume of 20.0 μL per sample, reversed phase C-18 column (Agilent, Zorbax ODS, 5 µm, 4.6 mm x 150 mm), flow rate of 0.3 mL/min, gradient mode of elution using a mobile phase composed of deionized HPLC grade water acidified with 0.10 % formic acid (solvent A) and a mixture of HPLC grade CH3CN/MeOH (1.5: 1; v/v), acidified with 0.1 % formic acid (solvent B). Elution was performed using a gradient of 5,00 % B for 0-2 minutes, 20.00 % B for 2-4 minutes, 70.00 % B for 4-6 minutes and 5.00 % B for 6-30 minutes. The chromatogram was monitored at 254 nm using a PDA detector. The chromatogram for the sample was processed using the Masslynx processing software and tentative identification was performed by comparing sample retention times with results from the standards.

III. Resultsand Discussion

3.1 Ethnobotanical Uses of Plecranthusesculentus

The common ethnobotanical uses of *Plecranthusesculentus* in Makoni district are shown in Table 1.

Table 1: Commonethnobotanical uses of *Plecranthusesculentus* in Makoni district, Zimbabwe.

Ethnobotanical use	**Plant part used**	**Frequency of quote**
Food eaten cooked	Tubers	85.00 %
Food eaten raw	Tubers	95.00 %
Treating stomach aches	Tubers	81.00 %
Treating nausea	Tubers	70.00 %
Treating menstrual cramps in women	Tubers	60.00 %
Increasing potency in man	Tubers	55.00 %

Only uses with a frequency quote greater than or equal to 50.00 % were considered.

As indicated in Table 1, *Plecranthusesculentus* is commonly used as food (raw or cooked), with more than 80.00 % quotes. The plant is also strongly believed to possess medicinal potential against stomach aches and nausea in the area. It used to treat stomach problems and nausea among the general populace of the area. *Plecranthusesculentus*is moderately believed to cure menstrual cramps associated with female reproductive system and to increase potency in man. The results coincide with findings by other researchers in Nigeria and Kenya [35, 36].

3.2 Yield of Extracted Phytochemicals

The extraction yield for phytochemicals from the tubers was calculated as per the formula for extraction yield (Equation 1).

Extraction yield (%) = 6.85 g ÷ 125.00 g × 100

= 5.48 %

The extraction yield for phytochemicals was found to be 5.48 %. The extraction yield was very low. This could be explained by the fact that phytochemical yields from plant materials using solvent extraction techniques are typically very low [21]. Other researchers have also recorded yields of phytochemicals within the same range [37, 38]. Usually very long extraction times and large amounts of solvents are required in order to increase the yields.

3.3 UV-Visible Spectrophotometric Scanning

The UV-Visible absorption profile for *Plecranthusesculentus* tuber extract is summarized in Table 2.

Table 2: UV-Visible absorption profile for *Plecranthusesculentus* tuber extract.

Wavelength of absorption (nm)	Absorbance
212	0.388
234	0.175
320	0.640

The tuber extract of *Plecranthusesculentus* gave three major distinctive UV-Visible absorption peaks at 212 nm, 234 nm and 320 nm (Table 2). Phenolic compounds, precisely flavonoids and their derivatives, have

characteristic maximum absorptions in the range 230-285 nm and 300-350 nm [29]. The *Plecranthusesculentus* tuber extract absorption bands are therefore characteristic for flavonoids and related compounds. UV-Visible peaks in the range 200-400 nm of a given sample explicitly indicates the presence of unsaturated groups and heteroatoms, such as S, N and O [28]. The UV-Visible spectrophotometric profile of *Plecranthusesculentus* tuber extract indicates the existence of polyphenolic organic chromophores.

3.4 Fourier Transform Infra-Red (FTIR) Analysis

The extract of *Plecranthusesculentus* tubers was analyzed using FTIR in the range 3800-400 cm^{-1}. The FTIR spectrum for *Plecranthusesculentus* tuber extract is shown in Figure 1.

