PHYTOCHEMICAL STUDY TO VALIDATE THE ETHNOBOTANICAL IMPORTANCE OF *Dioscorea steriscus* TUBERS OBTAINED FROM ZIMBABWE

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ABSTRACT

Modern people have embraced plants as a source of useful bioactive compounds. As such, plants with medicinal properties have become essential components in human life. The purpose of this study was to investigate the phytochemical profile of the extract of *Dioscorea steriscus* tubers obtained from Zimbabwe. Phytochemicals were extracted from tubers of *D. steriscus* using aqueous acetone. UV-Visible Spectrophotometry, Fourier transform infra-red (FTIR) spectrometry and high-performance liquid chromatography (HPLC) techniques were used to determine the phytochemical profile of the tuber extract. The yield of phytochemicals extracted from *D. steriscus* tubers was found to be 6.38 %. The presence of bioactive compounds possessing phenyl, organic hydroxyl, amine, carboxyl, carbonyl, acyl, alkyl and aromatic functional groups was confirmed using UV-Visible and FTIR analysis. The presence of substantial amounts of vanillic acid and kaempferol in *D. steriscus* tubers was confirmed using HPLC analysis. In support of the ethnobotanical values of *Dioscorea* species, the study confirmed the presence of potent phytochemicals in the extract of *D. steriscus* tubers obtained from Zimbabwe.

Keywords: *Dioscorea steriscus*, ethnobotany, medicinal plant, phytochemicals.

INTRODUCTION

Plants have been used as sources of food and medication by man since ancient times (Eleazu, Eleazu, & Ikpeama, 2012). Plants that are known to contain chemical substances with definite physiological action on biological systems are called medicinal plants or herbs. Medicinal plants have been discovered long back by ancient man through trial and error methods (Dar, Shahnawaz & Qazi, 2017). The presence of biologically active and potent chemical substances in these plants makes them ethnobotanically important. Various studies have shown that many plants are rich sources of phytochemical compounds that

possess useful medicinal properties (Altemimi, Lakhssassi, Baharlouei, Watson, & Lightfoot, 2017). A number of researchers have been extensively studying the possible applications of phytochemicals in resolving current health problems (Altemimi, Lakhssassi, Baharlouei, Watson, & Lightfoot, 2007; Sheikh, Kumar, Misra, & Pfoze, 2013; Dzomba & Musekiwa, 2014; Musila, Nguta, Lukhoba, & Dossaji, 2017; Abdel-Hady et al., 2018). Medicinal plants have been used for many years to treat medical conditions, as food flavorants, food preservatives and to combat disease epidemics (Dar et al., 2017). It is recommended that research on medicinal plants and tubers is performed to investigate their unknown agronomic qualities, physicochemical characteristics and other possible medicinal properties (Musila et al., 2017; Abdel-Hady et al., 2018; Tapera, 2019).

The *Dioscoreaceae* family members, also known as yam species are widely distributed medicinal plants in the world. Among the *Dioscoreaceae* family members, the *Dioscorea* genus is known to be one of the oldest yams found wildly or cultivated by man (Dutta, 2015). Other tubers of the same genus with *Dioscorea steriscus* include *Dioscorea alata*, *Dioscorea pentaphylla*, *Dioscorea bulbifera*, *Dioscorea villosa*, *Dioscorea orbiculata*, *Dioscorea hispida* and *Dioscorea pubera*. Among these members, *D. alata*, *D. pentaphylla*, *D. bulbifera*, *D. villosa* and *D. hispida* have been utilized as traditional medications especially for skin infections and wound healing (Dutta, 2015). *Dioscoreaceae* tubers have been reportedly used as sources of traditional medicine to treat several ailments including diarrhea and diabetes (Sakthidevi & Mohan, 2013).

