



Antibacterial Activity of Methanolic Extracts of *Euphorbia heterophylla* and *Tithonia diversifolia* against Some Microorganisms

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Authors' contributions

This work was carried out in collaboration between both authors. Authors BAO and TAO designed the study, performed the statistical analysis and wrote the protocol. Author TAO wrote the first draft of the manuscript, managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the antibacterial activities of methanolic extracts of *Euphorbia heterophylla* and *Tithonia diversifolia* on clinical isolates.

Place and Duration of Study: The study was carried out at Afe Babalola University and the plants were collected from the campus. The isolates used were collected from Ekiti State University Teaching Hospital, Ekiti state.

Methodology: Methanolic extracts of *Euphorbia heterophylla* and *Tithonia diversifolia* leaves were obtained by cold extraction method. The activity of the extracts were tested on *Staphylococcus aureus* from skin, *Staphylococcus aureus* from infection of the respiratory tract (RTI), *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* using agar well diffusion technique on Mueller Hinton Agar.

Results: For *Euphorbia heterophylla*, the extract had greatest activity on *Salmonella typhi*. The diameter of the zone of inhibition at 100 mg/ml was 25.00 mm and 17.00 mm at 30 mg/ml. The least activities were recorded for *Streptococcus pneumoniae* and *Escherichia coli*. The extract did

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not show activity against *Staphylococcus aureus* (RTI). The Minimum lethal Concentration (MLC) was between 80 mg/ml and 100 mg/ml. For *Staphylococcus aureus* (skin), *Klebsiella pneumoniae*, *Staphylococcus aureus* (RTI) and *Pseudomonas aeruginosa*, it was 80 mg/ml. For *Streptococcus pneumoniae*, and *Escherichia coli*, it was 100 mg/ml. The methanolic extract of *Tithonia diversifolia* showed greatest activity on *Pseudomonas aeruginosa*. The diameter of zone of inhibition was 30.00 mm at 100 mg/ml and 15.00 mm at 30 mg/ml. The least activity was recorded for *Klebsiella pneumoniae*. The zone of inhibition was 20.00 mm at 100 mg/ml and 13.00 mm at 30 mg/ml. The minimum lethal concentration (MLC) for *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* were 80 mg/ml. It was 100 mg/ml for *Staphylococcus aureus* (skin) and *Staphylococcus aureus* (RTI).

Conclusion: Extracts of *Euphorbia heterophylla* and *Tithonia diversifolia* showed activities on some microorganisms including *Pseudomonas aeruginosa* and *Salmonella typhi*. This is in agreement with the use of these plants in folkore medicine in the treatment of typhoid fever, wounds and boils.

Keywords: *Euphorbia heterophylla*; *Tithonia diversifolia*; antibacterial assay; extract; minimum lethal concentration (MLC).

1. INTRODUCTION

Plants or herbs have been found to have medicinal and therapeutic importance in the prevention, palliation, treatment or cure of diseases and ailments. This knowledge has been passed down from one generation to another either verbally or in writing [1]. The universal role of plants in the treatment of diseases is exemplified by their employment in all major systems of medicine [2]. An alternative to purchase of drugs by people for the treatment of diseases is found in the use of herbal treatment. For centuries, medicinal plants have been used as remedies for human diseases because of the presence of compounds with therapeutic values [3,4].

The active phytochemicals present in some plants used by most communities in Africa for the cure of many diseases have been documented. Secondary metabolites present in plants such as peptides, tannins, alkaloids, essential oils, phenols and flavonoids have therapeutic effects against bacteria, fungi and viruses pathogenic to man [5,6].

Euphorbia heterophylla is herbaceous, erect and 20-200 cm in height (depending on growing conditions). It belongs to the family of Euphorbiaceae and have been identified as plants widely used in traditional medicine in various parts of Africa [7] and are used in the treatment of skin infections, wart, respiratory tract infection, tumors and diseases of viral origin [8,9,10,11]. The most common size is 40-60 cm tall. Milky latex is present when most parts of the plant are broken. The stem is branched and

cylindrical, with nodes at regular intervals. The surface is smooth and reddish-green [12]. The plant is used in traditional medicine as laxative, in the treatment of gonorrhea, migraine and viral warts while the latex is used as fish poison, insecticide and ordeal poison [13,14], treatment of constipation, bronchitis, asthma and as purgative [15,16]. *Euphorbia heterophylla* with the common name "spurge weed" grows in semi-humid places especially in cassava, cowpea and soya beans plantations [17].

