

Phytochemical screening, Antioxidant and Antiangiogenic Properties of *Oncoba welwitschii* (Oliv.) Gilgn. and *Tetrorchidium oppositifolium*(Pax. and Khoffm.), Medicinal Plants from Gabon.

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ABSTRACT: Angiogenesis is essential for the continued growth of the tumor. The inhibition of tumoral angiogenesis is considered to be an attractive target in the development of anti-cancer agents. The aim of this study was to evaluate the antioxidant and antiangiogenic activities of bark extracts of *Oncoba welwitschii* and *Tetrorchidium oppositifolium*. The antioxidant efficacy was determined using the methods DPPH inhibition. The antiangiogenic activity was evaluated *in vivo* in a chick chorioallantoic membrane (CAM) model. The extracts demonstrated a high capacity to inhibit free radicals, with IC₅₀ values ranged from 7.140 to 16.090 µg/mL for *O. welwitschii* and 5.860 to 38.160 µg/mL for *T. oppositifolium*. The Antioxidant activity index (AAI) of plants ranged from 1.032 to 6.723. The crude extracts showed a very strong antioxidant activity. Moreover, these showed capable of antiangiogenic activity of 83.334% at 0.500 mg/mL. These data suggest that aqueous extracts of these plants may be a promising source material for the development of anticancer chemotherapeutics.

KEYWORDS: *Oncoba welwitschii*, *Tetrorchidium oppositifolium*, Antioxidant and antiangiogenic activities.

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I. INTRODUCTION

Cancer is a devastating disease with tremendous negative implications at the personal, health care, economical, and social levels. A cancer death account to about 8.7 million per year and stands as the second most common disease. There is still a malignant disease which is difficult to cure and with higher mortality rate. In cancer biology, angiogenesis is required to permit increased delivery of oxygen and nutrients to the nascent tumor [1]. Angiogenesis is a process of formation of new vessels from the arterial supply created by endothelial cells. Under physiological conditions, it is required for embryogenesis, tissue repair (wound healing) and organ generation [2]. This process must be finely balanced, since insufficient or excessive angiogenesis can cause several diseases such as diabetic retinopathy, sclerosis, rheumatoid arthritis and cancer [3]. Indeed it was that Folkman in 1971 hypothesized that tumor growth depended on the formation of new vessels. Tumor angiogenesis is initiated by issuing proangiogenic substances to form detectable tumors. It is essential for the continued growth of the tumor because it supplies the tumor with nutrients, oxygen and removes cellular waste, which can be toxic to cancer cells. And blocking the action of these substances proangiogenic must stop tumor growth [1]. To establish secondary cancers in other organs (metastasis), cancer cells secrete substances called reactive species oxygen (ROS) that will induce the synthesis of proangiogenic factors [4; 5]. Angiogenesis is a general hallmark of cancer; therefore, the inhibition of tumor-derived angiogenesis is considered to be an attractive target in the development of anti-cancer agents. Several studies have shown that medicinal plants are a major source of antioxidants, substances whose primary role is to neutralize ROS [6; 7; 8]. The neutralization of ROS results in the inhibition of angiogenesis, thus inhibition of metastasis, which is the leading cause of cancer death [9]. Natural products have been shown to be effective in cancer prevention or treatment on their own or in combination with chemotherapeutics [10; 11].

II. STATEMENT OF THE PROBLEM

Despite advances in medical technology in the treatment of cancer, it remains one of the leading causes of morbidity and mortality worldwide. According to the World Health Organization, the number of new cases is expected to increase by around 70% over the next two decades. In Gabon, incomplete data available due to the lack of actual registration of the cancer population indicate that the number of new patients is constantly increasing from 183 new cases in 2000 to more than 1,000 new patients in 2008 constituting a real public health problem. In African traditional medicine, plants or the prescription drugs derived from them, which have been used for disease management, can be promising source materials in the development of anticancer drugs because they are originally based on multi-target and multi-component strategies. Additionally, medicinal plants extracts can be prescribed as adjuvants to produce synergistic effects with conventional anti-cancer drugs or to relieve the adverse side effects of the anti-cancer drugs.

