



Phytochemical Constituents of *Cissus oliveri* growing in Pwani Region, Tanzania

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Abstract

Cissus oliveri (Engl.) Gilg ex Engl. is among the plant species of the family Vitaceae widely used in traditional medicine for management of different ailments. This study aimed at assessing the phytochemicals of *C. oliveri* parts in order to provide scientific validation for its use as a therapeutic agent. Qualitative analysis of water and ethanolic extracts was conducted by using standard methods and the results revealed that all the parts possess alkaloids, flavonoids, phenols and tannins. Similarly, glycosides and saponins were only present in leaf and root. Quantitative analysis performed by gravimetric and spectrophotometric methods showed that all parts contain alkaloids, phenol, and tannins in large quantities. The largest amount was found in leaf for alkaloids (6.47 ± 0.41 mg/g DW) and phenols (4.85 ± 0.90 mg/g DW) compared to stem and root. Alkaloid content was higher in root than in stem ($P < 0.01$), whereas phenol content was the same in root and stem ($p > 0.05$). Further, there was no significant difference in tannins content among all the plant parts assessed. The presence and quantities of active phytochemicals in *C. oliveri* call for further investigations on the bioactivities of the extracts and isolated chemical constituents for potential pharmacological applications.

Keywords: *Cissus oliveri*, Medicinal plants, Plant parts, Vitaceae, Secondary metabolites.

Introduction

Plants with medicinal values to human beings are the basis of primary health care for a larger part of the population in the world. The World Health Organization (WHO) estimates about 80% of people in developing countries depend on the traditional and complementary medicines (T & CM) for their primary health care (Tesfahuneyn and Gebreegziabher 2019), and that plant extracts make about 85% of these traditional medicines (Amanullah et al. 2011). In Africa and some developing countries, extensive use of T & CM is attributed to being much more available and readily affordable than modern

medicines (WHO 2013). In these areas, accessibility of conventional medicine-based health services is inadequate and the majority of people depend much on indigenous knowledge-based practices for treatment of their ailments. For instance, the ratio of traditional healers to population in Africa is 1:500, whereas that of medical doctors to population is 1:40,000 (WHO 2002, Abdullahi 2011). Other reasons for increase of acceptance of T & CM in both rural and urban settings include worry about the adverse effects of conventional drugs, changing values and reduced tolerance of paternalism (Abdullahi 2011) and increase in

resistance by several pathogens to various antibiotics (Doughari 2012, Vasantharaj et al. 2013).

Despite the acceptance and embrace of T & CM by the large population, developing countries face many difficulties to incorporate them into mainstream healthcare which include stigmatization to consumers due to poor perceptions and attitudes, complications in utilization of communities that own the traditional medicine knowledge, irrational uses and issues which distract safety, efficacy and quality (Abdullahi 2011, Gakuya et al. 2020). In order to integrate T & CM services into health care service delivery and self-health care researches are needed to improve and verify the safety, quality and efficacy of traditional medicines so that they can be accessed in a safe, respectful, low cost and effective manner (WHO 2013). Systematic evaluation of plants used in traditional medicine is required for permanent search and for development of new drugs and advance their use to compliment conventional drugs treatment (Parekh and Chanda 2006). Based on the global strategy of integrative approach to health care, there is a need to continue exploring plants which have medicinal values in order to identify their bioactive ingredients which can be extracted and isolated to foster their appropriateness and efficacy in disease control.

The medicinal values of plants are an outcome of non-nutritive chemical constituents called secondary metabolites (Geetha and Geetha 2014). These secondary metabolites have pharmacological effects to various ailments and are used as templates for the synthesis of useful drugs (Osugwu et al. 2013). Extracts of various plant species of the family Vitaceae (Grape family) possess secondary metabolites (Fernandes and Banu 2012), many of which have been used by human beings and other animals as medicines and food (Sani et al. 2014). The family Vitaceae consists of approximately 14 genera and about 900 species primarily distributed in tropical regions of Asia, Africa, Australia, Neotropics and the Pacific islands, with a few genera in temperate regions (Soejima and Wen 2006). *Cissus* is among the largest

genera of the family Vitaceae which comprises about 300 species (Liu et al. 2013). Different parts of *Cissus* plants are employed in the treatment of different ailments (Doughari 2012, Fernandes and Banu 2012, Sudmoon et al. 2016). Pharmacological effects of plant species in genus *Cissus* are due to active chemical ingredients found in their parts such as alkaloids, flavonoids, saponins, tannins, steroids, glycosides, stilbenes, coumarins and terpenoids (Soladoye and Chukwuma 2012, Fernandes and Banu 2012, Aguoru et al. 2014, Sudmoon et al. 2016).

