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The anticancer, antioxidant and phytochemical screening of *Philenoptera violacea* and *Xanthocercis zambesiaca* leaf, flower & twig extracts

P H Ntsoelinyane[*1], S S Mashele[2] and I T Manduna[2]

[1]*Department of Clinical Sciences, Central University of Technology, Free-state, Health extended curriculum programme Division, Private Bag X20539, Bloemfontein, 9300, South Africa*
[2]*Department of Health Sciences, Central University of Technology, Free-state, Biomedical Technology Division, Private Bag X20539, Bloemfontein, 9300, South Africa*

Corresponding author*:
P H Ntsoelinyane
Department of Clinical Sciences,
Central University of Technology, Free-state,
Health extended curriculum programme Division,
Private Bag X20539, Bloemfontein, 9300, South Africa
E-mail: pntsoeli@cut.ac.za

Abstract
The aim of this study was to investigate the antioxidant potential, anticancer activity, phytochemical constituents and total phenolic content of *Philenoptera violacea and Xanthocercis zambesiaca* leaves, flower & twig extracts. The anticancer activity of the extracts was tested in the 5-cell line panel consisting of MCF7 (breast cancer), HCT116 (colon cancer), TK10 (renal), UACC62 (melanoma) and PC3 (prostate cancer) by Sulforhodamine B (SRB) assay at the CSIR. The scavenging activities of the *Xanthocercis zambesiaca* extract and *Philenoptera violacea* was determined by 2, 2- diphenyl-1-picrylhydrazyl (DPPH) assay and was compared with standard antioxidant (ascorbic acid). *Xanthocercis zambesiaca* showed moderate (50%) free radical scavenging activity, while *Philenoptera violacea* extract had low free radical scavenging activity at the concentration of 2.5 mg/ml when compared to ascorbic acid. Qualitative phytochemical analysis of these plant extracts confirmed the presence of tannins, flavonoids, steroids, terpenoids, alkaloids and cardiac glycosides from *Philenoptera violacea* extract, while *Xanthocercis zambesiaca* extract showed the presence of flavonoids, saponnins, terpenoids and glycosides. Both extracts showed inactivity against all 5 cell lines with an IC_{50} of more than 100 µg/ml. Our results indicate that, *Xanthocercis zambesiaca* extract is a weak source of antioxidant and *Philenoptera violacea* extract has a number of phytochemical compounds. Future study will be done to isolate and identify the active compounds of both these plants.
Keywords: *Philenoptera violacea*, *Xanthocercis zambesiaca*, Anticancer activity, Phytochemical constituents, Antioxidant activity

1. Introduction
People have been relying on natural plants as a source of treatment for various diseases even today that is still the case especially in rural areas where traditional healers outnumber western doctors. Most medicines used by western doctors are also derived from natural plants. Phytochemicals or secondary metabolites are chemical compounds formed during the plants normal metabolic processes and plants use them to protect themselves[1-2]. Free radicals are important mediators that provoke inflammatory processes and are neutralized by antioxidant which exerts anti-inflammatory effect[3]. Free radical scavenging molecules such as flavonoids, tannins, alkaloids, quinones, amines, vitamins, and other metabolites possess anti-inflammatory, anti-carcinogenic, antibacterial and antiviral activities[4]. Most phytochemicals have antioxidant activity and protect human cells against oxidative damage. Plants with antioxidants properties are used for minimizing the severity of the inflammation related diseases and a health-promoting effect of antioxidants from plants is thought to arise from their protective effects by counteracting ROS[5].

Philenoptera and Xanthocercis genus both belong to the Fabaceae family[6]. Trees, herbs, vines and schrubs of this plant family are native to all regions of the world and are commonly cultivated[7]. *Xanthocercis zambesiaca* is found in Africa and is known as Muchetuchetu/ Musharo in Shona and as Nyala berry in English. *Xanthocercis zambesiaca* is traditionally used to treat diabetes mellitus and has been scientifically proven to have anti-hyperglycemic effects[8]. *Philenoptera violacea* is also found in Africa, known as Mohata in Shona, Mphata in Sotho and as Apple-leaf in English. It has been used in traditional remedies to treat gastro-intestinal problems, powdered root-bark for colds and snakebite treatment, root infusions as hookworm remedy and most part of the plant has been used to treat diarrhoea[9] but this plant has not yet been investigated for pharmaceutical uses before. In this study, antioxidant potential, anticancer activity and

phytochemical constituents of *Philenoptera violacea* and *Xanthocercis zambesiaca* extracts will be determined.

2. Materials and method

2.1 Plant material

The plant materials were authenticated by scientists at the National Botanical Gardens in Bloemfontein South Africa (MAS002). The collected materials were dried at room temperature and pulverized by mechanical mills and weighed. They were then stored in a cool place until analysis.

