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Investigation of the Phytochemistry and Antioxidant Activity of *Pterocarpus angolensis (Mubvamaropa)* from Mupandawana

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study investigated the phytochemistry and antioxidant activity of *Pterocarpus angolensis* (Mubvamaropa) from Mupandawana. *Pt. angolensis* has numerous medicinal uses. The bark, sap, leaves and roots are all used to treat different ailments. The sap is used to stop nose bleeds, treatment of ulcers and kill ringworms. Several studies have supported the use of the tree's sap to treat cataracts, malaria as well as skin inflammations. *Pterocarpus angolensis* bark was collected from Gutu, Masvingo province and authenticated by a plant taxonomist at the Zimbabwe National herbarium (Mr Chapano). The bark was cleaned using distilled water and oven dried at 40˚C for 4 hours to constant weight. Size reduction was achieved using a Thomas-Wiley laboratory mill model with a 2 mm sieve mesh. The phytochemical analysis revealed that the ethanolic extract had more phyto-constituents than the water extract which only four phyto-constituents present all of which were weakly positive. DPPH scavenging activity was 95.11% at a concentration of 500 µg/mL bark extract, while that of the control, ascorbic acid, was 97.60%. Ascorbic acid had an IC_{50} value of 4.35 µg/mL while the bark extract had a value of 150.64 µg/mL.

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1. INTRODUCTION

1.1 Background

Pterocarpus angolensis (Mubvamaropa) is a deciduous tree that can grow up to 30m high. It belongs to the *Leguminosae: Fabaceae* family. The genus *Pterocarpus* comprises of 30 species that are mainly found in the tropics except for Madagascar and Australia. Only four species of angolensis are found in Africa [1] *Pt. angolensis* is found in the Miombo woodlands of South and East Africa and the dry evergreen and deciduous forests [2]. The hardwood is popular for its practical as well as medicinal uses. Large trees for this species are becoming rare and in some areas extinct because of their high demand in furniture making and medicinal use [3,4]. In South Africa the tree is protected by state law. While in Zimbabwe, past and present exploitation has raised concerns regarding the tree's extinction. In Tanzania a minimum stem diameter at breast height (DHB) is prescribed to limit harvesting of small trees. This has however been difficult to enforce with locals in some areas ignoring size prescriptions that were given [5].

Pt. angolensis has numerous medicinal uses. The bark, sap, leaves and roots are all used to treat different ailments. The sap is used to stop nose bleeds, treatment of ulcers and kill ringworms. Several studies have supported the use of the tree's sap to treat cataracts, malaria as well as skin inflammations. The bark is taken orally for piles, and a cold infusion made from the bark is taken to relieve stomach disorders, headaches, blood in the urine, ear ache and mouth ulcers.
The tree bark and roots, when boiled with fresh meat, are used as a preliminary accelerator in the treatment of gonorrhea [6] Roots are burnt and the ashes are drunk in water to treat asthma and tuberculosis [7].

In Zimbabwe the tree is widely popular and the bark and roots are used to treat a variety of ailments [8]. The bark and roots are being used to treat various forms of cancer as well topical ulcers and gonorrhea. Testimonials by treated patients and witnesses allude to the high efficacy and potency of the concoctions by the herbalist leading to curative treatment outcomes. The herbalist uses the tree extracts in their crude form as the primary part of a triple herb treatment therapeutic regimen.

2. METHODOLOGY

2.1 Plant Collection and Preparation

Pterocarpus angolensis bark was collected from Gutu (19°38′12″S 031°10′06″E), Masvingo province and authenticated by a plant taxonomist at the Zimbabwe National herbarium (Mr Chapano). The bark was cleaned using distilled water and oven dried at 40˚C for 4 hours to constant weight. Size reduction was achieved using a Thomas-Wiley laboratory mill model with a 2 mm sieve mesh.

2.2 Extraction of Pt. Angolensis

The bark of *Pt. angolensis* (200 g) was macerated in 70 % ethanol (600 mL) for 48 hours. The sample was sonicated for 30 minutes before it was filtered using a mutton cloth. Removal of bulky material was followed by vacuum filtration using Whatman number 1 filter paper. Rotary vapour (Buchi Rotavapor R-114) apparatus was used to remove excess ethanol. The apparatus was set at 55˚C at 150 rpm. The obtained concentrate was lyophilized using a freeze dryer. The weight obtained was recorded. To reconstitute, the extract was dissolved, at 0.2 g/mL, in 12 % (v/v) dimethyl sulphoxide (DMSO) in water, to give a stock solution.

2.3 Qualitative Phytochemical Tests of Pt. Angolensis Extracts

Primary qualitative tests were carried out to determine the presence or absence of various class of phyto-constituents.

