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Phytochemistry and Antimicrobial Activity of Extracts from Medicinal Plants *Tithonia diversifolia* and *Olea africana*

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Authors' contributions

This work was carried out in collaboration between both authors. Author KD designed the study, wrote the protocol and wrote the first draft of the manuscript. Author KD managed the literature searches, analyses of the study and performed the spectroscopy analysis. Author JJ managed the experimental process. Author KD identified the species of plant. Both authors read and approved the final manuscript.

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ABSTRACT

In an increasing search of new antimicrobial agent to cope with the microbial resistance to antibiotics, scientists are searching from different sources including plants. Plants extracts of the leaves of *Tithonia diversifolia* and *Olea africana* were obtained using different solvents. *In-vitro* antibacterial activity of dichloromethane, ethyl acetate and methanol extracts of *Tithonia diversifolia* and *Olea africana* were evaluated against *Pseudomonas aeruginosa*, *staphylococcus aureus, Bacillus subtilis* and *Escherichia coli,* using micro-broth diffusion method. Zones of inhibition at different concentrations were recorded in millimeters. Growth of test pathogens (*Staphylococcus aureus)* were inhibited by dichloromethane leaf extract of *T. diversifolia* at higher concentration of 25 mg/mL (18 mm zone of inhibition), followed by inhibition of 14 mm (*Pseudomonas aeruginosa*). *E. coli* was found to be least sensitive to different plants extracts of *T. diversifolia. Pseudomonas aeruginosa* was susceptible to methanol extracts of the leaves of *O. africana* at a concentration of

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25 mg/mL (18 mm zone of inhibition) followed by *E. coli* with a zone of inhibition of 17 mm. *Staphylococcus aureus* growth was inhibited by ethyl acetate extracts of *O. africana* with a zone of inhibition of 19.20 mm. Results of the phytochemical screening revealed the presence of tannins, terpenoids, saponins, phenols and flavanoids in dichloromethane, ethyl acetate and methanol extracts of *T. diversifolia,* While alkaloids, terpenoids, tannins and flavones were screened in *O. africana* extracts. The activity of the crude extract was found to be concentration dependent on all the organisms tested. The occurrence of these phytochemicals in the selected plants of *T. diversifolia* and *O. africana* may justify their wide range usage in traditional medicine. However, other genera of Gram-positive and Gram-negative bacteria as well as fungi species should be tested in order to ascertain the broad spectrum activity of the crude extract.

Keywords: Phytochemistry; Olea africana, Tithonia diversifolia; triterpenoids; antibacterial activity.

ABBREVIATIONS

ATCC, American Type Culture Collection; BCC, Belgian Coordinated Collection Microorganisms; DCM, Dichloromethane; TLC, Thin Layer Chromatograph; WHO, World Health Organization.

1. INTRODUCTION

Medicinal plants are gifts of nature used to cure diseases among human beings. The World Health Organization [1] estimates that about 80% of the world population, settled in regions with low development and with low income makes use of Traditional Medicine as an alternative to attend to a variety of health problems. This is because pharmaceuticals currently available are very much expensive for most of the world's population [2]. When compared to pharmaceuticals, herbal medicines can be grown from seed or gathered from nature for little or no cost at all. These plants have been identified and used throughout human history because they have the ability to synthesize a wide variety of chemical compounds [3] Such compounds are used to perform important biological functions, and to defend the medicinal plants against attack from predators such as insects, fungus and herbivorous mammals [4] At least 12,000 such compounds have been isolated so far, a number estimated to be less than 10% of the total [5] Since drug resistant microorganisms have become prevalent in the current time, there is need to look for bioactive compounds from natural sources that can help in designing new and affordable antimicrobial agents. This research was aimed at extraction and determination of the antimicrobial activity of active components from the leaves of *Tithonia diversifolia* and *O. africana*.

