THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

Odeyemi, A. T et al, The *Experiment, 2014, Vol. 20(4), 1421-1426*

ANTIBACTERIAL ACTIVITIES OF CRUDE EXTRACTS OF TITHONIA DIVERSIFOLIA AGAINST COMMON ENVIRONMENTAL PATHOGENIC BACTERIA

ABSTRACT

In-vitro antibacterial activity of methanolic, ethanol and water extract of Tithonia diversifolia was evaluated against Pseudomonas aeruginosa, Shigella spp, Enterococcus spp, E. coli, and Salmonella spp using Agar well diffusion method. Minimum inhibitory concentrations (MIC) were performed on bacteria with inhibition zone greater than 14 mm by a modification of standard agar dilution method. E. coli was found to be more susceptible to the ethanol leaf extract of T. diversifolia at high concentration of 20 mg/mL (24 mm zone of inhibition) follow by Salmonella and Shigella spp both with zone of inhibition 18 mm each when compared to Enterococcus and Pseudomonad spp with zone of inhibition of (16 mm and 11 mm respectively). Enterococcus was found to be more susceptible to crude ethanol extracts of the root with zone of inhibition of 14 mm at high concentration of 20 mg/mL than the methanol and water extracts when compare to others bacteria tested in this study. The values of the MIC ranged between 1.25 mg/mL – 5 mg/mL. However, extracts of T. diversifolia flower did not show any antimicrobial effect in vitro on all the tested bacteria. The activity of the crude extract was found to be concentration dependant on all the organisms tested. Identified chemical compounds from phytochemical results indicated that saponin, alkaloid, flavonoids, phenol and triterpene were the active antibacterial compounds present. The result therefore indicated that T. diversifolia has a broad spectrum antibacterial activity and could serve as a good source of new antimicrobial agent. 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. Further research is underway to evaluate the effect of the crude extract on haematological parameters in-vivo

INTRODUCTION

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 from different genera and species were found to have antimicrobial potentials which lead to the discovery and development of new antimicrobials or drugs (Hammer et al., 1999; Sharififar et al., 2009; Ilesanmi and Olawoye, 2011). The detection of the antimicrobial properties of a plant indicates that, such plant could be a good source for the development of antimicrobial agent

From antiquity, nature has been a rich store of remedies for relief from various ailments affecting mankind. Plants, marine organisms and microorganisms produce structurally diverse compounds, which are useful as drugs, lead structures or raw materials (Adedapo et al., 2005). Plants have been used for thousands of years in traditional medicine. The earliest written records on Egyptian, Chinese, Indian, Greek and Roman traditional medicine have listed medicinal plants and prescriptions used in treating various ailments. In Africa, medicinal recipes from plants have been passed orally from generation to generation (Adedapo et al., 2005)

Presently, there is a wide range of antimicrobial drugs derived from microbial and synthetic sources available for the treatment of infectious conditions, at least for those in developed countries and the urban elites of developing countries (Adedapo et al., 2005). In resource poor communities, ignorance to good hygienic practices, poverty coupled with high cost of synthetic drugs and the circulation of drugs of questionable qualities and counterfeit pharmaceuticals combine to worsen the plight of the less privileged, forcing many to seek for the medicines of their ancestors. Herbs have been used as sources of food and medicinal purposes for centuries and this knowledge have been passed on from generation to generation (Adedapo et al., 2005). Even today, a significant proportion of the populace, particularly in the developing world depends on herbal medicines. This is particularly evident in the rural areas where infectious diseases are endemic and modern health care facilities are few and far between and where the people nurse their ailments back to health using local herbs. In Nigeria like in other parts of the African continent, practitioners of traditional system of medicine are still being consulted

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as a first choice before visiting western type health centre.

1. MATERIALS AND METHODS

2.1. PLANT MATERIALS

Fresh plant of Tithonia diversifolia was collected at different locations in Iworoko town, Ekiti State. Identification of the plant was done in the herbarium unit of the Department of Plant Science at the University of Ado-Ekiti, Ekiti State, Nigeria.