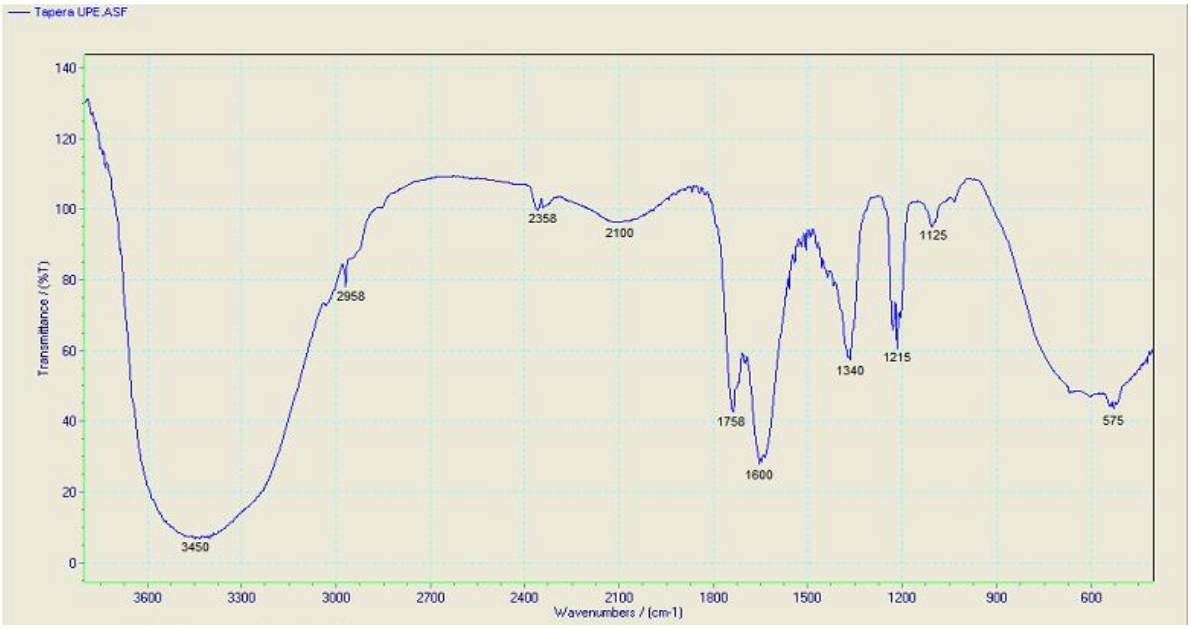

Figure 1: FTIR spectrum for *Plecranthusesculentus* tuber extract

As shown Figure 1, the IR absorption pattern of *Plecranthusesculentus*tuber extract showed the following major absorption peaks: 3450 cm^{-1}, 2958 cm^{-1}, 2358 cm^{-1}, 2100 cm^{-1}, 1758 cm^{-1}, 1600 cm^{-1}, 1340 cm^{-1}, 1215 cm^{-1}, 1125 cm^{-1} and 575 cm^{-1}. Table 3 summarizes the possible bond assignments to each of the characteristic FTIR peaks.

Table 3: Possible bond assignments to FTIR peaks for Plecranthusesculentus tuber extract sample.

FTIR Absorption peak	**Possible assignment**
3450 cm^{-1}	O-H vibrations (alcohol at 3200-3400 cm^{-1} and carboxylic acid at 3500 cm^{-1})
2958 cm^{-1}	CH_3, CH_2 and CH stretching vibrations
2358 cm^{-1}	No possible assignments
2100 cm^{-1}	Variable bond stretches
1758 cm^{-1}	C=O stretching vibrations in aldehydes
1600 cm^{-1}	C=O stretching vibrations in ketones
1340 cm^{-1}	CH_3 bending vibrations
1215 cm^{-1}	Acyl or phenyl C-O stretching vibrations
1125 cm^{-1}	C-C-C- bending vibrations
575 cm^{-1}	C=C (cis) bending vibrations and aromatic ring vibrations

As elaborated in Table 3, the results showed that *Plecranthusesculentus* tuber extract contains phenyl, organic hydroxyl, organic nitro, carboxyl, carbonyl, acyl, alkyl and aromatic compounds. The findings of the FTIR study are in line with several works carried out on other plant extracts. In *Viscum album* and *Allium sativum* herbal extracts, [39] determined flavonoids and phenolic compounds using FTIR and identified characteristic peaks for O-H groups, C=O groups and C=C systems. [40] also identified similar functional groups on FTIR analysis of Indian medicinal plant extracts.