Cissus oliveri (Engl.) Gilg ex Engl. is among the popular species of the genus *Cissus* that are widely used in traditional medicine. It has been described by Verdcourt (1993) as a climbing shrub liana, 1.2–6 m long; with grey stems and red roots growing primarily in seasonally dry tropical biome. According to Plants of the World Online-POWO (2023), this is distributed across East and Central Africa and is indigenous in Angola, Burundi, D.R. Congo, Kenya, Malawi, Mozambique, Rwanda, Tanzania and Uganda. Herbarium collections at the University of Dar es Salaam show that it is found in Kilimanjaro, Dodoma, Kigoma and Pwani regions in Tanzania.

C. oliveri is reported as medicinal plant consumed by chimpanzees in Tanzania and Kenya for the treatment of parasite and gastrointestinal upsets (Nakamura et al. 2015). Its leaves are used for the treatment of diarrhea, while the whole plant is designated as analgesic, antibacterial, antifungal and antiviral (Maurice 1993, Quattrocchi 2012). In Tanzania, *C. oliveri* is commonly known by native tribes in Pwani region (Doe and Kwere) as Mtamba/Zagamba. People occasionally drink its sap from freshly matured cut stems when there is no water during activities in the bush. It is also used by the traditional healers to treat different ailments including; back, knee and abdominal pains especially in women. Unlike other plants in the genus *Cissus*, the phytochemical profile of *C. oliveri* has not been established. Since medicinal value of plants lays in its secondary metabolites which produce a

positive physiological effect on the human body, this paper therefore reports on the screening for the presence of some selected classes of secondary metabolites with a main objective of determining the quantity of phytochemicals found in different parts of *C. oliveri* parts (i.e., leaf, stem and root) that could be responsible for traditional management of ailments.

Materials and Methods

Collection of plant samples

The matured fresh parts (leaves, stems and roots) of *C. oliveri* were collected randomly in May 2018 from Zaraninge forest, Gongo village, Msegele valley (GPS Location: 37 M 0459466/UTM 9316783 at Altitude of 250 m) in Bagamoyo District, Pwani Region, Tanzania. The plant species were identified in the field and authenticated. Voucher specimens were deposited in the Herbarium of Botany Department of the University of Dar es Salaam, Tanzania.

Sample preparation and extraction

The plant materials were washed with water to remove sand and debris, and air dried under shade for two weeks in the laboratory at the Department of Botany, University of Dar es Salaam. They were then ground to fine powder and stored in airtight dry containers before analysis. Water and alcohol were purposively selected as extracting solvents based on the anecdotal evidence that are also used by traditional healers in the community to administer the extracts to patients. Powdered samples (100 g) of each part of the plant (leaf, stem and root) were soaked for 48 hours, with occasional shaking in 1.5 L of distilled water, then after 48 hours, the extracts were first filtered off using clean and dry sieves to remove large plant particles followed by Whatman number 1 filter paper in a vacuum pump. Then, the filtrate was evaporated to dryness by using a rotary evaporator (HEIDOLPH, Essex Scientific Laboratory Supplies Ltd, SS72AN) to obtain crude extracts. The procedures above were repeated by using 95% ethanol to obtain ethanolic extracts. Crude extracts were placed in vials

and stored in the fridge at 4 °C before carrying analysis of phytochemicals.

Qualitative analysis of secondary metabolites

Water and ethanolic crude extracts from the leaf, stem and root of *C. oliveri* were qualitatively screened to determine the types of selected groups of secondary metabolites (alkaloids, anthraquinone, flavonoids, tannins, glycosides, saponins, phenols and steroids) present in the investigated samples by employing standard methods: The presence of alkaloids was determined using Wagner reagent, while that of steroids was achieved using Liebermann-Burchard's test as described by Njau et al. (2014). Determination of anthraquinones and phenols was carried out by Bontrager's test and lead acetate test, respectively as described by Geetha and Geetha (2014). Also, cardiac glycosides were screened out by the method described by Geetha and Geetha (2014). Determination of flavonoids was carried out using the acid alkaline test, saponins by froth test, and tannins by ferric chloride test as described by Zohra et al. (2012).

Quantitative analysis of secondary metabolites

Quantitative determination of selected groups of secondary metabolites in each part of the plant was conducted by gravimetric and spectrophotometric methods as per standard methods as described by Osuagwu et al. (2013).