1.2. EXTRACTION PROCEDURES

The parts of various plants were dusted and air dried at room temperature and then grounded into coarse powder using electric miller (Moulinex). The pulverised plant materials was weighted and then subjected to exhaustive Soxhlet extraction with methanol, ethanol and water. Extracts were collected and concentrated under reduced pressure using rotary evaporator at 40^{o}C, then reconstituted with 20% dimethy sulphoxide (DMSO). The stock extracts were kept in the refrigerator at 4^{0}C until use.

1.3. Phytochemical Screening

The plant contains alkaloids, saponins, cardiac glycosides, tannins, fixed oils, phenolics. Leaf contain a glucoside, flowers contain, sesquiterpene lactone, quercimeritin, and anthocyanin. this founding was in conformity with the work of Obafemi et al., 2006; Ogundare, 2007.

2.3.1. Test for Alkaloids

0.5 g of the extract was diluted to 10 ml with acid alcohol, boiled and filtered. 2 ml of dilute ammonia was added to 5 ml of the filtrate, followed by the addition of 5 ml of chloroform. The mixture was shaken gently to extract the alkaloidal base, and the chloroform layer was extracted with 10 ml of acetic acid. The chloroform layer was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

2.3.2. Test for Saponin

5 ml of distilled water was added to 0.5 g of extract in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The froth was mixed with three drops of olive oil and shaken vigorously, after which it was observed for the formation of an emulsion

2.3.3. Test for Phenolic Compounds

50 mg of extract was dissolved in distilled water and to this; 3 ml of 10% lead acetate solution was added. Formation of a bulky white precipitate indicated the presence of phenolic compounds (lead acetate test).
50 mg of extract dissolved in 5 ml of distilled water and to this; 2 ml of a 1% solution of gelatin containing 10% sodium chloride was added. The appearance of white precipitates indicated the presence of phenolic compounds (gelatin test).

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2.3.4. Test for Flavonoids

Three methods were used to test for flavonoids. (i) Dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was then added. A yellow colouration that disappeared on standing indicated the presence of flavonoids. (ii) A few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicated the presence of flavonoids. (iii) A portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered, and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicated the presence of flavonoids (Aiyegroro and Okoh, 2010).

2.3.5. Test for terpenoids

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

1. SOURCE OF TEST ORGANISMS

The test organisms include Shigella spp, Escherichia coli, Salmonella specie, Pseudomonas specie and Enterococcus specie. These organisms were obtained from the stock culture of the Microbiology Department.

3.1. Standardization of microorganisms

Culture was standardized according to the methods described by Baker and Thomsberg (1983) and the National Committee for Clinical Laboratory Standards (NCCLS, 2002). 0.2 mL of an 18 hrs old culture of each bacterium was suspended into sterile universal bottles containing 20 ml nutrient broth and incubated for 5 hrs at 37°C to obtain a logarithm growth phase. Normal saline was gradually added so as to compare its turbidity to McFarland Standard of 0.5 which corresponds to approximately 1.0×10^8 CFU/mL.

1.2. Susceptibility testing of bacteria species

Susceptibility was determined using the Agar cup diffusion technique. (Adeniyi et al., 2004) A 0.1 mL aliquot of logarithmic phase broth culture of each bacterium (optical density equivalent to 10^7-10^8 CFU/mL) was used to seed sterile molten Mueller-Hinton agar (Oxoid) medium. The seeded plates were allowed to dry in the dryer for 20 min. A standard cork borer (6 mm diameter) was used to cut uniform wells on the surface of the agar, into which was added increasing concentrations of reconstituted test extract. A pre-incubation diffusion of the extracts into the seeded medium was allowed for 1 hr. Bacteria plates were incubated at 37^{o}C in an incubator for 18- 24 hrs after which diameters of zones of inhibition (mm) were measured. Since each of the extracts was reconstituted in solvents, those diluents were included in each plate as controls.