2.3.1 Test for alkaloids

2.3.1.1 Mayer's test

To 2 mL of plant sample extract, two drops of Mayer's reagent were added along the sides of the test tube. The solution was checked for the appearance of white creamy precipitate which indicates the presence of alkaloids [9].

2.3.2 Identification of tannins

To a test tube, 1 mL of ethanol extract was added to 2 mL of distilled water. Followed by 2-3 drops of ferric chloride. The test sample was checked for the development of a green-blue colour which indicates the presence of catechic

tannins and blue-black indicated the presence of Gallic tannins [10].

2.3.3 Identification of Saponins

3 mL of extract was diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 minutes. The test sample was checked or the formation of 1 cm layer of foam which indicates the presence of Saponins [10].

2.3.4 Identification of cardiac glycosides

To 2 mL of plant extract, glacial acetic acid, one drop of 5 % $FeCl_3$ and conc. H_2SO_4 was added. If a red-brown colour appeared at the junction of the two liquid layers and the upper layer appears blue-green, it confirms the presence of glycosides [9].

2.3.5 Determination of terpenoids and steroids

4 mg of extract was treated with 0.5 mL acetic anhydride and 0.5 mL acetic acid. Then concentrated H_2SO_4 was added slowly. The sample was checked for the development of a blue green color which is an indication that terpenoids are present while a reddish brown color indicates the presence of steroids [11].

2.3.6 Determination of flavonoids

Three drops of dilute sodium hydroxide were added to 1 mL of both extracts. The test sample was observed for the development of an intense yellow colour development of colourless solution upon addition of a few drops of dilute acid which is an indication for the presence of flavonoids [12].

2.3.7 Anti-oxidancy evaluation of Pt angolensis extracts

The antioxidant activity of *Pt. angolensis* was determined using the DPPH free radical scavenging assay as per methods described by Mahdi-Pour et al., 2012. To identical bottles, 50 µL of *Pt. angolensis* extract in concentrations from 5 to 150 mg/mL were added followed by 5 ml of 0.004 % (w/v) solution of DPPH. The resultant mixture was vortexed and incubated for 30 minutes at room temperature in a dark cupboard and then read using a UV spectrophotometer (Lambda 35 UV/Vis-Spectrometer, Perkin Elmer Instruments) at 517 nm. The blank was 70 % (v/v) methanol. Ascorbic acid (Vitamin C) was used for comparison. Measurements were taken in triplicate.

DPPH scavenging effect was calculated using the following equation:

$$DPPH\ scavenging\ effect\ (\%) = \{\frac{A^\circ - A}{A^\circ}\}\ x\ 100$$

Where A^0 is the absorbance of negative control (0.004% DPPH solution) and A is the absorbance in presence of extract.

The results were reported as IC_{50} values and ascorbic acid equivalents (AAE, mg/g) of *Pt. angolensis* extracts.

2.3.8 Total phenolic content

The total phenolic content of the extracts were determined according to the Folin-Ciocalteu spectrophotometric method with some modifications. To prepare a calibration curve, phenol (Gallic acid) stock solution (5 mg/mL) was added into 100 mL volumetric flasks, and then diluted to volume with water. From each calibration solution, 0.25 mL was mixed with 1.25 mL of 10-fold diluted Folin-Ciocalteu's phenol (1mL Folin reagent and 9 mL deionized water) reagent and allowed to react for 5 min. Then, 1mL of 7.5 % Na_2CO_3 solution was added, and the final volume was made up to 5 mL with deionized water. After 1hr of reaction at room temperature, the absorbance at 760 nm was determined by spectrophotometer. The test was done in triplicate. A calibration curve was plotted to determine the level of phenolics in the samples. Same procedure was done for different parts of *Pt. angolensis* extracts in concentrations. The test was done in triplicate. The results were expressed as galic acid equivalents (GAE, mg/g) of *Pt. angolensis* extract (Rebelo *et al.*, 2009).

3. RESULTS

3.1 Extraction of Pt Angolensis Bark

The percentage yield, texture and colour of the extract obtained from extracting *Pt. angolensis* bark were recorded in Table 1 below. A higher yield was obtained using 70 % ethanol compared to using water. The texture and colour was however, the same for both extracts.