1. Objective of study

The present study reports results of a detailed analysis of antioxidant and antiangiogenic properties of *O. welwitschii* and *T. oppositifolium*. To the best of our knowledge, such investigation has not been conducted yet on *O. welwitschii* and *T. oppositifolium* species from Gabon.

2. Significance of study

Out of a plethora of Gabonese medicinal plants, we have selected only two most important medicinal plants such as *O. welwitschii* and *T. oppositifolium*, which are used for the treatment of number of diseases. *O. welwitschii* is a shrub growing in secondary forests or on the edge of the rain forest. The leaves are heart-shaped, fitted with a long petiole and grouped at the end of the branches. The flowers are white, fragrant, with yellow stamens numerous, recalling a wide flower rose hips, come on the trunk and branches. Bur dehiscent long thorns many seeds surrounded by a yellow pulp, sweet thesis contain oil seeds chaulmoogric specific to cure leprosy. The leaves are used to treat syphilis [12]. *T. oppositifolium* is a tree common in rural site of ancient cultures. Small laughing's yellowish along the branches. The fruits are small capsules. The bark has year unpleasant odor. The shredded bark and soaked in palm oil, are used in rubbing to treat rheumatism. This bark is a good remedy for the eyes [12]. *Oncoba welwitschii* and *Tetrorchidium*

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oppositifolium, have never been the subject of an antiangiogenic and antioxidant study.

3. Statistical analysis

The data were expressed as the mean \pm standard deviation (SD) of three independent experiments and analyzed using one-way analysis of variance and Student's t-test p-values of <0.05 were considered to be statistically significant.

III. MATERIAL AND METHODS

1. Plante material

The bark of *Oncoba welwitschii* and *Tetrorchidium oppositifolium* were collected in Oyem in June and August 2015 in Mitzi (WoleuNtem, Northern of Gabon), respectively. Identification of the species was carried out at the National Herbarium of IPHAMETRA, Libreville (Gabon). Voucher specimens have been deposited in the Herbarium of IPHAMETRA, at Laboratory of Natural Substances and Organometallic Synthesis (LASNSOM) and Laboratory of Biochemistry Research (LAREBIO) Department of Chemistry-Biochemistry, Faculty of Sciences of USTM in Franceville.

2. Preparation of plants extracts

Water-ethanol (50/50 v/v) extracts, ethanol extracts and water extracts were prepared from dry powder. 25 g of powder from each sample were soaked with 250 mL of the appropriate solvent mixture and left under shaking conditions at room temperature (25°C) for 24 h. Water extracts were prepared by decoction mixing 25 g of powder to 250 mL of distilled water. The mixture was boiled for 1 h. Each extract was filtered using Whatman N°1 filter paper and solvents were completely removed at low pressure with a rotary evaporator (Büchi, Labortechnik, Switzerland). The extracts were then concentrated, freeze-dried and stored at 4°C until analysis.

3. Phytochemical study

a. Phytochemical Screening

Each extract was then tested for the presence of flavonoids, coumarins, tannins, total phenolic, saponosids, cardiac glycosides, reducing sugar, sterols, triterpenes, oses, holosides, anthracenics, anthocyanins, alkaloids and anthracenosids as described elsewhere [13].