3.5 HPLC Analysis

3.5.1 Validation of the HPLC Method

To validate the HPLC method used in this study, linearity, limits of detection, limits of quantification and precision of the method were determined. The validation parameters for the HPLC process used in this analysis are summarized in Table 4.

Table 4: Regression equations, R^2 values and validation parameters for HPLC analysis of phenolic standards

Standard	**Equation**	**R^2**	**LOD (µg/mL)**	**LOQ (µg/mL)**
Kaempferol	y = 10739x+348.76	0.9941	0.0034	0.0102
Vanillic acid	y = 100435x+7961.4	0.9987	0.00085	0.0026

Precision for kaempferol =1.00 %

Precision for vanillic acid = 0.44 %

LOD = limit of detection and LOQ = limit of quantification

As shown in Table 4, high values of R^2 that are ≥ 0.9941 were obtained. The high values of R^2 for the standard calibration curves used for validation purposes indicate a good response linearity of the method. The repeatability of the standard retention times was below 1 %, indicating the method's accuracy. The relative standard deviations of all the peak areas for the standards were ≤ 1 %, confirming the precision of the method. The validation results in Table 4 show that the HPLC method was precise and sensitive for the analysis.

3.5.2 HPLC Analysis of Plecranthusesculentus Tuber Extract

Selected phenolic compounds in *Plecranthusesculentus* tuber extract were analyzed using the HPLC technique. Retention times for individual standards were used to identify selected phenolic compounds in *Plecranthusesculentus* tuber extract. Calibration curves for individual standards were used to determine the concentrations of the selected phenolic compounds in the tuber extract. Figure 2 and Figure 3 show the typical HPLC chromatograms for phenolic standards (kaempferol and vanillic acid) and the *Plecranthusesculentus* tuber extract respectively.

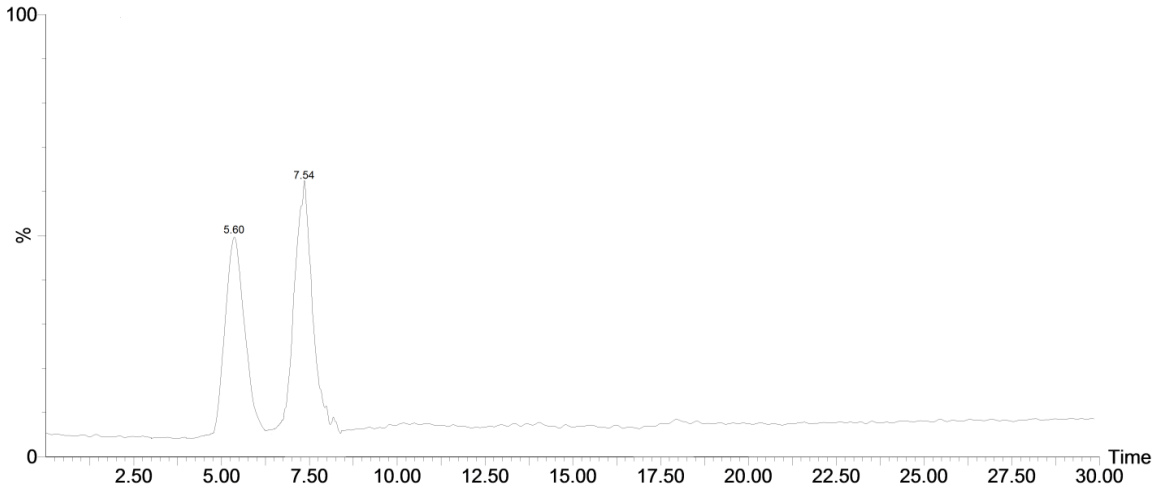

Figure 2: Typical HPLC chromatogram for phenolic standards (kaempferol and vanillic acid)