2. RESULTS

Phytochemical screening of leaves, roots, and flower extract of Tithonia diversifolia showed some active components namely; alkaloids, saponins, phenols, flavonoid, riterpenes, sesquiterpene, monoterpenes and diterpenes

The leave extracts of Tithonia diversifolia possesses active components which are, alkaloids, flavonoids, phenols sesquiterpenes, monoterpenes, and diterpenes. The roots extract of Tithonia diversifolia possesses alkaloids, flavonoids, and phenol as active components. The flower extracts of Tithonia diversifolia possesses active components of saponin, flavonoids and triterpenes.

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The result showed that the leave extracts of Tithonia diversifolia exhibited tangible activities on the test organisms especially at 20mg/ml. However, the ethanolic extracts had better antibacterial activities. The highest zone of inhibition was observed on Escherichia coli (16mm) followed by Shigella sp (12mm). Activities of the methanolic extract ranged from (1.0mm-12.0mm) at 20mg/ml. Distilled water extract exhibited poor activity against the test microorganisms in all the concentrations. Pseudomonas sp was the least susceptible. (Table 2)

The root extracts of Tithonia diversifolia had slight antibacterial activities at 20mg/ml. However, the methanolic extracts demonstrated fair antibacterial activities. The highest zone of inhibition was observed on Enterococcus specie (7.0mm), followed by E. coli (4.0mm). Ethanolic extract of the root exhibited poor activity against the test organisms. Distilled water extract also exhibited poor activities against test organisms. Salmonella specie was the least susceptible (Table 2).
Extracts of flowers of Tithonia diversifolia had no antibacterial activities on the test organisms in all the concentrations.

Test	Leaves	Flowers	Roots
Alkaloids	+	-	+
Saponins	-	+	-
Phenols	+	-	+
Flavonoids	+	+	+
Triterpenes	-	+	-
Sesquiterpenes	+	-	-
Monoterpenes	+	-	-
Diterpenes	+	-	-

Table 1: Phytochemical Screening of Extracts of Tithonia diversifolia

Minimum inhibitory concentrations (MIC) were performed by a modification of standard agar dilution method procedures as previously described (Adeniyi et al., 2009). Extracts were tested at various concentrations. The MICs were determined after 1-3 days of incubation at 37^0C under appropriate conditions suitable for bacteria growth. The MIC was regarded as the lowest concentration that showed no visible growth from a duplicate experiment

Organisms	20 mg/mL			10 mg/mL			5 mg/mL			2.5 mg/mL			DMSO
	Et	Me	Wa	Et	Me	Wa	Et	Me	Wa	Et	Me	Wa	
Leaves Extract of Tithonia diversifolia (mm)													
Escherichia coli	24.0	16.0	7.0	17.0	14.0	7.0	15.0	11.0	8.0	13.0	8.0	7.0	0.0
Shigella sp	18.0	16.0	6.0	14.0	11.0	7.0	14.0	12.0	7.0	10.0	8.0	7.0	0.0
Enterococcus sp	16.0	16.0	14.0	13.0	10.0	8.0	16.0	10.0	7.0	13.0	8.0	7.0	0.0
Salmonella sp	18.0	12.0	7.0	14.0	12.0	7.0	14.0	10.0	8.0	12.0	9.0	8.0	0.0
Pseudomonas sp	11.0	8.0	0.0	14.0	11.0	8.0	12.0	10.0	8.0	10.0	8.0	6.0	0.0
Roots Extract of Tithonia diversifolia (mm)													
Escherichia coli	12.0	8.0	0.0	10.0	8.0	0.0	14.0	12.0	0.0	10.0	7.0	0.0	0.0
Shigella sp	10.0	8.0	0.0	8.0	7.0	0.0	10.0	8.0	0.0	10.0	7.0	0.0	0.0
Enterococcus sp	14.0	11.0	8.0	12.0	9.0	7.0	13.0	10.0	1.0	14.0	8.0	0.0	0.0
Salmonella sp	10.0	7.0	0.0	9.0	7.0	0.0	12.0	9.0	7.0	7.0	7.0	0.0	0.0
Pseudomonas sp	10.0	8.0	7.0	9.0	7.0	0.0	14.0	10.0	8.0	10.0	8.0	0.0	0.0

