International Journal of Advance Study and Research Work (2581-5997)/ Volume 2/Issue 2/February 2019

Total Phenolic Content (TPC) Determination and Phytochemical Screening of *Plecranthus Esculentus* Tubers of Rusape, Zimbabwe

Masimba Tapera*[1], Selemawit Abebe Gitore[2], Tobias Shumba[3], Nomore P. Male[4], Sydney Kaseke[5]
*[1] Chemistry Department, Kenyatta University, Nairobi, Kenya
[2] Agriculture Resource Management Department, Kenyatta University, Nairobi, Kenya
[3]B.Tech Department, Harare Polytechnic, Harare, Zimbabwe
*[1, 4, 5] Department of Physical Sciences, Harare Polytechnic, Harare, Zimbabwe
*Email Id: masimbatapera@gmail.com

DOI: 10.5281/zenodo.2563674

Abstract

Plectranthus esculentus is a common tuberous, perennial plant widely distributed in Rusape district of Zimbabwe and other areas of eastern and central and southern Africa. Phytochemicals were extracted from Plectranthus esculentus tubers using the matrix solid-phase dispersion extraction technique with ethanol as the extraction solvent. The extracts were first screened for flavonoids, reducing sugars, saponins, glycosides, terpernoids, alkaloids, and tannins using well known phytochemical screening procedures. Plectranthus esculentus tuber extracts were found to contain for flavonoids, reducing sugars, saponins, glycosides, terpernoids, alkaloids, and tannins. Total phenolic content for the tuber extracts was found to be 11.03 ±0.05mgGAE/g fresh weight. The results revealed the presence of medicinally important phytochemicals in the tubers. The presence of these phytochemicals helps to explain the importance of Plectranthus esculentus. The tubers have potential uses in food preservation and natural medicine. There is a need for continued cultivation, preservation and more research on Plectranthus esculentus in Zimbabwe and abroad.

Keywords: Extracts, Medicinal, Phytochemicals, Plectranthus, Tuber.

Introduction

Focus on natural product researches, medicinal plants, in particular, has increased all over the world. Large volumes of evidence have been gathered and compiled to show the immense potential of different medicinal plants used in different traditional and indigenous systems [17]. *Plecranthus esculentus* is one of the plants which is widely used in Zimbabwean indigenous systems. It is an edible tuber that constitutes an additional source of food, vegetable and medication to most people in Zimbabwe's' Rusape district. *Plectranthus esculentus* is a perennial herb that is a member of the dicot family, Lamiaceae [5]. *Plectranthus esculentus* is indigenous to Southern Africa, Central and Eastern Africa [5, 13]. In Zimbabwe, It is widely produced in the Eastern districts. The major producing areas in Zimbabwe include Rusape, Nyanga, Makoni, and Mutasa. *Plectranthus esculentus* tubers are often used as a substitute or supplement for potatoes and sweet potatoes. The tubers can be eaten raw, boiled or roasted directly after cultivation [3]. The tubers can also be dried and stored for later use. The stems have been extensively used to sweeten porridge [15]. *Plectranthus esculentus* leaves can be used as a vegetable and cooked in sauces. *Plectranthus esculentus* has been said to help with digestive problems, also used to treat stomach ache and abdominal pain and has been used as anthelminthics [15]. It has been used in Eastern and Southern Africa to treat intestinal worms, its cytotoxic and anti-tumor promoting activity promotes its use in the treatment of cancer [14]. Applied topically, the plant is used to treat skin conditions [18]. It has been under scientific investigation as a possible treatment for congestive heart disease, glaucoma and chronic bronchial disease [7, 8].

The tubers of *Plectranthus esculentus* have been shown to contain substantial amounts of polyphenolic compounds with antioxidant properties [9]. The data obtained by Fredrick. M et al. (2017) [11], clearly demonstrated that tubers of the *Plectranthus* species are a potential source of natural antioxidants and may be a good candidate for pharmaceutical plant-based products. He also

suggested that studies on their mechanisms of action may yield potential compounds against oxidative stress. Recent studies have shown that tuber extracts of different *Plectranthus* species exhibits antioxidant activity and have substantial amounts of phenolic compounds [1, 26]. Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radicals induced by oxidative stress [16]. Oxidative stress is a harmful condition in which there is an imbalance between free radicals and their stabilizing agents in the body [24]. Phenolic compounds have an important role in preventing and restricting the progression of free radicals [6]. Generally, antioxidant phenolic compounds like phenolic acids, polyphenols, and flavonoids quench free radicals such as peroxides and hydroperoxides of lipid hydroxyl, thus inhibit the oxidative mechanisms that lead to degenerative diseases like atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies [22].