Table 1. Percentage yield, texture and colour of extracts

Extract	% Yield	Texture	Colour
Water	9.6	Powdery	Dark red-brown
70% ethanol	11.23	Powdery	Dark red-brown

Table 2. Secondary metabolites of Pt angolensis

Phyto-constituent	Ethanolic extract	Water extract
Alkaloids	+	+
Flavonoids	++	-
Terpenoids	+	-
Steroids	+	+
Tannins	++	-
Saponins	+	+
Phenols	++	+
Cardiac glycosides	-	-

Key: Strongly positive (+++); moderately positive (++); weakly positive (+); absent (-)

3.2 Qualitative Phytochemical Tests

Qualitative phytochemical analysis was carried out for both extracts and recorded in Table 2. The phytochemical analysis revealed that the ethanolic extract had more phyto-constituents than the water extract which only four phyto-constituents present all of which were weakly positive. The water extract was weakly positive for alkaloids, steroids, saponins and phenols. The ethanolic extract had phenols, tannins, and flavonoids which were moderately positive. While the rest were weakly positive except for cardiac glycosides which were absent in both extracts.

3.3 Anti Oxidancy Studies

3.3.1 Total phenolic content

The GAE calibration curve was done to help determine the total phenolic content of both extracts.

The ethanolic extract had a higher total phenolic content compared to the aqueous extract as a result it was expected that the ethanolic extract would have a higher antioxidant activity than the aqueous extract due to the greater concentration of bio actives.

3.3.2 Antioxidant assay

Fig. 3 was used to determine the DPPH scavenging activity. DPPH scavenging activity was 93.39 % for ascorbic acid (the standard used) at 150 µm/mL. While *Pt. angolensis* ethanolic extract was 90.17 % and 84.87 % for the aqueous extract. The IC_{50} values Fig. 4 were 28.51 µg/mL, 49.33 µg/mL and 64.08 µg/mL for ascorbic acid, *Pt. angolensis* hydro-ethanolic extract and the aqueous extract respectively.

4. DISCUSSION

4.1 Phytochemical Studies

200 g of *Pt. angolensis* bark powder was extracted using 70 % ethanol. A red brown powder was obtained after freeze drying the sample. The yield of crude extract obtained was 32.28 g (16.14 %). Phytochemical screening showed the presence of alkaloids, saponins, tannins, flavonoids and phenols. Anthraquinones were however absent. The phyto-constituents found in the crude extracts of *Pt. angolensis* have been reported to treat various medical conditions.

Alkaloids and their synthetic derivatives have antibacterial, antispasmodic and analgesic properties [13]. While Tannins exhibit anti-diarrheal activity. Saponins are known to boost a person's immune system as well as to lower cholesterol. Studies have also shown that they have anti-mutagenic activity, anti-hypoglycemic property as well as antitumor activity. This potentially justifies the plant's use by herbalists to treat cancer [14]. Flavonoids on the other hand prevent oxidative stress and have strong anticancer activity. They also protect against allergies, tumors and ulcers [15]. Plant phenolics are believed to play a key part as defense compounds when environmental stresses, such as high light, low temperatures and pathogenic infection lead to an increased production of free radicals and other oxidative species in plants [16].

4.2 Antioxidant activity

The formation of cancer cells is partly influenced by cellular oxidative damage. As a result there has been increasing interest in the mechanism of action of antioxidants and whether they specifically intercept or remove free radicals from cells in the human body. Ames et al. (1993) reported that antioxidants prevent injury to blood vessel membranes, optimize blood flow to the heart and brain, and prevent cancer-causing DNA damage. Jo et al. (2006) also indicated that antioxidants can prevent or slow the oxidative damage linked to various diseases such as carcinogenesis, atherogenesis and aging.

Free radicals are a result of normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals [17]. The antioxidant activity of *Pt. angolensis* stem bark was assessed using DPPH free radical scavenging and reducing power assays. Antioxidant activity is normally determined with more than one assay because plants exhibit antioxidant activity through different mechanisms due to the variety of chemical compounds present [18]. The DPPH assay measures the ability of a sample to reduce the DPPH radical by donating the hydrogen atom which is monitored through the dis-coloration of the mixture from purple to yellow coloured diphenyl picryl hydrazine [19]. DPPH scavenging activity was 95.11 % at a concentration of 500 µg/mL bark extract, while that of the control, ascorbic acid, was 97.60 %. Ascorbic acid had an IC_{50} value of 4.35 µg/mL while the bark extract had a value of 150.64 µg/mL.