b. Total Phenolic, Total Flavonoid, Tannins and Proanthocyanidins contents

The total phenolic contents of the different extracts were determined according to the Folin-Ciocalteu Method [14] with minor modifications as described by Zongo [15] using gallic acid as standard. The absorbance was measured at 735 nm using a multiwell plate reader (μ Quant Bio-Tek Instrument, Inc, USA). All analyses were done in triplicate and results (average of triplicate analysis) were expressed as gallic acid equivalent per gram of lyophilized sample. Total flavonoid contents were determined by the aluminum chloride ($AlCl_3$) colorimetric assay method [16] adapted to 96-well plate, using quercetin as a standard [7]. The total flavonoid contents were expressed as quercetin equivalents in milligrams per gram sample (average of the triplicate analysis). The reference method of European community was used to measure total amount of tannins [17]. The method consists on the hydrolysis of proanthocyanidins in a hot acid-alcohol medium into anthocyanidins. This method allows taking into account all the units of flavans-3-ols constituting the polymers [18]. The assay is performed by mixing 50 μ L of the extract with 700 μ L of 30% HCl-butanol solution (v/v). The mixture was put in tightly closed 1.5 mL Eppendorf tube and vortexed for 1 min. Subsequently, the tube was heated at 100°C for 2 h and after cooling, 200 μ L aliquots were put in triplicate into a 96-well plate and the absorbance were read at 550 nm. Apple procyanidins (DP \approx 7.4) treated as aforementioned were used as a standard. Results were expressed as apple procyanidins equivalent (APE).

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4. Antioxidant Activity Index

The Antioxidant Activity Index (AAI) was assessed according to the method described by Scherer and Godoy [19]. This method is based on the DPPH radical test. Briefly, the working reagent was prepared by dissolving 10 mg of DPPH in 100 mL ethanol. Graded concentrations of extracts ranging from 0.781 to 100 µg/mL obtained by two-fold dilutions were prepared and 100 µL of each dilution were mixed with 100 µL of the working solution of DPPH in a 96-well plate. Absorbencies were measured at 517 nm after 15 min incubation at room temperature in the dark. Ascorbic acid (Vitamin C) and Butylatedhydroxytoluene (BHT) were used as references. The ability to scavenge DPPH radical was calculated by the following equation: %RSA = $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$.

A = Absorbance at 517 nm.

The IC₅₀ (concentration providing 50% inhibition) of extracts and standards was determined using regression curves in the linear range of concentrations. The AAI was then calculated as follows: AAI = [DPPH] (µg/mL) / IC₅₀ (µg/mL). [DPPH] is the final concentration of DPPH. We considered criteria of Scherer and Godoy [19] according to which plant extracts show poor antioxidant activity when AAI < 0.5, moderate antioxidant activity when AAI between 0.5 and 1.0, strong antioxidant activity when AAI between 1.0 and 2.0, and very strong when AAI > 2.0.

5. Evaluation of antiangiogenic activity

In this assay, Chick Chorioallantoic Membrane (CAM) Model was used to determine the antiangiogenic efficacy of the water extracts of *O. welwitschii* and *T. oppositifolium* according to previously reported methods [20; 21]. Fertilized chicken eggs were purchased from a local poultry farm, were sterilized with 70° ethanol and incubated at 37°C in an egg incubator (Lab. Incubator, Digisystem Laboratory Instruments Inc.), with 80% relative humidity. On day 3 of post incubation, 2 to 3 mL of albumin were withdrawn through a small opening at the large blunt edge of the egg. A square window of 1 cm² was opened in the egg shell at the opposite to blunt edge and sealed with an adhesive tape to prevent dehydration. The eggs were returned for further incubation. At the 8th day, the experimental groups were divided into 3 of each containing 30 numbers of eggs. Group 1 and 2 were treated with water extracts. Sterile discs (diameter: 10 mm) of Whatman N° 1 soaked of 10 µL of the water extract at concentrations ranging from 0.25 to 0.50 mg/mL was applied to the CAM. In parallel Group 3 treated with phosphate buffer saline (PBS) alone as control, a paper disc Whatman N° 1 soaked of 10 µL PBS at pH 7 was placed on the CAM of egg. On the 9th day, a volume of 10 µL of para-formaldehyde was applied to the CAM. The CAM was cut around the disk and all disc (CAM) was placed in a Petri dish containing agarose gel 1.6%. Then the photos were taken with a cannon digital camera of 16 × 5.0 Mega Pixel (made in China) and the images were subsequently analyzed with the software Image J. The percentage of vascularization (density) is measured relative to a normal control vascularization.