2.2 Extraction methods

Plant materials (10g of the dried leaf, twig and flower) were dried, weighed and soaked in methanol for 72 hours with occasional stirring. The extracts were filtered and lyophilized.

2.3 Scavenging ability towards 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical

The DPPH assay was performed as described by Shirwaikar[10]. Hundred µl of various concentrations of each sample were added to 2 ml solution of 0.1 mM DPPH. Hundred µl of methanol and 2 ml DPPH served as control. After 60 min of incubation at 25°C in the dark, the absorbance was recorded at 517 nm wavelength. The experiment was performed in triplicates. The DPPH radical scavenging activity was calculated according to the following equation:

$$\% \text{ DPPH radical scavenging activity} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

where Asample and Acontrol are absorbance of sample and control. The SC_{50} (concentration of sample required to scavenge 50% of DPPH radicals) values were determined. The decrease of absorbance of DPPH solution indicates an increase of the DPPH radical scavenging activity.

2.4 Anticancer screening

The growth inhibitory effects of the compounds were tested in the 5-cell line panel consisting of MCF7 (breast cancer), HCT116 (colon cancer), TK10 (renal), UACC62 (melanoma) and PC3 (prostate cancer). Cell lines were routinely maintained as monolayer cell cultures at 37 °C, 5% CO_2 and 100% relative humidity in RPMI containing 5% fetal bovine serum, 2mM L-glutamate and 50µg/ml gentamicin. The primary anticancer assay was performed at the CSIR in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute[11-12]. The extracts or compounds were tested at a single concentration (100 ppm) and the culture was incubated for 24 h. After 24 h the cells were treated with the experimental drugs which were previously dissolved in DMSO and diluted in medium to produce 5 concentrations.

The plates were incubated for 48 h after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed and protein-bound dye was extracted with 10mM Tris base for optical density determination at the wavelength 540 nm using a multiwell spectrophotometer. Data analysis was performed using GraphPad Prism software 50% of cell growth inhibition (IC_{50}) was determined by non-linear regression. The blank contained complete medium without cells and cells without drug addition served as control. Parthenolide nd etoposide were used as a standard.

2.5 Phytochemical analysis

A small portion of dry extracts were used for the phytochemical tests for compounds which include tannins, flavonoids, alkaloids, saponins, terpenoids, steroids and cardiac glycosides[13-15].

2.5.1 Test for tannis

0.2 ml of extract was added to few drops of 1% $FeCI_3$. A green/blue-black colour formed indicated presence of tannins.

2.5.2 Test for flavonoids

0.1 ml extract was added to 5 ml ammonium hydroxide solution and 3 drops of H_2SO_4. The formation of a yellow colour solution and a white precipitate indicates the presence of flavonoids.

2.5.3 Test for saponins:

Foam test: 0.1 ml extract will be shaken vigorously with 3 ml water. The foam formation indicates the presence of saponins.

2.5.4 Test for steroids

To 2 ml of the extract, 2 ml of chloroform and 2 ml of concentrated H_2SO_4 was added and then the solution shaken well. Presence of steroids is indicated by chloroform (upper) layer turning reddish and the acid layer showing yellow colour with greenish fluorescence.

2.5.5 Test for terpenoids

1 ml of extract was mixed with 2 ml of chloroform and 2 ml H_2SO_4 was carefully added to form a layer. A yellow layer at the interface indicates presence of terpenoids.

2.5.6 Test for alkaloids:

Exactly 0.1 ml extract was added to 1 ml Mayer's reagent. The solution turning creamy was an indicative of the presence of alkaloid.

2.5.7 Test for cardiac glycosides

In a test tube, 5ml of the extract was mixed with 2 ml glacial acetic acid, 1 drop of 5% $FeCI_3$ and 1 ml concentrated H_2SO_4. Formation of a brown ring at the interface indicates de-oxysugar characteristic of cardenolides. A

violet ring may appear below the brown ring, while in acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