Tithonia diversifolia (Hemls.) A. Gray is used widely in folk medicine to treat various illnesses such as skin diseases with skin products formulated with extracts of *Tithonia diversifolia* [18]. It is known as Mexican sunflower, tree marigold, "sepeleba" in Yoruba. Ethno botanical surveys have shown that this plant extracts exhibited antimalarial, anti-diarrhoeic, anti-inflammatory, antibacterial antiproliferation properties, treatment of haematomas and wounds [19,20,21,22,23]. The various parts are used in Nigeria for the treatment of malaria, diabetes mellitus, sore throat, liver and menstrual pains [24,25,26]. Dried leaves are applied externally to wounds in Costa Rica [27] while leaves infusion is used for the treatment of measles in Cameroun [28]. The identified plant constituents include tagitinins ABC and F with diversifol, tirtundin, tithonine and sulphurein [29,23,30].

The aim of this study was to determine the activities of extracts of *Euphorbia heterophylla* and *Tithonia diversifolia* on some selected clinical bacterial isolates that are causative agents of some infections.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The fresh leaves of *Euphorbia heterophylla* and *Tithonia diversifolia* plants were collected from Afe Babalola University, Ado Ekiti, Ekiti state and identified by Mr Donatus Esimekhuai of the Department of Botany, University of Ibadan, Oyo state, Nigeria. Specimens were deposited at the herbarium of University of Ibadan with specimen numbers given to the plants- *Euphorbia heterophylla*: uih 22541 and *Tithonia diversifolia*: uih 22540.

2.2 Preparation of Plant Extracts

The leaves were cut into bits and blended. The blended plant samples were weighed. Afterwards, cold maceration method was carried out on the plants.

2.3 Cold Maceration Method

Methanol extraction: Six hundred grams of each pulverized plant samples were extracted with methanol and allowed to stand for 24 hours, after which the sample was filtered. The rotary evaporator at 65°C, was used to recover the solvent from the filtrate and the extract was subsequently obtained, dried, weighed and then stored in bottles and kept in the refrigerator until needed.

2.4 Test Organisms

The bacteria used were *Staphylococcus aureus* from skin, *Staphylococcus aureus* from infection of the respiratory tract (RTI), *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. The bacteria were clinical isolates obtained from Ekiti State University Teaching Hospital, Ado Ekiti, Ekiti State. The organisms were maintained on Nutrient agar slants at 4°C in the refrigerator.

Staphylococcus aureus Gram positive cocci organism found in the human respiratory tract and on the skin and implicated in skin infections, respiratory disease, food poisoning and also resistant to antibiotics such as methicillin [31]. *Streptococcus pneumoniae* is a Gram positive α -hemolytic facultative anaerobe belonging to the genus *Streptococcus* and implicated in infections such as pneumonia, meningitis in children and

the elderly and septicemia in HIV infected persons [32]. *Escherichia coli* is a Gram negative facultative rod shaped anaerobe and a normal flora of lower intestine of warm blooded animals [33]. It is harmless but can cause food poisoning in their host [34]. *Pseudomonas aeruginosa* is a Gram negative shaped organism which can respire aerobically and anaerobically on nitrate or other alternative electron acceptor [35]. It is an opportunistic organism and found to cause infections such as cystic fibrosis, cancer or AIDS in immunocompromised patients [36].