IV. RESULTS AND DISCUSSION

1. Phytochemical study

a. Phytochemical screening

The phytochemical screening of the extracts was first performed to detect the major chemical groups occurring in the extracts. In view of the results in table 1, it appears that two plants studied *O. welwitschii* and *T. oppositifolium* contain very abundant coumarins and polyphenols. This abundance in phenolic compounds would confirm the therapeutic properties that there are assigned in ethnotherapy. Indeed, several works have demonstrated that phenolic compounds confer to the plant several biologic activities [22].

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Table 1: Chemical groups detected in *O. welwitschii* and *T. oppositifolium* extracts

Chemical groups	<i>Oncoba welwitschii</i>			<i>Tetrorchidium oppositifolium</i>		
	Water	Water-ethanol	Ethanol	Water	Water-ethanol	Ethanol
Saponosids	+++	++	-	+++	++	-
Polyphenols	+++	+++	+++	+++	+++	+++
Sterols and triterpenes	-	-	-	-	+	++
Oses and holosides	-	-	-	-	-	-
Tannins	Gallics	+++	++	+	+	++
	Catechics	++	+++	++	+	++
Alkaloids	+++	++	++	+++	++	++
Cyanidins	Flavons	-	-	-	-	-
	Flavanons	+	+	-	-	+
	Flavonols	-	-	-	-	-
	Flavanonols	-	-	-	-	-
Total flavonoids	+	+	+	++	+	+
Anthocyan	-	-	+	-	-	-
Proanthocyanidins	-	+	-	++	+++	+++
Anthracenics	++	+++	+	++	++	+
Coumarins	+++	+++	+++	+++	+++	+++
Cardiac glycosides	Digitoxine	+++	+++	-	-	+
	Digitoxigenine	-	-	-	-	-
	Gitoxine	-	-	-	-	-
	Gitoxigenine	-	-	-	-	-
Reducing sugar	++	++	++	++	++	++

+++ = Very abundant; ++= Abundant; + = Not abundant; - = Not detected.

The abundance of biologic antioxidant compounds such as phenolic compounds, flavonoids and tannins suggests that *O. welwitschii* and *T. oppositifolium* may have a preventive effect on diseases like cancer, cardiovascular diseases and diabetes. Moreover, concentrations in phenolic compounds in the two plants are similar those met in certain medicinal plants used in traditional medicine in certain regions of Gabon [6; 7; 8; 23].

b. Total Phenolic, Total Flavonoid, Tannins and Proanthocyanidins contents

The contents of total phenolic, total flavonoids, total tannins and total proanthocyanidins of extracts from *O. welwitschii* and *T. oppositifolium* are presented in table 2. The contents of total phenolic in terms of gallic acid equivalent (standard curve equation: $Y = 0.0012X - 0.0004$, $R^2 = 0.9902$) [24]; ranged from 126.400 ± 0.750 to 9735.100 ± 23.650 mg GAE/g of drug and were abundants in water-ethanol extracts of *O. welwitschii*. Total flavonoids (standard curve equation: $Y = 0.0032X + 0.0077$, $R^2 = 1$) [24]; ranged from 40.900 ± 4.400 to 102.150 ± 1.700 mg EQ/g and were abundants in water-ethanol extracts of *T. oppositifolium*. There were abundant in ethanol extracts than water-ethanol and water extracts. Levels of tannins were expressed in terms of tannic acid equivalent (TAE). The equation of the right-hand side of the proportioning of the total tannins by the reference method of European Community [17] gave $Y = 0.0009X + 0.2088$ with $R^2 = 1$. Total tannins are ranged from 15.600 ± 5.150 to 287.050 ± 0.834 mg EQ/g and were abundant in water extracts of *T. oppositifolium*. There were abundant in water extracts than water-ethanol and ethanol extracts. Levels of proanthocyanidins were expressed in terms of apple proanthocyanidins equivalent (APE). The equation of the right-hand side of the proportioning of the proanthocyanidins by the HCl-butanol method gave $Y = 0.0006X + 0.0024$ with $R^2 = 0.9869$ [24]. Among extracts, proanthocyanidin contents had ranged between 95.433 and 185.267 mg APE/g of drug. The content of proanthocyanidins is very abundant in water-ethanol extract of *T. oppositifolium*. Proanthocyanidins have been reported to exhibit many beneficial health effects such as antioxidant and