Determination of alkaloid content

Alkaline precipitation gravimetric method as described by Osuagwu et al. (2013) was used to determine concentrations of alkaloids in the crude extracts of each plant part. Five grams (5 g) of each of the powdered samples were soaked in 20 ml of 10% ethanolic acetic acid and left to dissolve for four hours at room temperature before being filtered through Whatman filter paper (No 42). The filtrate was concentrated by evaporation over a steam bath to a quarter of its initial volume and then precipitated by addition of concentrated ammonium hydroxide solution

drop wise then in excess. The alkaloid precipitate was filtered in a vacuum pump using a pre-weighed filter paper. The obtained precipitate was washed with 9% ammonia solution and dried in the oven at 60 °C for 30 minutes then cooled in a desiccator and reweighed. The experiments were performed in triplicate to obtain an average weight. The weight of alkaloid content was determined by obtaining the difference in weight and expressed in mg/g DW sample as shown below;

Weight of alkaloids in the sample = $W_2 - W_1$

Where: W_1 = weight of filter paper, W_2 = weight of filter paper + alkaloid precipitate.

Determination of phenolic content

The concentrations of phenols in the parts of the plant were determined using the Folin-Ciocalteu colorimetric method described by Osuagwu et al. (2013). To a 0.2 g of the powdered sample of each of the investigated plant part in different test tubes, 10 ml of methanol was added and the mixture was shaken thoroughly and left to stand for 15 minutes. Then the mixture was filtered using Whatman (No 42) filter paper. About 1 ml of the extract obtained was placed in a test-tube followed by 1 ml Folin-Ciocalteu reagent, and then 5 ml of distilled water was added into the mixture. The mixture was left for 2 hours at room temperature for the colour to develop. The absorbance of the developed colour was measured at 760 nm wavelength. The phenol content was calculated as;

$$\% \text{ Phenol} = \frac{100}{W} \times \frac{AU}{AS} \times \frac{C}{100} \times \frac{VF}{VA} \times D$$

Where, W = Weight of sample analysed, AU = Absorbance of test sample, AS = Absorbance of standard solution, C = Concentration of standard in mg/ml, VF = total filtrate volume, VA = Volume of filtrate analysed, D = Dilution factor (where applicable). The experiment was done in triplicate to get the percentage average for each plant part, and the results are presented as Mean \pm SD. However, the contents were estimated and expressed in mg/g DW.

Determination of tannins content

The tannins content of each of the plant parts was determined by using the Folin-Dennis spectrophotometric method as described by Osuagwu et al. (2013). Powdered sample (2 g) of each part of the plant was mixed with 50 ml of distilled water and shaken for 30 minutes in the shaker. The mixture was filtered using Whatman (No 42) filter paper to obtain the filtrate which was used for experiments. Five (5) ml of the filtrate was poured into a 50 ml volume flask and diluted with 3 ml of distilled water. In another 50 ml volume flask, 5 ml of standard tannic acid solution was added followed by 5 ml of distilled water. Then, 1 ml of Folin-Dennis reagent was added to each of the flask followed by 2.5 ml of 7.5 % (w/v) saturated sodium carbonate solution. The content of each flask was made up to mark and incubated for 90 minutes at room temperature. The absorbance of the developed colour was measured at 760 nm wavelength with the reagent blank at zero. The tannin content was calculated using the following formula:

$$\% \text{ Tannins} = \frac{100}{W} \times \frac{AU}{AS} \times \frac{C}{100} \times \frac{VF}{VA} \times D$$

Where, W = Weight of sample analysed, AU = Absorbance of test sample, AS = Absorbance of standard solution, C = Concentration of standard in mg/ml, VF = total filtrate volume, VA = Volume of filtrate analysed, D = Dilution factor (where applicable). The experiment was performed in triplicate to get the percentage average and the results are presented as Mean \pm SD. However, the contents were estimated and expressed in mg/g DW.

Statistical analysis

The quantitative analysis results are given as mean \pm standard deviation. The significant differences of quantities of phytochemicals in parts of the plant (i.e., leaf, stem and root) were evaluated by applying Newman-Keuls Multiple Comparison Test ANOVA-in GraphPad InStat software version 3.0. The Fisher's least significance difference (L.S.D.) was used to compare treatment means. Values with $p \leq 0.05$ were taken to be significant.