2.5 Antimicrobial Sensitivity Test

2.5.1 Agar well diffusion technique

Prior to the microbiological testing, a loop full of each of the bacterial isolates was transferred aseptically from stock slant onto Nutrient broth in a bijoux bottle and incubated for 18 – 24 hours at 35°C. The turbidity was adjusted to McFarland turbidity standard by adding sterile distilled water. The McFarland turbidity standard was prepared by adding 0.5 ml aliquot of 0.048 mol/L BaCl₂ in 99.5 ml of 0.18 mol/L H₂SO₄ with constant stirring to maintain a suspension and the density was verified using a spectrophotometer. A sterile swab was immersed into the organism and was used to make a lawn on the already prepared Mueller Hinton Agar plate. One gram of each of the semi-solid extracts was diluted in 10 mls of extracting solvent to give a standard concentration of 100 mg/ml, from which other concentrations- 80, 50 and 30 mg/ml were obtained. Five millimeter diameter wells were cut into the agar using sterile cork borer and 0.2 ml of the different plant extracts of different concentrations were introduced into the wells. Methanol in the cut wells served as control. The plates were incubated at 37°C for 24 hours after which the plates were examined for presence or absence of zone of inhibition around the wells. The degree of sensitivity was expressed as a measure of the diameter of growth of the test organism. The antibiotic cefuroxime (100 mg/ml) was also used on the isolates.

Statistical analysis was carried out on the results obtained for the zones of inhibition using SPSS 16.

2.6 Determination of Minimum Lethal Concentration (MLC)

The minimum Lethal Concentration (MLC) is the lowest concentration of an antimicrobial

compound /agent that is able to kill or completely inhibit the growth of a microorganism. Based on the sensitivity of the plants extract, the MLC of the plant extract was determined by preparing dilutions (100, 80, 50 and 30 mg/ml) of the extracts in peptone water with the bacterial isolates. The test tubes were incubated at 35°C for 24 hrs. The broth cultures were then subcultured on Nutrient agar plates, incubated and observed for growth. Plates that had no growth for each of the isolates were identified as the minimal lethal concentration.

3. RESULTS AND DISCUSSION

The methanolic extract of leaves of *E. heterophylla* was tested on seven clinical isolates implicated in various diseases to know their activity.

Table 1 shows the antibacterial activity of methanolic extract of *Euphorbia heterophylla* against some microorganisms. The highest activity was observed against *Salmonella typhi* at all the concentrations. At 100 mg/ml, diameter of zone of inhibition was 25.00^b mm. At 80 mg/ml, 50 mg/ml and 30 mg/ml, it was 20.00^b mm, 18.00^{cd} mm and 17.00^d mm respectively, the zone of inhibition at 100 mg/ml being significantly different from that obtained from the other concentrations. Also, the zone of inhibition at 80 mg/ml was also significantly different from that obtained at 30 m/ml. The least activities were observed for *Streptococcus pneumoniae* and *Escherichia coli*. At 100 mg/ml, the diameters of zone of inhibition were 14.00^b mm each with no activity observed at 30 mg/ml. For *Streptococcus pneumoniae*, the zone of inhibition at 100 mg/ml was significantly different from the ones obtained at 80 mg/ml and 50 mg/ml. The extract did not show any activity on *Staphylococcus aureus* (RTI).

The methanolic extract of leaves of *Tithonia diversifolia* was tested against seven clinical isolates implicated in various diseases to determine its activity. Table 2 shows the antibacterial activity of methanolic extract of *Tithonia diversifolia*. The extract was active against all the test organisms at different concentrations. The highest activity was observed on *Pseudomonas aeruginosa* with diameter of zone of inhibition of 30.00^b mm at 100 mg/ml. At 80 mg/ml, it was 28.00^p mm and not significantly different while it was 22.00^c mm and 15.00^d mm at 50 mg/ml and 30 mg/ml respectively. The values obtained for these lower

concentrations were significantly different from those obtained at 80 mg/ml and 100 mg/ml. For *Streptococcus pneumoniae*, the diameters of zone of inhibition was 27.00^b mm at 100 mg/ml and 24.00^c mm at 80 mg/ml and were significantly different. The least inhibition zones were observed for *Klebsiella pneumoniae*, it was 20.00^b mm at 100 mg/ml and 13.00^c mm at 30 mg/m and significantly different. The antibiotic cefuroxime showed highest activity on all the clinical isolates at 100 mg/ml when compared to the different concentrations of the extracts. The highest activity was observed on *Pseudomonas aeruginosa* with zone of inhibition of 36.00^a mm followed by *Streptococcus pneumoniae* and *Salmonella typhi* having zone of inhibition of 32.00^a mm each.