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anti-carcinogenic effects. Thus, Bak [25] has reported that taken together, the proanthocyanidins from wild grape (*Vitisamurensis*) seeds could be used as a potential natural cancer chemopreventive agent through Nrf2/ARE mediated phase II detoxifying/antioxidant enzymes induction via p38 and PI3K/Akt pathway.

Table 2. Total phenolic content (TPC), Total flavonoid content (TFC), Total Tannins Content (TTC) and Total Proanthocyanidins Content (TPC) of extracts from *O. welwitschii* and *T. oppositifolium* .

Extracts		Total phenolic content (TPC) (mgGAE/g of drug)	Total flavonoid content (TFC) (mgEQ/g)	Total tannins content (TTC) (mgEAT/g)	Total proanthocyanidins content (TPC) (mgAPE/g of drug)
<i>Oncoba</i>	Aq	232.050 ± 2.200	60.200 ± 0.010	136.350 ± 1.250	110.767
<i>welwitschii</i>	Eth-Aq	735.150 ± 23.650	52.150 ± 0.700	122.700 ± 10.200	134.600
	Eth	693.100 ± 9.450	69.100 ± 8.100	39.700 ± 17.250	178.100
<i>Tetrorchidium oppositifolium</i>	Aq	230.75 ± 15.00	40.900 ± 4.400	287.050 ± 0.834	95.433
	Eth-Aq	126.400 ± 0.750	47.500 ± 27.500	16.800 ± 4.450	185.267
	Eth	718.100 ± 0.100	102.150 ± 1.700	15.600 ± 5.150	168.267

Aq = Aqueous; *Eth-Aq* = Ethanol-aqueous; *Eth* = Ethanol,

Phenolic substances have been suggested to play a preventive role in the development of chronic diseases such as cancer and heart disease [7; 26]. The phytochemical screening had indicated that the extracts of *O. welwitschii* and *T. oppositifolium* had total polyphenol, flavonoids and tannins abundances, the study quantitative of polyphenols confirm those results.

2. Antiradical activity

Phenolic compounds have beneficial biological effects to scavenge free radicals [27]. Free radical scavenging of phenolic compounds is an important property underlying their various biological and pharmacological activities. The free radical-scavenging activities of various extracts were evaluated at their initial concentration. All extracts show free radical scavenging activity (Table 3), lower IC₅₀ indicating the higher antiradical activity of the extract. The crude extract of *O. welwitschii* and *T. oppositifolium* show best DPPH free radical-scavenging activities (IC₅₀ values ranged from 7.140 to 16.090 µg/mL for *Oncoba welwitschii* and 5.860 to 38.160 µg/mL for *T. oppositifolium*. As it can be seen, the AAI of extracts ranged from 1.032 to 6.723 and can be compared to AAI of Vitamin C and BHT (AAI values of 11.320 and 7.850, respectively).

Table 3. Antioxidant Activity Index (AAI) of extracts by DPPH free radical scavenging method.

Plants extracts		Equations	R ²	IC ₅₀ (µg/mL)	AAI	Activity
<i>Oncoba</i>	<i>Aq</i>	Y = -5.755X + 91.081	0.998	7.140	5.518	very strong
<i>welwitschii</i>	<i>Eth-Aq</i>	Y = -3.234X + 91,045	0.991	12.690	3.100	very strong
	<i>Eth</i>	Y = -3.019X + 98,589	0.997	16.090	2.450	very strong
<i>Tetrorchidium oppositifolium</i>	<i>Aq</i>	Y = -4.234X + 74.805	0.937	5.860	6.723	verystrong
	<i>Eth-Aq</i>	Y = -3.240X + 78.822	0.967	8.890	4.431	verystrong
	<i>Eth</i>	Y = -1.259X + 98.032	0.990	38.160	1.032	strong
Vitamin C		Y = 14.559X - 0.613	0.999	3.480	11.320	verystrong
BHT		Y = 5.659X + 11.513	0.996	6.300	7.850	verystrong