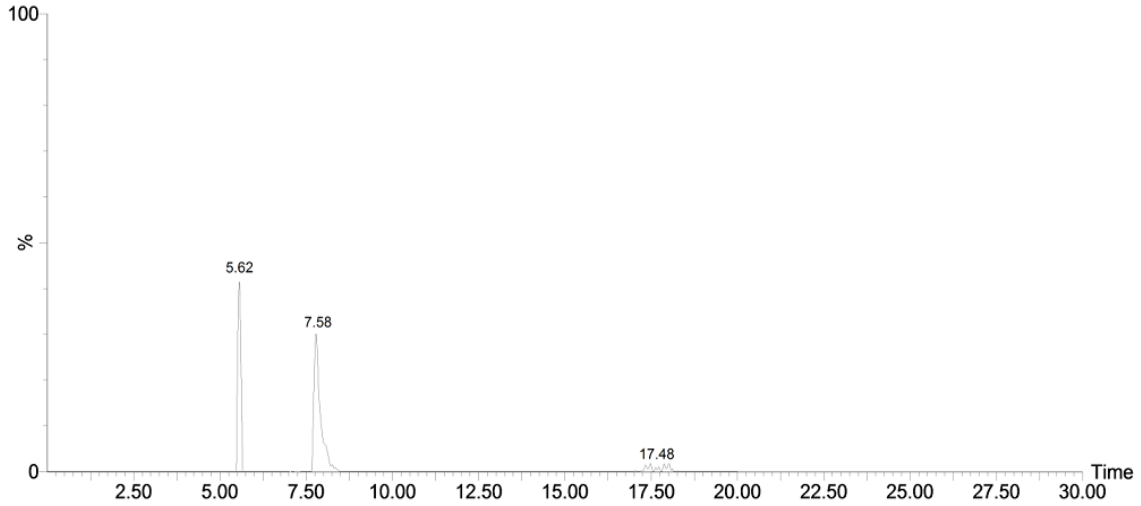

Figure 3: Typical HPLC chromatogram for *Plecranthusesculentus* tuber extract

As shown in Figure 3, kaempferol and vanillic acid in the *Plecranthusesculentus* tuber extract was detected at 5.62 minutes and 7.58 minutes respectively. *Plecranthusesculentus* tuber extract was found to contain 0.0166 ±0.002 µg/g kaempferol and 0.1241 ±0.004 µg/g vanillic acid. The results indicate the presence of substantial quantities of kaempferol, a flavonoid and vanillic acid, a phenolic acid in *Plecranthusesculentus* tuber extract. Both kaempferol and vanillic acid are potent phytochemical compounds that have wide applications in the food, pharmaceutical and allied industries. Other flavonoids and phenolic acids which are known to exhibit potent biological activities are also likely to be present in *Plecranthusesculentus* tubers. Kaempferol and vanillic acid were also identified and quantified in other plant extracts [31, 33, 41]. The presence of kaempferol and vanillic acid in *Plecranthusesculentus* clearly shows that the plant is a potential source of important medicinal compounds.

IV. Conclusions

The study confirmed the common ethnobotanical uses of *Plecranthusesculentus* in Makoni district, Zimbabwe. The investigation has given fundamental information on the phytochemical composition of *Plecranthusesculentus* using UV-Visible spectrometry and FTIR techniques. The HPLC technique further confirmed the presence of kaempferol and vanillic acid in *Plecranthusesculentus* tuber extract. The presence of bioactive compounds in *Plecranthusesculentus*tuber extract authenticates its wide use as a folklore medication. *Plecranthusesculentus*is therefore recommended as a plant of phyto-pharmaceutical importance. Further studies will need to be undertaken to fully ascertain the bioactivity and toxicity profile of *Plecranthusesulentus* tuber extract.

Acknowledgements

The authors would like to acknowledge the chemistry laboratory personnel at Harare Polytechnic and Kenyatta University for the support on the execution of laboratory work and the local people of Makoni district, Zimbabwe who participated in the interviews for giving valuable information.

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MasimbaTapera, et. al. "Phytochemical profile of *Plecranthusesculentus* N.E.Br obtained from Zimbabwe authenticates its medicinal uses." *IOSR Journal of Applied Chemistry (IOSR-JAC)*, 13(9), (2020): pp34-41.