Table 3 shows the minimum lethal concentration (MLC) of *Euphorbia heterophylla* on some isolates. The MLC for *Klebsiella pneumoniae*, *Staphylococcus aureus* (skin), *Staphylococcus aureus* (RTI) and *Pseudomonas aeruginosa* were 80 mg/ml. It was 100 mg/ml for *Escherichia coli* and *Streptococcus pneumoniae*.

Table 4 shows the minimum lethal concentration (MLC) of *Tithonia diversifolia* on some isolates. The MLC for *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* were 80 mg/ml. It was 100 mg/ml for *Staphylococcus aureus* (skin), and *Staphylococcus aureus* (RTI).

The methanolic extracts were used to determine the activity of the plants on some clinical isolates and exhibited varying degree of activity. The report of this study shows that the extract was effective against some of the organisms such as *Staphylococcus aureus* (Skin), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi* at all concentrations used (30-100 mg/ml). [14] had previously investigated antibacterial activity of the petroleum ether, butanolic and ethanolic extracts of the leaves of *E. heterophylla* against *E. coli*, *Klebsiella pneumoniae*, *S. aureus*, *P. aeruginosa* and *Bacillus subtilis*. The butanolic extract showed a broad spectrum of antibacterial activity against the test organisms at concentration of 100, 150 and 200 mg/ml. The petroleum ether and ethanolic extracts did not show any antibacterial activity against any of the test organisms. In traditional medicine, it is the aqueous extract or decoction of the leaves that is used to prepare food such as yam porridge or taken directly to "wash out the bowels" or as a purgative [37,14]. According to [38], the leaves of

E. heterophylla contain quercetin, saponins, tannins and flavonoids. Antimicrobial activity of medicinal plants has been linked with the presence of these chemical substances [39,40,41] which were also present in the plants used for this study. Studies carried out on aqueous and ethanolic extracts of *E. heterophylla* showed activity against *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* [42,43] and not on *Enterococcus faecalis* and *Pseudomonas aeruginosa* [43]. The antibacterial activity of *E. heterophylla* may be due to the presence of these chemical substances in the leaves [17].

Table 1. Antibacterial activity of methanolic extract of *Euphorbia heterophylla* against some microorganisms

Organisms	Cefuroxime	100 mg/ml	80 mg/ml	50 mg/ml	30 mg/ml
		Zone of inhibition (mm)			
<i>Staphylococcus aureus</i> (skin)	28.00 ^a	19.00 ^b	16.00 ^c	14.00 ^c	10.00 ^d
<i>Klebsiella pneumoniae</i>	24.00 ^a	16.00 ^b	14.00 ^{bc}	12.00 ^{cd}	10.00 ^d
<i>Streptococcus pneumoniae</i>	32.00 ^a	14.00 ^b	11.00 ^c	11.00 ^c	-
<i>Escherichia coli</i>	26.00 ^a	14.00 ^b	12.00 ^b	12.00 ^b	-
<i>Staphylococcus aureus</i> (RTI)	26.00 ^a	-	-	-	-
<i>Pseudomonas aeruginosa</i>	36.00 ^a	22.00 ^b	20.00 ^{bc}	18.00 ^{cd}	16.00 ^d
<i>Salmonella typhi</i>	32.00 ^a	25.00 ^b	20.00 ^c	18.00 ^{cd}	17.00 ^d

Key: a b c d= means on the same row but with different superscripts are statistically significant ($p < 0.05$)

Reading in duplicates

Diameter of cork borer 5.00 mm

RTI- Respiratory Tract infection

Table 2. Antibacterial activity of methanolic extract of *Tithonia diversifolia* against some microorganisms