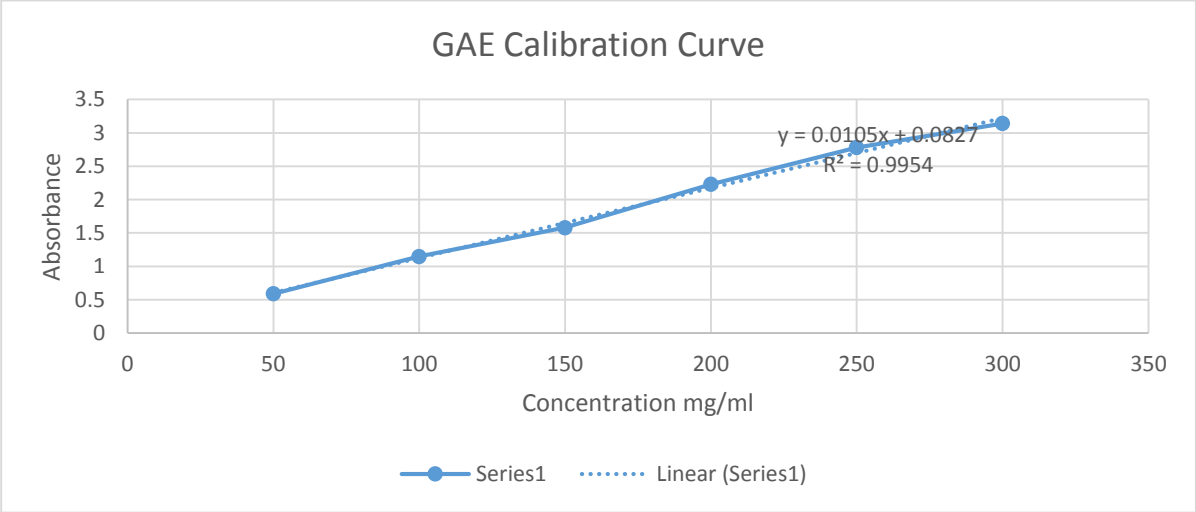

Fig. 1. Gallic acid standard curve for Folin-Ciocalteu assay

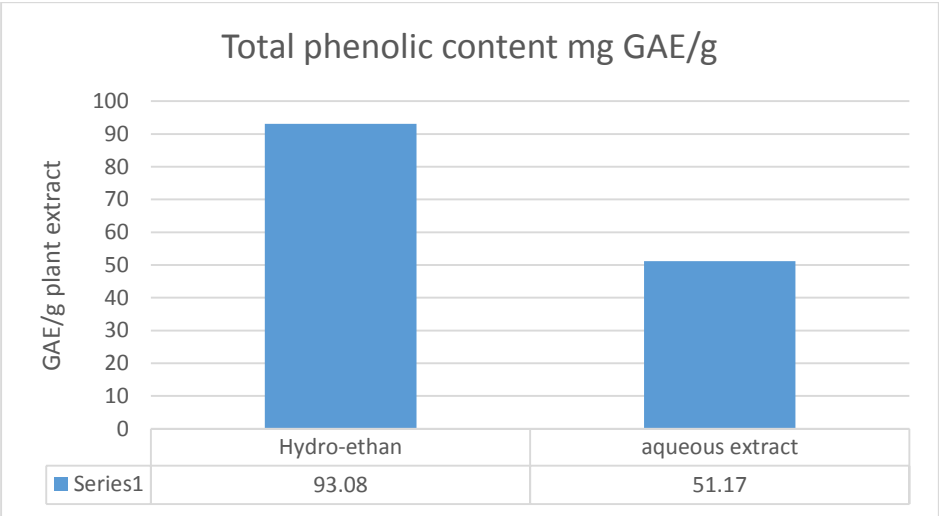

Fig. 2. Total phenolic content for Pt. angolensis

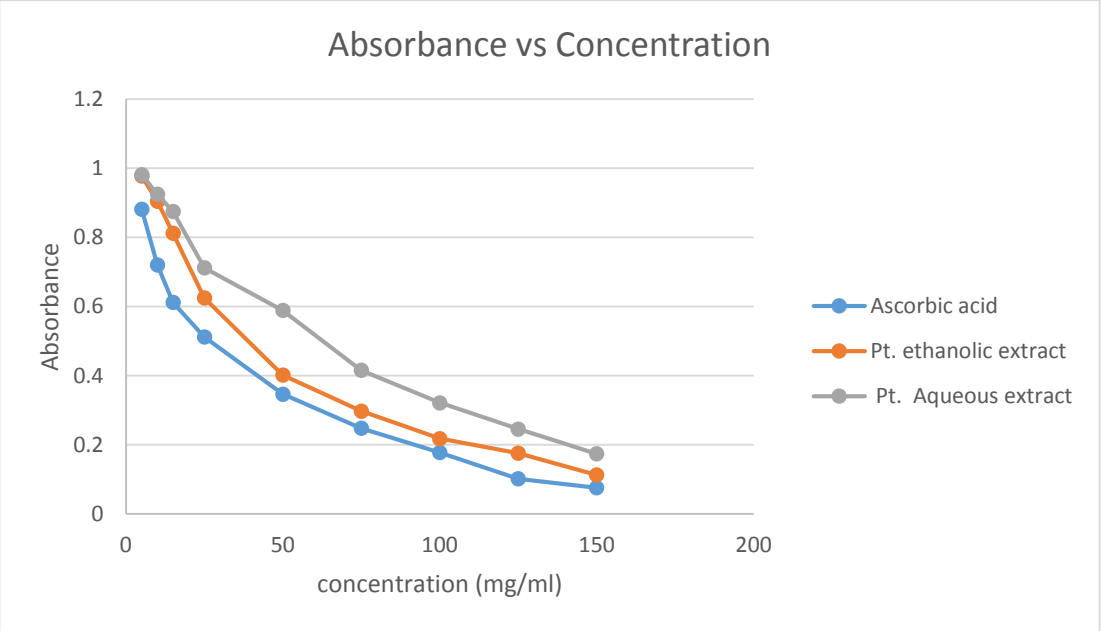

Fig. 3. DPPH scavenging assay of bark extracts compared to ascorbic

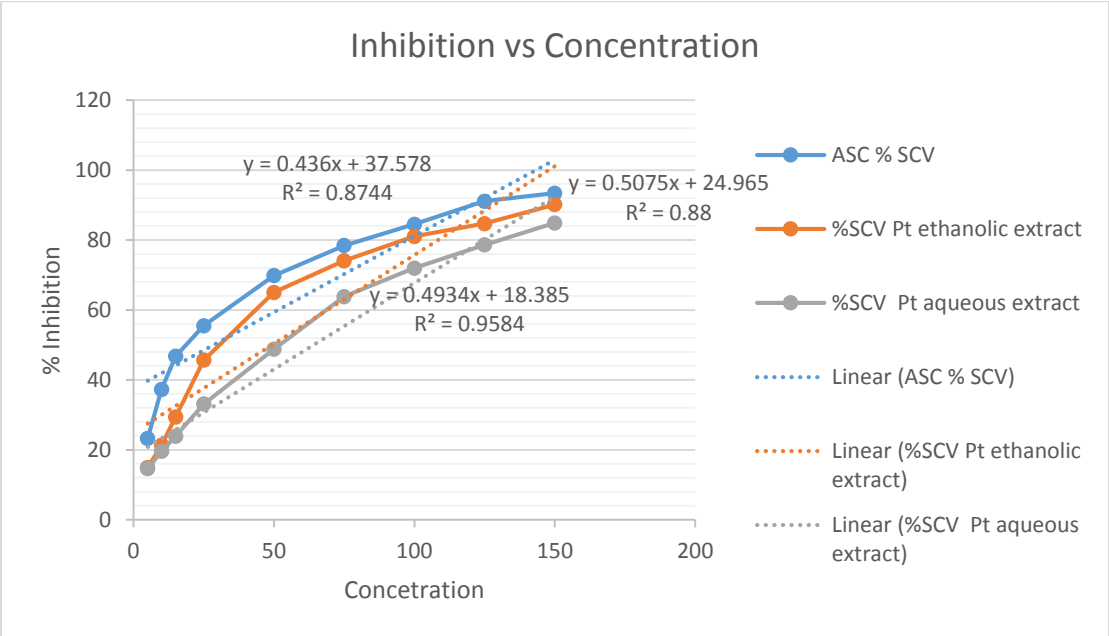

Fig. 0. Evaluation of IC50 of Pt. angolensis and ascorbic

The total phenolic content was assayed using Folin-Ciocalteu reagent. This method which is routinely employed to study phenolic antioxidants is fast, convenient, simple and most importantly reproducible [20]. The value of the TPC of the extract obtained confirms that the extract is very rich in phenolic contents. Phenolics have received much scientific attention because they are the most widely-spread secondary metabolites in the plant kingdom and aside this, they are also known as sources of potential natural antioxidants because of their abilities to act both as efficient radical scavengers and metal chelators [21]. The total phenolic content was determined to be 20.1 mg GAE/g of extract.

5. CONCLUSION

Ethanol proved to be a better extraction solvent for the extraction of bio-active compounds of *Pt.*

angolensis leaves. This conclusion was supported by the higher yield that was obtained as well as the presence of more phyto-constituents. Both extracts exhibited antioxidant activity. Higher activity was however, observed in the 70% ethanolic extract. The presence of phyto-constituents and antioxidant activity therefore validates its use by traditional healers to various conditions. The ethanolic extracts should be investigated further for drug development of bio-active compounds from *Pt. angolensis.*

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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