Aq = Aqueous; *Eth-Aq* = Ethanol-aqueous; *Eth* = Ethanol, BHT= Butylatedhydroxytoluene: Positive control.

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The crude extract of *O. welwitschii* and *T. oppositifolium* showed a very strong activity. A number of studies conducted on plant samples in order to evaluate their antioxidant efficacy have confirmed that plant-based organic extracts rich in phenolic compounds exert potent antioxidant activities [7; 28; 29]. Phenolic extracts have been reported to retard lipid oxidation in oils and fatty foods [30], decrease the risk of heart diseases by inhibiting the oxidation of low-density lipoproteins. They are also known to possess antibacterial, antiviral, antiangiogenic and anticarcinogenic properties [7; 31; 32].

3. Antiangiogenic Activity

The antiangiogenic potential of the extracts was evaluated *in ovo* with the chicken chorioallantoic membrane (CAM) the eighth embryonic day. The fertilized eggs were treated with aqueous extracts (0.25-0.5 mg/mL). The degree of vessel formation on CAM was scored 1 day later. The vessel density is the percentage of blood supply to the analysis area. It is inversely proportional to the degree of inhibition; plus the value of the density, the lower the degree of inhibition of angiogenesis is strong. In the presence of phosphate buffer saline (PBS) used as a control, the target area has a vascularization percentage of 100%, corresponding to a normal vasculature (Figure 1a, 1b).

Control (PBS) Water extract of *Oncoba welwitschii* Water extract of *Tetrorchidium oppositifolium*
0.25 mg/mL 0.50 mg/mL 0.25 mg/mL 0.50 mg/mL



Figure 1(a). Inhibitory effects of water extracts of *O. welwitschii* and *T. oppositifolium* on angiogenesis.

The CAM of an 8 days old chick embryo was separately exposed to PBS (control). Extracts were introduced on top of the CAMs. After 24 h of incubation, the CAM tissue directly beneath each filter disk was resected, and digital images of the CAM sections were captured.

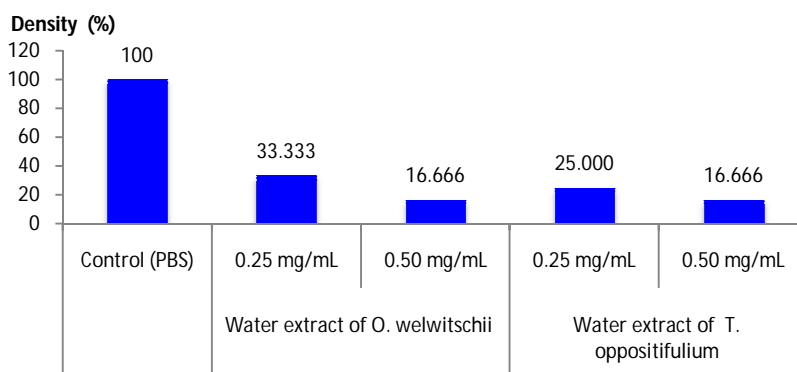


Figure 1(b). Inhibitory effects of water extracts of *O. welwitschii* and *T. oppositifolium* on angiogenesis.

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The bar graph represents the number of branches after action of extracts. Photographs were imported into an image software program to visualize the new vessel branch points. Data are shown as the mean \pm SD: $p < 0.05$ compared with untreated control. None of the tested concentrations (0.25 and 0.50 mg/mL) of the water extract display statistically significant differences with respect to each other.