Physical and chemical characteristics of plant tubers vary from one variety to another and within the same variety depending upon many factors during growth [19]. Despite being an important medicinal plant, researchers in Zimbabwe have largely ignored *Plectranthus esculentus* and there are no agronomic recommendations being developed for growing, preserving and using it in Zimbabwe [4]. The death of general information on the crop has led to the loss of interest in its cultivation and consequently, a gradual loss of its germplasm [4]. The present study seeks to determine total phenolic compounds and to screen for phytochemicals in *Plectranthus esculentus* tubers of Rusape district, Zimbabwe. The study goes a long way in promoting use, cultivation and further research on *Plectranthus esculentus* in Zimbabwe.

Methodology

I. Chemicals

All chemicals and reagents used in the experimental procedures were analytical reagent grade procured from Skylabs, South Africa and Sigma Aldrich, Germany.

II. Plant Sample Collection

Plectranthus esculentus (tsenza) tubers and whole plants were obtained from local farmers in Rusape, Mashonaland East Province in Zimbabwe. The plants and the tubers were first taken to the Harare Polytechnic's Horticultural Section for identification and authentication. The tubers were then washed using clean tap water to remove dirt and weighed to obtain the fresh mass.

III. Extraction of Phytochemicals

Extraction of phytochemicals from the fresh tubers was performed using Matrix Solid Phase Dispersion Extraction (MSPD) technique according to the methods outlined by Karasova et al. (2003) [12] and Tapera et al. (2017) [23]. Briefly, fresh *Plectranthus esculentus* tubers were sliced into tiny pieces using a small knife. The sliced tubers (10g per sample) were mixed with 20 g of clean sand in a porcelain mortar and then ground to homogeneity using a pestle. Three other separate samples of fresh *Plectranthus esculentus* tubers (20 g per sample) were homogenized with sand in separate mortar and pestles. Clean 10ml syringes were used to perform MSPD extraction of the homogenized samples using methanol as the elution solvent. Extracts for the samples were separately collected into test tubes.

IV. Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined by spectrophotometry, using Gallic acid as standard, according to the method described by Singleton et al. (1999) [20], with some modifications. Briefly, 1.0 mL of the sample extract was transferred to separate tubes containing 5.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.5 mL of sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperature for 60 min before absorbance at 765 nm was measured using a UV/Vis spectrophotometer. The TPC was expressed as Gallic acid equivalents (GAE) in mg/g fresh material. The concentration of phenolics in the samples was derived from a standard curve of Gallic acid. All measurements were done in triplicate.

V. Phytochemical Screening

Phytochemical screening was performed using known phytochemical screening procedures according to Firdouse et al. (2011) [10] and Sofowara. A (1993) [21] as illustrated in the table below.

Table 1: Phytochemical Screening Procedures

Constituents	Test	Positive Observation
Alkaloids	Mix sample with a little amount of dilute sulphuric acid and Mayer's Reagent.	White precipitate
Flavonoids	Mix sample with concentrated sulphuric acid (1 ml) and 0.5 g of Magnesium.	Pink or red coloration
Glycosides	Add 5% hydrochloric acid, boil in a water bath, and filter. Shake filtrate with an equal volume of chloroform and allow to stand. Shake the lower chloroform with dilute ammonia.	Rose pink to red color of the ammoniacal layer.
Phenols	Add a few drops of ferric chloride solution to the sample.	Bluish green or black coloration.
Reducing sugars	Add Benedicts Reagent and warm.	Red precipitate.
Saponins	Shake sample with an equal volume of water.	Frothing or foaming.
Terpernoids	Shake sample with an equal volume of chloroform. Add concentrated sulphuric acid.	Reddish brown coloration.

Results and Discussion

Figure1 below is a pictorial view of some of the *Plectranthus esculentus* tubers which were used for the study
.

Fig1: Sample Plectranthus esculentus tubers

I. **Total Phenolic Content (TPC)**

A calibration curve was plotted using the TPC working standard concentrations in milligrams per liter (x-axis) against the average absorbencies at the 765nm wavelength (y-axis) as shown in Figure 2 below.