Dioscorea steriscus is a yam commonly found in Zimbabwe and southern Africa. It is popularly used as food as well as a source of traditional medications in the northern parts of Zimbabwe. Preliminary phytochemical screening assays on *D. steriscus* tuber extracts confirmed the presence of flavonoids, terpernoids, saponins, alkaloids, polyphenols, tannins, steroids and glycosides (Tapera & Machacha, 2017). Moreover, total phenolic content of above 69.00 mg/g was recorded from extracts of *D. steriscus* tubers obtained from Zimbabwe (Tapera & Machacha, 2017). Similar studies on different *Dioscorea* yams confirmed the presence of potent phytochemicals that possess medicinal potential (Sheikh et al., 2013; Roy & Geetha, 2013; Narkhede et al., 2013). Figure 1 shows a pictorial view of *D. steriscus* tubers obtained from Zimbabwe.

Figure 1. A pictorial view of D. steriscus tubers

Enormous evidence shows that *D steriscus* is widely used as a source of supplementary food and traditional medicine in Zimbabwe (Dzomba & Musekiwa, 2014; Tapera & Machacha, 2017; Washaya, Mupangwa, & Muranda, 2016). *D. steriscus* is acclaimed to treat a variety of diseases which include hypertension, diabetes, heart attacks, stomach pains, erectile dysfunction in man and obesity (Dzomba & Musekiwa, 2014). *D. steriscus* has been categorized as an underutilized or neglected plant (Washaya et al., 2016). Underutilized plants are those crops that were once grown more extensively but their cultivation has dwindled due to economic, agronomic or genetic reasons (Czarapata, 2005). Moreover, *Dioscorea* species are also listed under priority underutilized root and tuber crops of Africa, Asia and Latin America (Wagner, Herbst, & Sohmer, 1999).

The frequency of use of *D. steriscus* tubers among residents of Bindura, Zimbabwe has been reported (Washaya et al., 2016). The plant is known to be an integral component of the traditional medicine and food system of some tribes in Zimbabwe. Washaya et al., confirmed that *D. steriscus* is a popular food and medicinal tuber consumed in Bindura, Zimbabwe (Washaya et al., 2016).

There is need to verify traditional and oral information regarding the chemical properties and biological activities of plant materials. The most appropriate approach towards authentication of chemical and biological properties of plant materials is through chemical analysis. Several analytical chemistry instruments and methods have been developed and validated for the purpose of analyzing different sample matrices. Advancement in science and technology has also seen the development of several methods and techniques for extraction, isolation and identification of medicinal compounds in plant materials (Naczk & Shahidi, 2004).

In this work, UV-Visible spectrophotometry, FTIR and HPLC techniques have been employed to evaluate the phytochemical profile of *D. steriscus* tuber extract.

EXPERIMENTAL

Chemicals and reagents

Analytical reagent grade methanol, acetone, hexane, chloroform, formic acid, potassium bromide and acetonitrile were obtained from Labcraft, Zimbabwe. Vanillic acid and kaempferol standards were procured from Sigma Aldrich, Germany.

Plant material and preparation of extract

Fresh and mature *D. steriscus* tubers (voucher specimen number 43) were collected in July 2019 from Bindura, Zimbabwe. Identification and authentication of the plant was done by a Botanist at Harare Polytechnic's horticultural section. The phytochemical extract of the plant tubers was prepared as per the protocol reported earlier (Adeogun, Maroyi, & Afolayan, 2017; Zhang, Lin, & Ye, 2018). In short, the fresh plant tubers (250.0 g) were reduced to a pulp using a porcelain mortar and pestle. The ground material was subjected to cold extraction with 60 % acetone (v/v) in distilled water. The extraction process was accelerated by shaking for 24 hours on a mechanical shaker. The extract obtained was sequentially extracted using chloroform and hexane in a separating funnel to get rid of lipids and other non-polar components. The crude extract solution was evaporated to dryness under reduced pressure at 50 °C using a rotary evaporator. The yield of the phytochemical extract was determined using the formula in Equation 1 as recommended (Ruwali, Ambwani, Gautam, & Thapliyal, 2015). The dried crude *D. steriscus* tubers extract was refrigerated at 4 °C before further use.

$$\text{Extraction yield (\%)} = \frac{\textit{Final dry weight of extract}}{\textit{Initial weight of fresh plant material}} \times 100 \quad (1)$$

UV-Visible spectrophotometric analysis

For UV-Visible spectrophotometric analysis, the dried extract (2.0 g) was dissolved in methanol and diluted appropriately with the same solvent. UV-Visible spectrophotometric analysis was then performed on the extract solution using a UV-Visible spectrophotometer (*Specroquant 300*) at ambient temperatures. The UV-Visible spectrophotometric spectrum of the tuber extract in the wavelength range 200-800 nm was captured.