Tithonia diversifolia (Hamsley) Gray is a plant of the family *Asteracea*. The common name of the plant is wild sunflower. The Luo call it Maua madungo. The plant grows in many tropical countries and is found along roadsides, crop fields and waste areas. The plant has been used by the Luo community of Kenya to treat gastrointestinal disorders [6]. Extracts of parts of the plant have been reported to exhibit antimalarial properties [7] anti-inflammatory [8] anti-proliferation [9], insecticidal [10] analgesic and antibacterial [11] activities. Mostly, the active ingredients of medicinal plants are commonly more concentrated in organs like roots, seeds, barks and leaves but less in flowers [12,13] *Olea africana* is a shrub or a small to medium sized tree 5-10 m in height, occasionally reaching 18 m. Bark is grey to brownish to blackish, smooth to rough when old. Leaves are narrow having an elongated form with slightly parallel sides, oval with a short point, 2-10 cm x 0.7-1.7 cm, grey-green to shiny dark green above, greyish or yellowish with a dense covering of silvery, golden or brown scales on the under surface; apex and base narrowly tapering, apex sharp tipped; margin entire, rolled under and curved back from the midrib, petiole slender, up to 1.0 cm long, so the leaves tend to drop. It is native in the ranges of China, Eritrea, Ethiopia, France, India, Italy, Kenya, Mozambique, South Africa, Spain, Swaziland, Tanzania, Uganda, and Zimbabwe among others. This does neither suggest that the species can be planted in every ecological zone within that country, nor that the species cannot be planted in other countries other than those mentioned. Since some tree species are invasive, one need to follow biosafety procedures that apply to planting site [14].

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves and twigs of *O. africana and T. diversifolia* were collected in January, 2015 from

University of Kabianga Botanical garden, Kericho County in Kenya. The plants were identified by a taxonomist, University of Kabianga, Kenya and a voucher specimen (kemboi 012 and Janet 0122 respectively) was deposited in the Herbarium, University of Kabianga, Kericho County, Kenya.

2.2 Extraction and Isolation

The plant materials were air dried at room temperature and grounded. The ground plant materials (1000 g leaves and 1000 g twigs) were sequentially extracted with organic solvents in the order of increasing polarities including; dichloromethane, ethyl acetate and methanol, using cold extraction for 24 hours in each case. The extracts were then concentrated under a reduced pressure to a minimum volume using a Rotavapor. They were then allowed to evaporate to dryness at room temperature of 25℃ to a solid mass.

2.3 Phytochemical Analysis

Qualitative tests for terpenoids, tannins, flavanoids, flavones, carotenoids and alkaloids were carried out by standard method as described by Edeoga [2]. The tests were based on the visual observations of color change or formation of a precipitate after addition of specific reagent.

2.4 Test for Flavones

Flavones were tested by adding 5 mL of ammonium solution to 1 mL of aqueous filtrate of the plant extract followed by addition of 2 mL sulphuric acid. A yellow coloration indicated the presence of flavones.

2.5 Test for Alkaloids

Alkaloids were tested for by mixing 5 g of the powder sample extract with 25 mL of 1% sulphuric acid. It was then allowed to stand and then filtered. About 10 mL of the filtrate was shaken and Meyer's reagent added. Formation of a white precipitate indicated the presence of alkaloids.

2.6 Test for Saponins

Tests for saponins was carried out according to the method described by Evans, [15]. About 2 g of the twigs and leaves powder extracts were boiled in 20 mL of distilled water on a water bath and then filtered. About 10 mL of the filtrate was then mixed with 5 mL of distilled water and shaken vigorously to give a stable persistent froth. Three drops of olive oil were then added and the mixture shaken vigorously, then observed for the formation of emulsion.

2.7 Test for Flavonoids

The test for flavonoids was carried out according to the method described by Geissman [16]. Whereby 5 mL of dilute ammonia solution was added to a portion of the aqueous filtrate of the stem and leaves powder extracts. This was followed by addition of concentrated sulphuric acid. Yellow coloration indicated the presence of flavonoids.

Twigs and leaves extracts were subjected to a test for carotenoids whereby 1 g of each extract was boiled in 20 mL water. The filtrate was then treated with concentrated sulphuric acid. Blue color indicated the presence of carotenoids.

2.8 Test for Terpenoids

Test for terpenoids was carried out by adding 1 mL chloroform to the 1 mL of extract and then an equal volume of concentrated sulphuric acid was added. Formation of the bluish red coloration indicated presence of terpenoids.