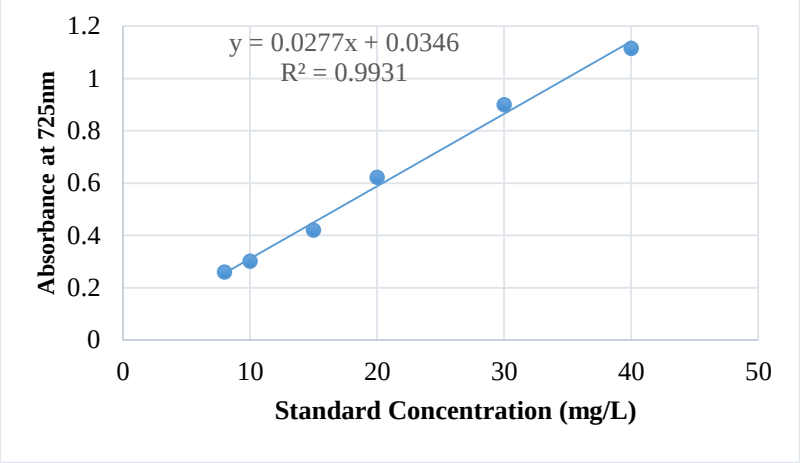

Figure 2: Calibration Curve for TPC working standards

TPC for the tuber extracts was determined by Folin- Ciocalteau assay using Gallic acid as a standard phenolic compound.
The Folin-Ciocalteau assay for Total Phenolic Content (TPC) is a fast and simple method that relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes to form blue complexes [2]. Sodium carbonate provides alkaline conditions which are favorable for the reaction to occur. The quantity of phenolic compounds is reflected by the color intensity of the reaction mixture that can be determined spectrophotometrically at approximately 745– 765 nm against a known phenolic compound equivalent [2]. Gallic acid is widely used as the comparison standard and values are usually compared as milligram of Gallic acid equivalent per gram or milliliter of extract among samples. The TPC calibration curve of Gallic acid was plotted in the working standard concentration range of 8 mg/l - 40 mg/l. A linear calibration curve was obtained, with a coefficient of determination (R^2) value of 0.9931and the regression equation was found to be y=0.0277x+0.0346 (Figure 2). The calibration curve was used to calculate the mean total phenolic content of the tuber extracts. Mean TPC of the tuber extracts of *P.Esculentus* in milligrams Gallic acid equivalent per gram of fresh sample was found to be 11.03 ±0.05mgGAE/g fresh weight.

II. Phytochemical Screening

The results for the qualitative Phytochemical Screening tests are illustrated in Table 2 below.

Table 2: Phytochemical Screening Results

Phytochemical	**Present (++) / Absent (--)**
Alkaloids	++
Flavonoids	++
Glycosides	++
Phenols	++
Reducing sugars	++
Saponins	++
Terpernoids	++

Phytochemical screening tests were conducted to test the presence of Alkaloids, Flavonoids, Glycosides, Phenols, Reducing sugars, Saponins and Terpernoids in the *Plectranthus esculentus* tuber extracts. The tuber extracts were found to contain all the seven phytochemicals which were tested. *Plectranthus esculentus* tuber extracts were found to contain a variety of important

phytochemical and the possibility of identifying other phytochemicals which were not tested is very high since phytochemicals are broadly distributed in the plant kingdom and are known to be the most abundant secondary metabolites in plants. [23]

Conclusion

Plectranthus esculentus tubers of Rusape district, Zimbabwe were found to contain substantial amounts of phenolic compounds and a variety of phytochemicals. Mean TPC of the tuber extracts of *Plectranthus Esculentus* was found to be 11.03 ±0.05mgGAE/g fresh weight. It was also found that the tubers contain Alkaloids, Flavonoids, Glycosides, Phenols, Reducing Sugars, Saponins, and Terpernoids. Due to its significantly higher TPC, and the various phytochemicals it possesses, *Plectranthus esculentus* can be used as a source of natural antioxidants. There is a need for preservation of *Plectranthus Esculentus* through cultivation and domestication. Further studies on *Plectranthus esculentus* tubers are recommended to ascertain their unknown agronomic characteristics, physicochemical properties, and other potential medicinal properties.

Acknowledgments

Authors appreciate the essential role played by the following laboratory personnel in the Science Technology Division at Harare Polytechnic: Mr. D. Mangi, Mrs. A. Faro, Byron Musekiwa, and Bridgette Tatenda Hwata.