Fourier transform infra-red (FTIR) analysis

FTIR analysis of *D. steriscus* tuber extract was conducted using an *Infra 3000A* FTIR instrument system. Briefly, a small portion of the tuber extract was thoroughly mixed with previously dried potassium bromide salt in a ratio of 1:10 using a mortar and pestle. The mixture was pressed into thin disks prior to FTIR analysis. FTIR analysis was then performed to identify characteristic peaks and their functional groups in the wavenumber range of 400-4000 cm^{-1}. The FTIR spectrum for the extract was captured.

High performance liquid chromatography (HPLC) analysis

In order to confirm the presence of potent phytochemical compounds in *D. steriscus* tuber extract, HPLC analysis of the tuber extract was performed on an *Agilent 1100* HPLC system equipped with a binary pump (G1312A), degasser (G1379A) and a photodiode array detector (PDA G1315A). Two reference phenolic compounds (vanillic acid and kaempferol) were used for HPLC analysis. Before conducting HPLC analysis, the *D. steriscus* tuber extract was further cleaned using column purification. Column purification of the crude tuber extract was conducted according to the process described by Bajpai et al., with small changes (Bajpai, Majumder, & Park, 2016). A column was filled with a silica gel paste made in hexane-ethyl acetate mixture of ratio 1:1. The packed column was given enough time to settle. Organic solvents with different polarities were used to sequentially fractionate the crude tuber extract following the order petroleum ether, acetone, ethyl acetate, methanol and finally water. The different fractions were collected and preserved for further use.

The HPLC method was validated by determination of linearity, limits of detection, limits of quantification and precision as recommended in similar works (Nour, Trandafir, & Cosmulescu, 2013). For the purpose of validation, six different standard concentrations in the range 0.05-0.5 ug/mL were prepared for both vanillic acid and kaempferol. Concentrations of the standards versus their HPLC peak area values were used to plot calibration curves. The correlation coefficients (R^2) values were used to indicate the linearity of the method. The precision of the method was calculated from the standard deviations determined from the analysis of a series of replicate standards. The precision was reported as percent relative standard deviation (% RSD) and was calculated using Equation 2.

$$\text{Precision} = \frac{SD}{Mean} \times 100 \qquad (2)$$

where, SD = standard deviation of replicate standard measurements

Limits of quantification and limits of detection for both standards were calculated using equation 3 and 4 respectively.

$$\text{Limit of quantification} = \frac{10 \times \delta}{S} \qquad (3)$$

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

$$\text{Limit of detection} = \frac{3.3 \times \delta}{S} \qquad (4)$$

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

Conditions for HPLC analysis

HPLC analysis was conducted as recommended by other researchers (Abdel-Hady, 2018; Theerasin & Baker, 2009). HPLC grade acetonitrile, methanol and water were used as solvents. The volume of injection for each sample was 20 µL and a reversed phase C-18 column (Agilent, Zorbax ODS, 5 µm, 4.6 mm x 150 mm) was used. The sample flow rate was set at 0.3 mL/min under gradient mode of elution employing 0.1 % formic acid in HPLC grade water as solvent number 1 and a blend of methanol and acetonitrile (1:1.5; v/v), acidified with 0.1 % formic acid as solvent number 2. The gradient of elution was maintained as follows: 50 % solvent 2 for 0-2 minutes, 20 % solvent 2 for 2-4 minutes, 70 % solvent 2 for 4-6 minutes and finally 5 % solvent 2 for 6-30 minutes. The chromatogram was monitored at 254 nm using a PDA detector. The HPLC chromatogram obtained was processed using the Masylynx software. The HPLC chromatogram was provisionally interpreted by comparison of retention times of the sample chromatogram with that of the reference compounds.