2.6 Determination of total phenolic content

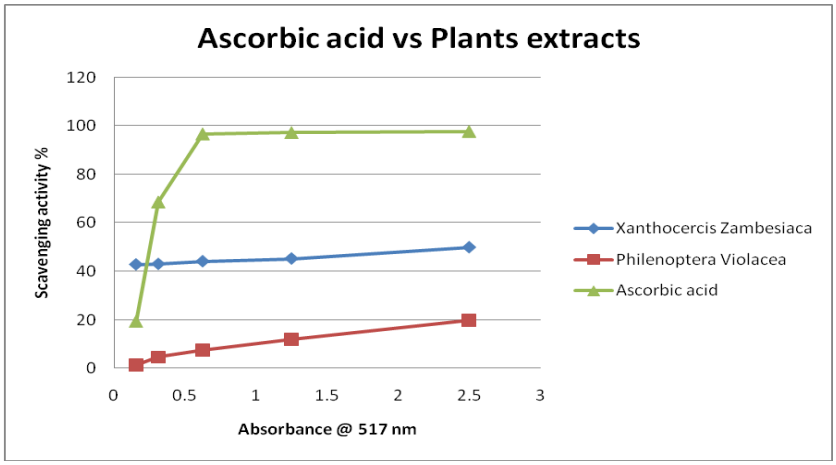
Total phenols content in the obtained extracts were estimated by a colorimetric assay based on procedures described by Singleton and Rossi[16] with some modifications. Briefly, 1 mL of sample was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm wavelength. Gallic acid was used for constructing the standard curve. The results were expressed as mg of gallic acid equivalents/g of extract (GAEs).

3. Statistical analysis

All analyses were run in triplicate and the results expressed as mean ± standard deviation (SD). Statistical analysis was carried out using the Statistic software Graphpad prism version 5.00. Fifty percent of cell growth inhibition (IC_{50}) was determined by non-linear regression and for antioxidant activity a two-tailed test was used and p-values of less than 0.05 were considered significant.

3. Results

Figure 1: DPPH scavenging activity of *Philenoptera violacea, Xanthocercis zambesiaca extracts* and Ascorbic acid



The free radical scavenging activities of *Philenoptera violacea* and *Xanthocercis zambesiaca* extracts were performed using DPPH assay and compared with the scavenging activity of ascorbic acid in figure 1. Ascorbic acid showed a high activity with SC_{50} (68.5%) from a concentration of 0.313 mg/ml. Free radical scavenging activity of *Philenoptera violacea* (19.8%) showed scavenging activity lower than 50% at a high concentration of 2.5 mg/ml. *Xanthocercis zambesiaca* extracts scavenging activity of 50% was at the concentration of 2.5 mg/ml. P values were determined using two-tailed test, and for both extracts $p < 0.05$, thus were statistically significant with *Philenoptera violacea* (p= 0.0010) and *Xanthocercis zambesiaca* (p= 0.0011). Total phenolic content of *Xanthocercis zambesiaca* extracts (0.0895 ± 0.006 mg/GAE) showed a correlation with the determined antioxidant activity.

Table 1: **IC_{50} of methanol extract of *Philenoptera violacea* and *Xanthocercis zambesiaca* on five human cell lines: MCF7, HCT116, TK-10, UACC-62 and PC3 against Etoposite and Parthenolide**

IC_{50}	MCF7	HCT116	TK-10	UACC-62	PC3
Philenoptera violacea	>100	>100	>100	>100	>100
Xanthocercis zambesiaca	>100	>100	>100	>100	>100
Etoposide	<6.25	19.96	-	-	34.64
Parthenolide	<6.25	-	<6.25	<6.25	-

Table 2. CSIR criteria

IC_{50},µg/ml	Status
> 100 µg/ml	Inactive
< 100 µg/ml	Weak Activity
>15 µg/ml	
< 15 µg/ml	Moderate Activity
> 6.25 µg/ml	
< 6.25 µg/ml	Potent Activity

The results of five dose screening were reported as 50% of cell growth inhibition (IC_{50}). The biological activities were separated into 4 categories: inactive (IC_{50} >100 µg/ml), weak activity (15 µg/ml < IC_{50} <100 µg/ml), moderate activity (6.25 µg/ml < IC_{50} <15 µg/ml) and potent activity (IC_{50} <6.25 µg/ml). According to CSIR criterion (Table 2) both samples were considered inactive if parameter IC_{50} for two or all five cell lines was higher than 100 µg/ml. Therefore both samples can be estimated as inactive against the 5 cell lines.

Table 3: Phytochemical screening of *Philenoptera violacea and Xanthocercis zambesiaca extracts*

Phytochemical test	A	B
Tannins	+	--
Flavonoids	+	+
Saponnins	--	+
Steroids	+	--
Terpenoids	+	+
Alkaloids	+	--
Cardiac glycosides	+	+

+ = present, -- = absent
A- *Philenoptera violacea* (leaves, flower & twig)
B- *Xanthocercis zambesiaca* (leaves, flower & twig)

The qualitative phytochemical screening above revealed the presence of tannins, flavonoids, steroids, terpenoids, alkaloids and cardiac glycosides from *Philenoptera violacea* (leaves, flower & twig) extract, while *Xanthocercis zambesiaca* (leaves, flower & twig) extract showed the presence of flavonoids, saponnins, terpenoids and glycosides.