2.9 Test for Tannins

Tannins were tested by mixing 0.5 g of the sample with 20 mL of water in a test a beaker. About 2 mL of 0.1 M $FeCl_3$ was added. Formation of blue black coloration indicated presence of tannins.

2.10 Test for phenols

The procedure was carried out according to the method described by chon [17]. Equal volumes of aqueous extract and 5% $FeCl_3$ were mixed. A dark green color indicated the presence of phenolic compounds.

3. BIOLOGICAL STUDIES

Antibacterial activity of the extracts were determined by micro-broth dilution assay as described by Buwa and Staden [18]. The four preserved strains of bacteria used were *Escherichia coli ATCC 25922*, *Pseudomonas aeruginosa ATCC 87853*, *Staphylococcus aureus ATCC 25923* and *Bacillus subtilis BCCM 1735.* The bacterial strains were cultured for 18 hours at 37℃ and were standardized to a cell density of 1.5×10^8 cfu mgL^{-1} equivalent to 0.5 McFarland Standard. About 1.0 mL of

standardized bacterial strains (*Escherichia coli* ATCC 25922, *Bacillus subtilis* BCCM 1735, *Pseudomonas aeruginosa* ATCC 87853, and *Staphylococcus aureus* ATCC 25923) were seeded in petri-plates containing MH agar by streaking entire MH media surface using sterilized cell spreader under luminous flame. The mixture was then allowed to dry for five minutes. This was followed by the application of plant extract filter paper discs which were placed over the numbered divided parts on culture media. Two-fold Serial dilutions was used. Concentration was made in range of 25 to 12.5 mgL^{-1}. Plates were covered to avoid contamination and were incubated for 24 hours at 37℃. DMSO impregnated discs were used as the negative control and standard reference antibiotic streptomycin was used as a positive control. Zones of inhibitions of growth were measured in millimeters. Tests were done in triplicates and average values recorded. The minimum inhibitory concentration (MIC) was described as the lowest concentration of the test compounds that completely inhibited the growth of microorganisms.

4. RESULTS AND DISCUSSION

Phytochemical tests of the extracts revealed the presence of alkaloids, terpenoids, tannins and flavones in *T. diversifolia* and *O. africana* (Tables 1 and 2). Alkaloids were absent in all the extracts of *O.africana* while carotenoids were absent in all leaves extracts of *T. diversifolia*, this was the main difference in the phytochemical profile of the twigs and leaves in *T. diversifolia* and *O. africana*. Other researches have shown that quinolone group of alkaloids, cinchonine and cinchonidine were found to be present in the plant ethanol leaves extracts of *Olea europea* [19]. While studies on stems showed presence of tannins, alkaloids and flavones [16].

The MIC values recorded for the crude extracts suggested moderate antibacterial activity (Tables 3 and Table 4). The most active extracts was dichloromethane of *O. africana* with zone of inhibition of 19.40 mm at a concentration of 25 mg/mL against *streptococcus aureus*. *Bacillus subtilis* was susceptible to methanol extracts of *O. africana* with a zone of inhibition of 19.20 mm. *Pseudomonas aeruginosa* showed susceptibility for DCM extracts of *T. diversifolia* with a zone of inhibition of 18.20 mm at 25 mg/mL followed by ethyl acetate extracts with a zone of inhibition of 16.00 mm at same concentration. *Bacillus substilis* was least susceptible with a zone of inhibition of 16.00 mm at a concentration of 25 mg/mL against methanol extracts of *T. diversifolia.* This could be because plant-based constituents have also been reported to exhibit different modes of action against bacterial strains which range from interference with the phospholipoidal cell membranes, this has a consequence of increasing the permeability profile and loss of cellular constituents, damage of the enzymes involved in the production of cellular energy and synthesis of structural components and destruction or inactivation of genetic material [20]. The study revealed that the extracts of *T. diversifolia* and *O. africana* showed significant activity against Gram negative bacteria *Escherichia coli.* This could be due to the fact that *E. coli* is reported to be multiresistant to antibiotics [21]. They have frequently been reported to have developed multi-drug resistance to many of the antibiotics currently available in the market. It is therefore not suprising to learn that *E. coli* was the least responding bacteria strain to the tested plant extracts. Previous study using hot water extracts of *Olea europea* leaves at concentration of 62.5 mg/mL were also found to be inactive against *Staphylococcus aureus* and *E. coli* [19].