Results and Discussion

Composition of secondary metabolites in extracts of *Cissus olerivi* parts

Phytochemicals have continued to be important sources for development of pharmaceutical drugs due to their various medicinal potentials. Qualitative analysis carried out on ethanol and water crude extracts of leaf, stem and root of *C. olerivi* revealed the presence of alkaloids, flavonoids, phenols and tannins in all of the studied parts as shown in Table 1. These results are similar to those reported in other species of *Cissus* (Fernandes and Banu 2012, Soladoye and Chukwuma 2012, Aguoru et al. 2014). Glycosides and saponins were found in both water and ethanol extracts from the leaf and root, while they were absent in the stem extracts. Anthraquinone was absent in all of the plant extracts. Leaf extracts strongly contained abundant flavonoids, phenols glycosides and saponins than those in stem and root extracts (Table 1). These phytochemicals have various biological activities in plants and produce many

physiological actions on human body (Fasola and Iyamah 2014, Madike et.al. 2017), therefore it is possible to harvest leaves of *C. olerivi* and use them like its roots, which have been long been used as a folklore medicine to treat different ailments.

Another secondary metabolite detected in leaf and stem extracts was steroids. Similar findings were observed in Muhamad et al. (2022) studies, albeit from leaves of other *Cissus* species, *C. hastata* and *C. sicyoides*. Irrespective of the species, phyto-steroids are important for plant growth and reproduction, yet they exert responses to many biotic and abiotic stresses (Muhamad et al. 2022). They also possess insecticidal and antimicrobial properties, are used in nutrition, herbal medicine and cosmetics (Gowri and Vasantha 2010). Presence of these phytochemicals in the parts of *C. olerivi* (leaves, stems and roots) supports their usage in herbal medicine, and that if isolated and tested pharmacologically could be rich and potential sources of drugs.

Table 1: Composition of secondary metabolites in extracts of *Cissus olerivi* parts

Secondary metabolites	Leaf		Stem		Root	
	WE	EE	WE	EE	WE	EE
Alkaloids	+	++	++	++	++	++
Anthraquinone	-	-	-	-	-	-
Flavonoids	++	++	+	+	+	+
Glycosides	++	++	-	-	+	+
Phenols	++	++	+	+	+	+
Saponins	++	++	-	-	+	++
Steroids	+	+	+	+	-	-
Tannins	+	+	+	+	+	+

Key: (+) = Present, (++) = Strongly present and (-) = Absent, (WE) = Water extracts and (EE) = Ethanol extracts.

Quantitative comparison of selected secondary metabolites in extracts of *Cissus olerivi* parts

Phytochemical analysis of the parts of *C. olerivi* extracts revealed the presence of secondary metabolites, which have been previously reported in other plants of the genus *Cissus* to possess medicinal properties. This study has revealed that there are varied quantities of alkaloids and phenols with significant differences ($p \leq 0.05$), between

the plant parts. The study showed that leaves had the highest values (6.47 ± 0.41 mg/g DW) of alkaloids followed by roots while the stems been the lowest as shown in Table 2. Alkaloids and phenols contents are abundant in the leaves and roots of *C. olerivi* plant, this is contrary with the results of *Cissus populnea* a plant within the same genus in the work of Aguoru et al. (2014) in which alkaloids and tannins were abundant in the stems and roots compared to leaves. Soladoye

and Chukwuma (2012) argued that plants containing alkaloids in higher concentrations are characteristically toxic but may be safe when used under controlled amount, for example, Onyeka and Nwambekwe (2007) reported that alkaloid content of 12.8–29.6 mg/g DW was harmless in green leafy of edible vegetables. Usually, plants use alkaloids in chemical defense against their enemies. They could also be used as a natural source of insecticides, fungicides and antibacterials (Wadood et al. 2013), similarly, they may be used as pain killers for reducing headache and fevers and act as stimulants for central nervous system (Doughari 2012).

The results in Table 2 show that the phenolic content is higher in the leaves (4.85 ± 0.90 mg/g DW) compared to the stems and roots of *C. oliveri*. So far, there is no previous study about the phenolic content of *C. oliveri*. Phenolic compounds are normally present in many plants and are associated with increasing their antioxidant capacity, for example, small quantities of these compounds have also been reported in the leaves of *Cissus hastata* (Muhamad et al. 2022). Apart from antioxidant properties, there are several biological activities of phenols which include; antitumor, antiviral, antimicrobial, and hypotensive effects (Madike et al. 2017). In this study, stems and roots of *C. oliveri* plant contained the low phenolic contents (Table 1). Fasola and Iyama (2014) maintained that low amounts of any phytochemical compound in the plant parts does not mean its medicinal properties are not good, therefore the intensive uses of roots of *C. oliveri* as a traditional medicine for treatment of different illness attest to this.