RESULTS AND DISCUSSION

Extraction yield

The yield of phytochemicals extracted from *D. steriscus* tubers was calculated using the formula for yield of extraction in Equation 1.

Extraction yield (%) = 15.96 g ÷ 250.00 g × 100= 6.38 %

The phytochemical yield was very low. The low yield can be attributed to the use of the solvent extraction technique, which typically gives poor yields (Ajila et al., 2011). Costly methods could be used in order to improve the yields; however, the focus of the work was not on the yield of extraction, and rather it was on the quality of the extract. Low yields of phytochemicals in the range 5 %-15 % have also been reported in other researches (Acharyya, Patra, & Bag, 2009; Joy & Siddhuraju, 2017).

UV-Visible analysis

From the UV-visible analysis, *D. steriscus* tuber extract showed characteristic UV-Visible spectrophotometric absorption peaks at wavelength 216 nm, 288 nm and 326 nm. Phytochemicals, specifically flavonoids and their derivatives, have characteristic absorption peaks in the range 230-290 nm and 300-360 nm (Dhivya, 2017). The UV-Visible spectrophotometric absorption bands of *D. steriscus* tuber extract are therefore typical for flavonoids and their derivatives. The precise location and relative intensities of the UV-Visible absorption bands may vary a bit and gives important data on the quality and nature of the flavonoids present (Saxena & Saxena, 2012). The occurrence of UV-Visible spectrophotometric bands in the 200-400 nm range for a given sample typically shows the presence of unsaturated groups and hetero-atomic compounds (Jain, Soni, Jain, & Bhawsar, 2016). The UV-Visible spectrophotometric profile of *D. steriscus* tuber extract showed the presence of organic chromophores characteristic of polyphenolic compounds.

Fourier transform infra-red (FTIR) analysis

D. steriscus tuber extract was analyzed using FTIR in the range 400-3800 cm^{-1}. Figure 2 displays the FTIR spectrum for *D. steriscus* tuber extract.