Table 1. Compounds present in stems and leaves of *Olea africana*

	Twigs extract				Leaves extract			
	Hex.	**DCM**	**EtoAc**	**Meth.**	**Hex.**	**DCM**	**EtoAc**	**Meth.**
Alkaloids	-	-	-	-	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+
Flavanoids	+	+	+	+	+	+	+	+
Flavones	+	+	+	+	+	+	+	+
Carotenoids	+	+	+	+	-	-	-	-
Phenols	+	+	+	+	+	+	+	+

Key: + denotes present, - denotes absent
Hex: Hexane, Meth: Methanol, EtoAc: Ethyl acetate, DCM; Dichloromethane

Table 2. Compounds present in stems and leaves of *Titholia diversifolia*

	Twigs extract				Leaves extract			
	Hex.	**DCM**	**EtoAc**	**Meth.**	**Hex.**	**DCM**	**EtoAc**	**Meth.**
Alkaloids	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+
Flavanoids	+	+	+	+	+	+	+	+
Flavones	+	+	+	+	+	+	+	+
Carotenoids	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+

Key: + denotes present, - denotes absent.
Hex: Hexane, Meth: Methanol, EtoAc: Ethyl acetate, DCM; Dichloromethane

Table 3. The antibacterial activity of crude extracts of *Olea africana* leaves

Extract	**Zones of inhibition (mm)**				
	Conc.	***E.c***	***P.a***	***S.a***	***B.s***
DCM	25.00	15.10	16.20	11.20	19.00
	15.00	15.40	15.40	19.40	16.40
	12.50	15.40	15.20	16.40	16.20
Ethyl acetate	25.00	16.10	16.10	10.10	17.50
	15.00	16.20	16.00	19.20	15.60
	12.50	16.30	15.20	16.20	15.10
Methanol	25.00	17.20	17.40	12.40	19.20
	15.00	17.30	17.10	18.10	16.20
	12.50	17.00	15.50	16.20	16.00

Key: E.c: Escherichia coli; P.a: Pseudomonas aeruginosa; S.a: Staphylococcus aureus; B.s: Bacillus subtilis.
Conc: concentration in mgmL^{-1}

Table 4. The antibacterial activity of crude extracts of *Tithonia diversifolia* leaves

Extract	**Zones of inhibition (mm)**				
	Conc.	***E.c***	***P.a***	***S.a***	***B.s***
DCM	25.00	12.10	18.20	15.00	14.00
	15.00	11.10	12.40	15.10	12.20
	12.50	9.40	10.20	11.20	12.20
Ethyl acetate	25.00	16.00	16.00	13.10	15.50
	15.00	11.20	10.00	8.20	12.00
	12.50	16.30	15.20	16.20	15.10
Methanol	25.00	15.20	16.40	14.40	17.20
	15.00	13.30	15.10	11.10	16.20
	12.50	13.00	12.50	10.20	16.00

Key: E.c: Escherichia coli; P.a: Pseudomonas aeruginosa; S.a: Staphylococcus aureus; B.s: Bacillus subtilis.
Conc: concentration in mgmL^{-1}

5. CONCLUSION

Olea africana and *Tithonia diversifolia* are used in Kenyan traditional medicine as a chewing sticks and for treatment of other periodontal diseases. Our study has revealed that the leaves crude extracts contains terpenoids, tannins, flavones and flavanoids while carotenoids were absent in *O. africana* but were present in *T. diversifolia*. Stem extracts tested positive for terpenoids, tannins, flavones and flavanoids while alkaloids tested negative. Growth of test pathogens were inhibited by dichloromethane leaf extract of *T. diversifolia* at higher concentration of 25 mg/mL *E. coli* was found to be least sensitive to different plants extracts of *T. diversifolia. Pseudomonas aeruginosa* was susceptible to methanol extracts of the leaves of *O. africana* at a concentration of 25 mg/mL followed by *E. coli*. The phytochemicals have been reported to be used as antibacterial, antifungal, anti-inflammatory,

anti-leishmanial and antimalarial. The plant could therefore be used in traditional medicine to treat the symptoms of inflammation and infections by bacteria and as a chewing sticks upon further investigation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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