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Flowers Extract of Tithonia diversifolia (mm)													
Escherichia coli	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Shigella sp	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Enterococcus sp	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Salmonella sp	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas sp	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Key: Size of the cork borer = 6mm Et = Ethanol, Me = Methanol Dw = Distilled Water DMSO = dimethyl sulphoxide

Table 2: Antimicrobial Activities of Extract of Tithonia diversifolia (mm)

1. DISCUSSION

The results obtained showed that, methanol, ethanol and aqueous extracts of leaves, and roots of Tithonia diversifolia possesses appreciable antimicrobial activities against some common pathogenic microorganisms such as; Escherichia coli, Shigella specie, Enterococcus sp, Salmonella sp and Pseudomonas sp. The presence of the active components in leaves extract led to the appreciable antibacterial activities on the test organisms. While flower extract did not have antibacterial effect on the test organism.

The antimicrobial activities of the crude extracts of leaf and root of Tithonia diversifolia appeared to be a broad spectrum since both Gram-negative and Gram positive bacterial were sensitive to the extracts. From the result, it shows that the ethanolic extract of the leaf appeared to be the most effective in inhibiting the growth of test organisms at the range of 12 mm to 24 mm. This was due to the fact that the leaf contain higher amount of antimicrobial active components than the other part; this is in conformity with the work of Ogunfolakan et al. (2010). Phytochemical results also confirmed the presence of the active component such as alkaloids, phenol, flavonoids, sesquiterpene, monoterperus and diterpenes in the leaf extract of Tithonia diversifolia.

The presence of these active components especially alkaloids is responsible for the fair antibacterial activities demonstrated on the test organism. Even in very small amounts, alkaloids produce strong physiological effect on the body. For instance, quinine (an alkaloid) is a specific remedy for malaria this founding was supported by the work of Oyewole and Ibidapo (2008). Phenol formerly known as carbolic acid, aromatic organic compound; is another component in the leaves extract; dilutions of which are useful as antiseptics.

Methanol roots extract appeared to be fairly effective in inhibiting the growth of the test organisms with zones of inhibition raging between (11 mm - 8 mm). This may be due to the fact that the roots extract contain some active components such as alkaloids, phenols and flavonoids but this Phytochemical components are lesser than those present in the leafs extract which makes the leaf extract more effective.

However, the inability of the flowers and the aqueous extracts from the plant to show or exhibit no inhibition effect on the test organisms could be attributed to the fact that active components present might not be polar solvent and or in the aqueous phase and might not be available for extraction by the solvent.

2. CONCLUSION/RECOMMENDATIONS

Several plants are currently being investigated to know their antimicrobial and medicinal properties. The present study reveals that leaf and root of T. diversifolia have great potentials as antimicrobial and as medicinal plants due to the presence of secondary metabolites and their ability to inhibit the growth of most of the selected organisms in vitro.

It is therefore, recommend that:
1) Further studies on the in vivo activity to include histopathology of the animal used, isolation and structural elucidation of the active component(s) and toxicological studies of the plant extracts.

2) The potentially useful phytochemical structures present in these plants could be isolated, modified and formulated and used as antibiotics, expectorant, and preservatives.
3) A need for further study to ascertain other extractive measure to improve yield of crude extracts

7. DECLARATION OF CONFLICT OF INTEREST

The authors wish to declare that there is no conflict of interest.

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