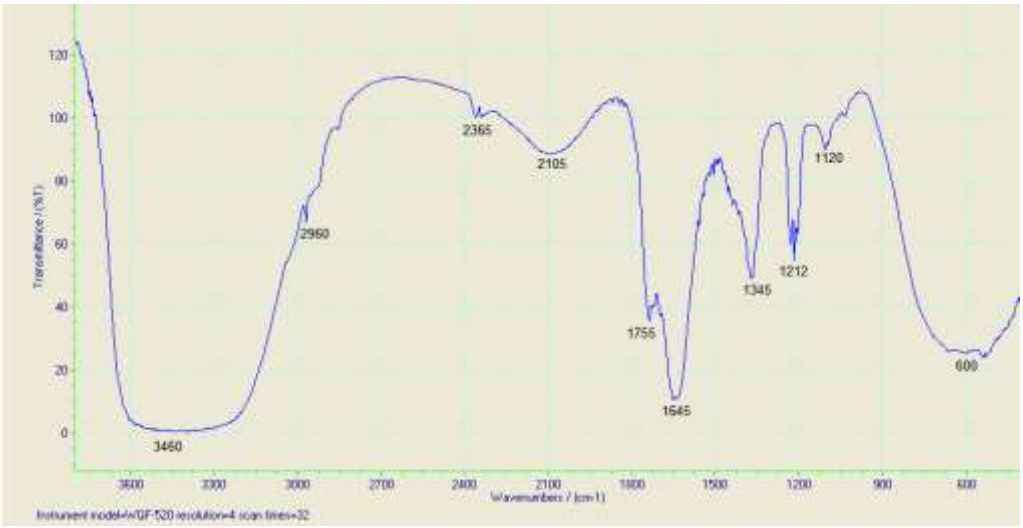

Figure 2. FTIR spectrum for *D. stericus* tuber extract

As shown in Figure 2, the following key peaks were found in the FTIR absorption pattern of *D. steriscus* tuber extract: 3460 cm^{-1}, 2960 cm^{-1}, 2105 cm^{-1}, 1755 cm^{-1}, 1645 cm^{-1}, 1345 cm^{-1}, 1212 cm^{-1}, 1120 cm^{-1} and 600 cm^{-1}. Table 1 summarizes the probable allocations of bonds to each of the distinctive peaks. The findings showed that the *D. steriscus* tuber extract contains phenyl, organic hydroxyl, organic nitro, carboxyl, carbonyl, acyl, alkyl, and aromatic compounds, as elaborated in Table 1. The FTIR study results are consistent with other studies performed on other natural products.

Trifunschi et al., determined flavonoids and phenolic compounds in *V. album* and *A. sativum* herbal extracts using FTIR analysis and confirmed the presence of O-H groups, C=O groups and C=C systems (Trifunschi, Munteanu, Agotici, Pintea, & Gligor, 2015). Similar functional groups were also described on FTIR analysis of Indian medicinal plant extracts (Ashokkumar & Ramaswamy, 2014).

Table 1. Probable bond assignments to FTIR peaks

Absorption peak	Possible assignment
3460 cm^{-1}	Vibration due to O-H (alcohol at 3200-3400 cm^{-1} and carboxylic acid at 3500 cm^{-1})
2960 cm^{-1}	Stretching vibrations of CH_3, CH_2 and CH
2105 cm^{-1}	Stretching vibration of variable bonds
1755 cm^{-1}	Stretching vibration of C=O in aldehydes
1645 cm^{-1}	Stretching vibration of C=O in ketones
1345 cm^{-1}	Bending vibration of CH_3
1212 cm^{-1}	Stretching vibrations of acyl or phenyl C-O
1120 cm^{-1}	Bending vibrations of -C-C-C-
600 cm^{-1}	Bending vibrations and aromatic ring vibrations due to C=C

High performance liquid chromatography (HPLC) analysis

HPLC method validation

Table 2 summarizes the validation parameters for the HPLC method used in this study. As indicated in Table 2, high R^2 values that are greater than 0.99 were obtained. The high R^2 values for the vanillic acid and kaempferol calibration curves used for validation purposes indicate a good response linearity of the method. The repeatability for both vanillic acid and kaempferol retention times was below 1.0 %, indicating the accuracy of the method. The percentage relative standard deviations for the peak areas of both standards were less than 1 .0 %, indicating the precision of the method. The results on the validation parameters in Table 2 indicate that the HPLC technique was reliable and sensitive enough for the study.

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

Phenolic standard	Regression equation	R^2 value	Limit of detection (µg/mL)	Limit of quantification (µg/mL)	Precision (% RSD)
Vanillic acid	y = 100435x+7961.4	0.999	0.00085	0.0026	0.44
Kaempferol	y = 10739x+348.76	0.994	0.00340	0.0102	0.98

HPLC analysis of D. steriscus tuber extract

Vanillic acid and kaempferol in *D. steriscus* tuber extract were determined using the HPLC method. The characteristic retention times for vanillic acid and kaempferol were used to identify them from the HPLC chromatogram of *D. steriscus* tuber extract. To determine their concentrations in the tuber extract, calibration curves for both vanillic acid and kaempferol were used. The typical HPLC chromatogram for the standards (vanillic acid and kaempferol) and the *D. steriscus* tuber extract are shown in Figure 3 and Figure 4 respectively.