Organisms	Cefuroxime	100 mg/ml	80 mg/ml	50 mg/ml	30 mg/ml
		Zone of inhibition (mm)			
<i>Staphylococcus aureus</i> (skin)	28.00 ^a	24.00 ^b	22.00 ^{bc}	20.00 ^{cd}	18.00 ^d
<i>Klebsiella pneumoniae</i>	24.00 ^a	20.00 ^b	18.00 ^b	15.00 ^c	13.00 ^c
<i>Streptococcus pneumoniae</i>	32.00 ^a	27.00 ^b	24.00 ^c	20.00 ^c	14.00 ^d
<i>Escherichia coli</i>	26.00 ^a	21.00 ^b	17.00 ^c	15.00 ^{cd}	13.00 ^d
<i>Staphylococcus aureus</i> (RTI)	26.00 ^a	22.00 ^b	20.00 ^b	16.00 ^c	13.00 ^d
<i>Pseudomonas aeruginosa</i>	36.00 ^a	30.00 ^b	28.00 ^b	22.00 ^c	15.00 ^d
<i>Salmonella typhi</i>	32.00 ^a	23.00 ^b	20.00 ^c	17.00 ^d	14.00 ^e

Key: a b c d= means on the same row but with different superscripts are statistically significant ($p < 0.05$).

Reading in duplicates

Diameter of cork borer 5.00 mm

RTI- Respiratory Tract infection

Table 3. The minimum lethal concentration (MLC) of extract of *Euphorbia heterophylla*

Organisms	100 mg/ml	80 mg/ml	50 mg/ml	30 mg/ml
<i>Staphylococcus aureus</i> (skin)	-	-	+	+
<i>Klebsiella pneumoniae</i>	-	-	+	+
<i>Streptococcus pneumoniae</i>	-	-	+	+
<i>Escherichia coli</i>	-	+	+	+
<i>Staphylococcus aureus</i> (RTI)	-	+	+	+
<i>Pseudomonas aeruginosa</i>	-	+	+	+
<i>Salmonella typhi</i>	+	+	+	+

Key: RTI- Respiratory Tract infection

+ = Minimum lethal concentration

Table 4. The minimum lethal concentration (MLC) of extract of *Tithonia diversifolia*

Organisms	100 mg/ml	80 mg/ml	50 mg/ml	30 mg/ml
<i>Staphylococcus aureus</i> (skin)	-	-	+	+
<i>Klebsiella pneumoniae</i>	-	+	+	+
<i>Streptococcus pneumoniae</i>	-	+	+	+
<i>Escherichia coli</i>	-	-	+	+
<i>Staphylococcus aureus</i> (RTI)	-	-	+	+
<i>Pseudomonas aeruginosa</i>	+	+	+	+
<i>Salmonella typhi</i>	-	-	+	+

Key: RTI- Respiratory Tract infection

+= Minimum lethal concentration

Tithonia diversifolia relieve rheumatism and the flowers used in the treatment of eye diseases [44]. Methanolic extract of *T. diversifolia* was active against *Salmonella typhi* and *Staphylococcus aureus* and showed no activity against *Escherichia coli*, *Proteus mirabilis*, *Shigella dysenteriae* and *Streptococcus pneumoniae* [45]. [46] reported antibacterial activity of extracts of leaf flower and roots of *Tithonia diversifolia* as a result of presence of methanolic toxins such as flavonoids, steroids and alkaloids. Anti-salmonella activity of *Tithonia diversifolia* was reported by [47] on the broad spectrum activity of the plant. This is in agreement with this work whereby the extract was active against *Salmonella typhi*.

4. CONCLUSION

The methanolic extracts of *Euphorbia heterophylla* and *Tithonia diversifolia* exhibited varying degree of activities against selected clinical isolates. This has also confirmed the use of the leaves of these plants in traditional medicine in Nigeria, whereby the extracts or decoction of the leaves are administered orally in the treatment of infections such as typhoid fever. The results obtained from this study are in agreement with the use of these plants in folklore medicine in the treatment of typhoid fever, wounds and boils. The extracts of different parts of plants can be used in the treatment of some infections that affect man.

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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