There is no statistical difference in the tannins contents in the leaves, stems and

roots of this plant ($p \geq 0.05$). These results are different from those of *C. populnea* in which there were variations in the concentrations of tannins in stem and root (Soladoye and Chukwuma 2012). In plants, tannins are reported to discourage feeding by herbivores and protect leaves against predators because they reduce palatability. They also have metabolic effects on plant predators or pathogens, thus provide protection against granivores, insects, fungal, bacterial and viral infections (Achakzai et al. 2009).

More results in Figure 1 illustrate the trend in which stems possess the least content of phytochemicals. It is also evident that leaves possess the largest content of phytochemicals (alkaloids and phenols) followed by roots of the plant. There is no single reason which contributes to the variations of phytochemicals in plants and its parts. The variations of the presence and quantities of secondary metabolites discussed between different plants are described by species differences, differences in geographical locations of plants which in turn determine environmental parameters such as light (Achakzai et al. 2009), variations of soil nutrients (Andzouana and Mombouli 2011), solubility properties of solvents used in the extraction, harvesting period, state of storage and differences in the plant materials (Shaik et al. 2011, Senguttuvan and Paulsamy 2014). In addition, the variations of these phytochemicals to various concentrations between parts (leaves, stems and roots) within one plant can be influenced by the degree of development or state of maturity of the collected samples (Fasola and Iyama 2014).

Table 2: Quantitative comparison of selected secondary metabolites in *Cissus oliveri* parts in mg/g DW

Phytochemicals	Leaf	Stem	Root	Comparison test
Alkaloids	6.47 ± 0.41^a	2.60 ± 0.14^b	3.66 ± 0.22^c	F = 156.00 ($p < 0.0001$)
Phenols	4.85 ± 0.90^a	1.46 ± 0.32^b	1.49 ± 0.34^b	F = 33.167 ($p = 0.0006$)
Tannins	0.12 ± 0.01^a	0.10 ± 0.01^a	0.12 ± 0.01^a	F = 2.5850 ($p = 0.1550$)

Values presented are mean \pm SD of triplicate tests; Means between parts followed by different superscript letter(s) in a row are significantly different from each other at $p \leq 0.05$ according to the Fisher's least significant difference (L.S.D.).

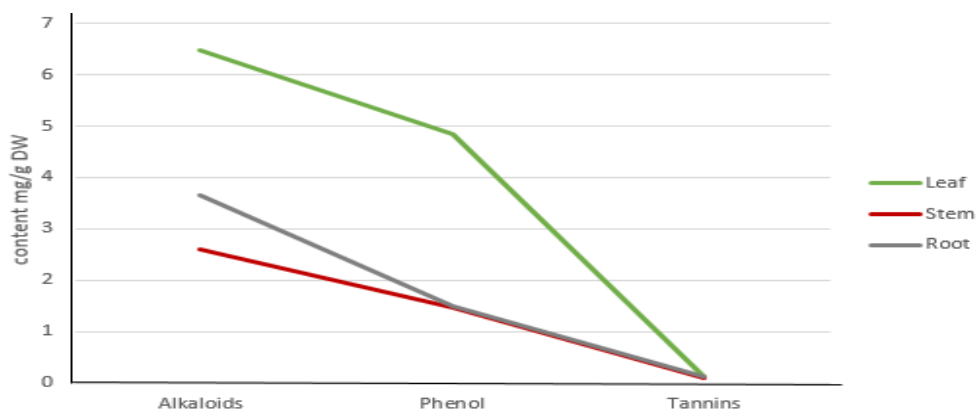


Figure 1: Trend of phytochemical contents in leaf, stem and root of *Cissus oleriveri*.

Conclusion

This research has clearly revealed that parts of *Cissus oleriveri* (leaf, stem and root) contain secondary metabolites including alkaloids, flavonoids, phenols and tannins. Glycosides and saponins were found in leaf and root, whereas steroids were present in leaf and stem. Leaves and roots have shown significant quantities of alkaloids, phenols and tannins as compared to stems; therefore they can be good sources of such compounds. The presence of these phytochemicals in *C. oleriveri* corroborates its uses and values in T & CM, and thus vantage the studied parts as potential raw materials for development of useful drugs if further pharmacological or pharmacognostic studies are conducted. Nevertheless, it is necessary for these secondary metabolites to be isolated, purified and characterized. Furthermore, we also suggest that the toxicity, bio-larvicide and antimicrobial activities of this plant should be investigated.

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Conflict of Interest

The authors declare no conflict of interest.

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