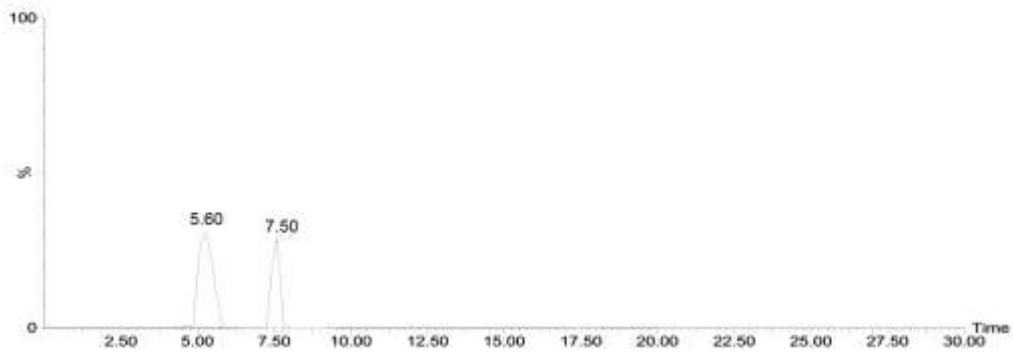

Figure 3. Typical HPLC chromatogram of the standards (kaempferol and vanillic acid)

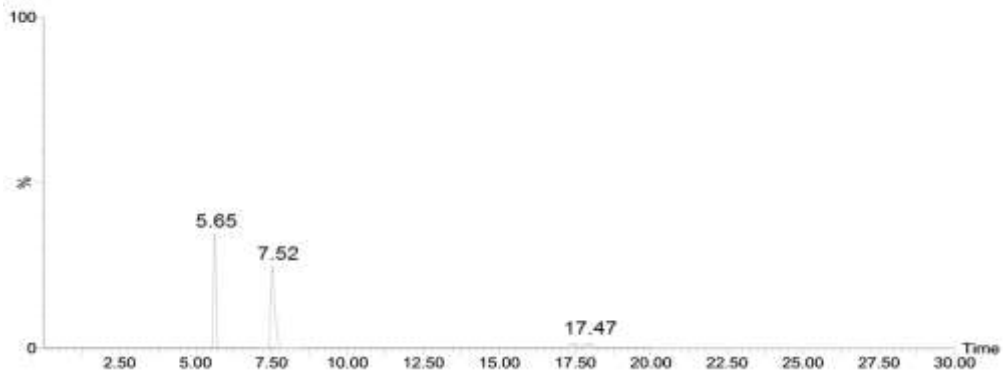

Figure 4. Typical HPLC chromatogram of *D. steriscus* tuber extract

As can be seen in Figure 4, vanillic acid and kaempferol in the *D. steriscus* tuber extract were detected at 7.52 minutes and 5.65 minutes respectively. The concentration of vanillic acid in *D. stersicus* tuber extract was found to be 0.0829 ±0.003 µg/g whilst that of kaempferol was found to be 0.0108 ±0.001 µg/g. The findings confirmed the presence of vanillic acid and kaempferol in the *D. steriscus* tuber extract. Many other polyphenolic compounds proven to possess potent biological activity can also be found in *D. steriscus* tubers.

The reported compounds (vanillic acid and kaempferol) are powerful bioactive compounds which have extensive applications in the food, medical and related industries. Other researchers also detected and quantified vanillic acid and kaempferol in other plant extracts (Theerasin & Baker, 2009; Calderon-Montano, Burgos-Morón, Pérez-Guerrero, & López-Lázaro,, 2011; Seal, 2016). It is evident from the presence of vanillic acid and kaempferol in *D. steriscus* that the plant is a possible source of other essential medicinal compounds.

CONCLUSION

This investigation provided basic evidence on *Dioscorea steriscus's* phytochemical composition using UV-Visible spectrophotometry and FTIR techniques. The HPLC analytical approach additionally verified the presence of vanillic acid and kaempferol in *D. steriscus* tuber extract. *Dioscorea steriscus* can be concluded to contain several active phytochemicals. The presence of potent phytochemicals in *D. steriscus* tuber extract authenticates its ethnobotanical importance. The use of *D. steriscus* tubers as a source of folk medicine is therefore highly recommended. However, there is need to further isolate, identify and ascertain the bioactivity and toxicity of phytochemicals from *D. steriscus* tubers.

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