References

[1] Abdel-Mogib M, Albar HA, Batterjee SM. Chemistry of the genus Plectranthus. Molecules. 7:271-301 (2002).
[2] Adedapo A.A., Jimoh F.O., Koduru S., Afolayan A.J., Masika P.J. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of Calpurnia aurea. BMC Complement. Altern. Med.;53:1-8 (2008).
[3] Allemann, James; Pieter Snyman Hammes. "Effect of photoperiod on tuberization in the Livingstone potato (Plectranthus esculentus NE Br. Lamiaceae)". Field Crops Research. 98(1): 76–81. Doi:10.1016/j.fcr.2005.12.011(2006).
[4] Andrew Mambondiyani. (3 Aug 2015). Naturally Zimbabwean, Tsenza makes Rusape tick - http://naturallyzimbabwean.com/2015/08/13/tsenza-makes-rusape-tick/ (2015).
[5] Ben-Erik, Van Wyk. Food Plants of the World. Timber Press, Oregon. p.302 (2005).
[6] Boro, H., Rava, M., Ali, R. and Das, S. Phytochemical Screening, Total Phenolic and Antioxidant Capacity of Root Extracts of Morus indica L., Int. J. Pure App. Biosci. 5(5): 1582-1589. doi: http://dx.doi.org/10.18782/2320-7051.3052 (2017).
[7] Bown. D. Encyclopedia of Herbs and their Uses. Dorling Kindersley, London. ISBN 0-7513-020-31 (1995).
[8] Chevallier. A. The Encyclopedia of Medicinal Plants, Dorling Kindersley. London, ISBN 9-780751-303148 (1956).
[9] Eleazu C.O., Eleazu K.C., and Ikpeam A.I. Phenolic Content, Antioxidant Capacity, and Toxicity of 3 Varieties of Living Stone Potato (Rizga). Journal of Pharmacology and Toxicology, 7: 206-212. DOI: 10.3923 (2012).
[10] Firdouse S, Alam P. Phytochemical investigation of an extract of Amorphophallus campanulatus tubers. Int J Phytomed 3: 32-35 (2011).
[11] Fredrick M. Musila, Joseph M. Nguta, Catherine W. Lukhoba, Saifuddin F. Dossaji. Antibacterial and antifungal activities of 10 Kenyan Plectranthus species in the Coleus clade, Journal of Pharmacy Research, Vol 11.Issue 8. (2017).
[12] Karasova G, Brandsteterova E, Lachova M. Matrix solid-phase dispersion as an effective preparation method for food samples and plants before HPLC analysis. Czech J Food Sci 6: 219-234 (2003).
[13] Kyesmu, P.M. "Plectranthus Esculentus n.e.br.a minor Tuber Crop in Dire Need of Rescue from Extinction". Lamiales Newsletter. pp. 3-4 (1994).
[14] Lukhoba C.W, Simmonds M.S.J.; Paton A.J. Plectranthus: A review of ethnobotanical uses, Journal of Ethnopharmacology 103 1-24 (2006).
[15] Lukhoba, Catherine W.; Monique S.J. Simmonds; Alan J. Paton. "Plectranthus: A review of ethnobotanical uses". Journal of Ethnopharmacology. 103 (1): 1–24. doi:10.1016/j.jep.2005.09.011. ISSN 0378-8741. PMID 16289602 (2006).
[16] Ozsoy N, Can A, Yanardag R, Akev N. Antioxidant activity of Smilax excelsa leaf extracts. Food Chem., 110: 571-583 (2008).
[17] Reena Sinku and Manoj Ranjan Sinha. Preliminary phytochemical screening and physiochemical analysis of Tinospora cordifolia Miers, Journal of Medicinal Plants Studies; 6(1): 177-180 (2018).
[18] Rice L.J.; Brits G.J.; Potgieter C.J.; Van Staden J. Plectranthus: A plant for the future? South African Journal of Botany 77 947-959 (2011).
[19] Sengul M, Keles F, Keles MS. The effect of storage condition (temperature, light, time) and variety on the glycoalkaloid content of potato tubers and sprouts. Food Control; 15:281–286. doi: 10.1016/S0956-7135(03)00077-X (2004).
[20] Singleton V. L, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent. Methods Enzymol. 299: 152-178 (1999).
[21] Sofowra A. Medicinal plants and traditional medicine in Africa, Spectrum Books Ltd. Ibadan, Nigeria, 191-289 (1993).
[22] Subramanion L. Jothy1, Zakaria Zuraini1, and Sreenivasan Sasidharan. Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitory activities of Cassia fistula seeds extract, Journal of Medicinal Plants Research Vol. 5(10), pp. 1941-1947 (2011).

[23] Tapera M, Machacha S. Matrix Solid Phase Dispersion Extraction and Screening of Phytochemicals from Dioscorea steriscus Tubers of Mashonaland Central Province, Zimbabwe, Pelagia Research Library, Der Chemica Sinica, 8(1):117-122 (2017).
[24] Toshikazu Yoshikawa and Yuji Naito. What Is Oxidative Stress? Journal of the Japan Medical Association (Vol. 124, No. 11, 2000, pages 1549–1553 (2000).
[25] Uddin G, Rauf A, Siddiqui B.S, Shah S.Q. Preliminary comparative phytochemical screening of Diospyros lotus Stewart. Middle-East. J Sci Res 1: 78-81 (2011).
[26] Waldia S, Joshi BC, Pathak U, Joshi MC. The genus Plectranthus in India and its chemistry. Chem Biodivers; 8:244-52 (2011).