Image analysis revealed that the degree of blood vessel formation in the presence of the extract was decreased compared with the normal vasculature (in the absence of the extract), and the avascularised area has been increased in a manner dependent on the concentration of the extract (Figure 1a). In the presence of the aqueous extract of *O. welwitschii*, the number of blood vessels is reduced to 8 to a concentration of 0.25 mg/mL to 4 to a concentration of 0.50 mg/mL, respectively densities of 33.333% and 16.666% (Figure 1a, 1b) either an antiangiogenic activity of 66.667% and 83.334%. In the presence of the aqueous extract of *T. oppositifolium*, the number of blood vessels is reduced to 6 to a concentration of 0.25 mg/mL to 4 to a concentration of 0.50 mg/mL, respectively densities of 25% and 16.66% (Figure 1b), that is to say an antiangiogenic activity of 75% and 83.334%, respectively.

Table 4: Antiangiogenic effect of *Oncoba welwitschii* and *Tetrorchidium oppositifolium*

	Dose mg/mL per eggs	Tested eggs (n)	Embryos status after 24 h	Antiangiogenic effect	Vessels numbers	Density (%)
PBS (control)		6	living	-	24	100
<i>T. oppositifolium</i>	0.500	6	living	+	4	16.666
	0.250	6	living	+	6	25.000
<i>O. welwitschii</i>	0.500	6	living	+	4	16.666
	0.250	6	living	+	8	33.333

The status of embryos after action on vascularization extracts information on toxicity (Table 4). No embryo death was recorded in the concentration range tested indicating that the observed antiangiogenic activity is not due to the toxicity of the extracts.

These extracts have antiangiogenic activity much greater than that of mannose 6-phosphate with a 38% density at 60 mg/mL and of the reference molecules, sunitinib (Sutent™). With 16.666% for aqueous extract of *T. oppositifolium* at 0.5 mg/mL against 82% for sunitinib at 60 mg/mL [33]. However a combination of four extracts called SMYGT (*Sargassumfusiforme*, *Laminaria japonica*, *OstreagigasandPrunella vulgaris*) at 0.4 mg/mL, has a 10% density, so an antiangiogenic activity than that of the aqueous extract of *Oncoba welwitschii* [21]. Recent studies show that plants with high antioxidant activity also have a strong antiangiogenic effect [20; 32].

V. CONCLUSION

This study provides the first convincing and integrated evidence that *O. welwitschii* and *T. oppositifolium* bark extracts have health-protecting effects as confirmed by their potent antioxidant effects and inhibitory activity towards angiogenesis. Specifically, the water, ethanol-water and ethanol bark extracts of *O. welwitschii* and *T. oppositifolium* were found to be strong sources of total phenolic, total flavonoids, total tannins and total proanthocyanidins showed potent antioxidant effects in terms of their reducing power and with a significant capacity to scavenge DPPH. In addition, the water extracts of *O. welwitschii* and *T. oppositifolium* displayed inhibitory activities towards CAM angiogenesis *in vivo*. The phytoconstituents identified in the water, ethanol-water and ethanol bark extracts of *O. welwitschii* and *T. oppositifolium* were found to be biologically active, thus confirming their role in preventing severe infections arising from microbial pathogenicity and oxidative stress caused by the over production of free radicals.

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Hence, we conclude that extracts of *O. welwitschii* and *T. oppositifolium* can be used as easily accessible sources of natural antioxidants for potential preventative therapies against angiogenesis related diseases.

VI. ACKNOWLEDGEMENTS

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VII. ABBREVIATION

CAM (Chick Chorioallantoic Membrane), DPPH (1,1-Diphenyl-2-picrylhydrazyl), PBS (Phosphate Buffer Saline), AAI (Antioxidant Activity Index), BHT (Butylatedhydroxytoluene), TPC (Total Phenolic Content), TFC (Total Flavonoid Content), TTC (Total Tannins Content) and TPC (Total Proanthocyanidins Content).

VIII. CONFLICT OF INTEREST

The authors declare that there are no competing interests. All the authors read and approved the final version.

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