Table 4: Total phenolic content of *Philenoptera violacea and Xanthocercis zambesiaca extracts*

Sample	Absorbance Mean	Polyphenol concentration (mg/GAE)
***Philenoptera violacea* (1 mg/ml)**	0.114	0.152 ± 0.0269
***Xanthocercis zambesiaca* (1 mg/ml)**	0.063	0.0895 ± 0.006

In table 4 are estimated total phenol content values of *Philenoptera violacea* and *Xanthocercis zambesiaca* extracts which were analysed at 1mg/ml against gallic acid. *Philenoptera violacea* extracts showed more polyphenols than *Xanthocercis zambesiaca*. The gallic acid equivalents of the estimated phenolic concentrations ranged from 0.152 ± 0.0269 to 0.0895 ± 0.006 mg/GAE.

5. Discussion

5.1 Antioxidant Agents

In normal conditions, the human body possesses many defence mechanisms against oxidative stress, including antioxidant enzymes and non-enzymatic compounds. The natural antioxidant mammalian mechanism sometimes become insufficient and then the excess of free radicals can damage both the structure and function of a cell membrane in a chain reaction leading to many degenerative diseases[17]. Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases. Natural plants are a cheap source for the extraction of antioxidant compounds, thus providing important economic advantage.
The DPPH radical is a stable organic free radical with an absorption maximum band around 515-528 nm. It is therefore a useful reagent for evaluation of antioxidant activity of compounds[10]. In the DPPH test, the antioxidants reduce the DPPH radical to a yellow-colored compound, diphenylpicrylhydrazine, and the extent of the reaction depends on the hydrogen donating ability of the antioxidants. The methanol extract of both *Philenoptera violacea* and *Xanthocercis zambesiaca* demonstrated a concentration dependent scavenging activity by quenching DPPH radicals. The hydrogen donating activity, measured using DPPH test, showed that the concentration of *Xanthocercis zambesiaca* needed for 50% scavenging (SC_{50}) was found to be 2.5 mg/ ml, for *Philenoptera violacea* was >2.5 mg/ml, whereas <0.313 mg/ml (Figure 1) was needed for ascorbic acid.

5.2 Anticancer screening

Cancer is one of the most prominent diseases in humans. Plants still remain a prime source of drugs for the treatment of cancer and can provide leads for the development of novel anticancer agents. The pace of research in the continuing discovery of new anticancer agents from natural product sources has been staggering lately[18]. Recently, intensive research has been focused on developing tumor therapies from saponins. Xanthocercis zambesiaca extract had saponins and glycosides. Saponins exhibits potent anticancer activity in several human cancer cells through apoptosis-inducing pathways[19] and glycosides are compounds that strongly influences the anticancer activity of the plant extract[20].
According to CSIR criteria shown in Table 2, both *Philenoptera violacea* and *Xanthocercis zambesiaca* extracts can be estimated as inactive (Table 1) against 5 selected cell lines. *Xanthocercis zambesiaca have been* proven to have

Isoflavones[21] and this compound regulates estrogen levels. As it is known that estrogen reduced risks of ovarian and endometrial cancer[22-23], anticancer screening on these cell lines might show some positive results. The obtained results have stimulated us to carry out on-going work to determine the anticancer activity against other cell lines.

5.3 Phytochemical properties

The medicinal values of plants lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body[24]. Phytochemical screening of *Philenoptera violacea* extract showed the presence of tannins, flavonoids, steroids, terpenoids, alkaloids and cardiac glycosides, while *Xanthocercis zambesiaca* extract showed the presence of flavonoids, saponnins, terpenoids and glycosides (Table 3). The beneficial health effects of plants are attributed to flavonoids, a class of secondary metabolites which protect the plant against ultraviolet light and even herbivores[25]. The protective effects of flavonoids are due to their capacity to transfer electrons to free radicals. Alkaloids and their synthetic derivatives are used as basic medicinal agents for their antispasmodic and bactericidal effects[26-27]. Alkaloids in *Xanthocercis zambesiaca* may be responsible for its antibacterial effect[28]. Tannins in *Philenoptera violacea* have astringent properties, hastens the healing of wounds (snakebite) and inflamed mucous membranes (due to tuberculosis/ colds)[9]. The phenolic compounds are commonly found in the plant kingdom, and they have been reported to have multiple biological effects including antioxidant activity[29].

We therefore conclude that *Xanthocercis zambesiaca* exhibited a moderate dose dependent antioxidant activity and is correlated with polyphenolic content of this plant extract. Both *Philenoptera violacea* and *Xanthocercis zambesiaca* extracts showed no anticancer activity against human cell lines*:* MCF7, HCT116, TK-10, UACC-62 and PC3. *Philenoptera violacea* extracts showed more polyphenols and phytochemical contents than *Xanthocercis zambesiaca* but this did not correlate with its antioxidant activity. Further testing using other cell lines is required and also to identify the active compounds of both *Philenoptera violacea* and *Xanthocercis zambesiaca*.

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