INTERNATIONAL JOURNAL OF BIOASSAYS
ISSN: 2278-778X
CODEN: IJBNHY
ORIGINAL RESEARCH ARTICLE
OPEN ACCESS

Bioassay screening of the ethanolic extract of *Tithonia diversifolia* leaves on selected microorganisms

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Received for publication: September 21, 2015; **Revised**: October 24, 2015; **Accepted**: October 27, 2015

Abstract: The study was conducted to analyze the antibacterial activity of *Tithonia diversifolia* leaves. The plant was extracted using Ethanol and water in the ratio 9:1. From the study the plant *Tithonia diversifolia* was found to inhibit the growth of *Staphilococcus epidermidis* (36.80mm), *Enterobacter aerogenes* (31.20mm), *Streptococcus a-hemolytic* (29.60mm), *Bacillus cereus* (26.2mm), *Escherichia coli* (10.40mm) and *Streptococcus γ-hemolytic* (0.000mm)The data collected and documented in this paper is a scientifically justification that the plant can be used to treat against various diseases caused by *Staphilococcus epidermidis* and *Enterobacter aerogenes*. However, further studies need to be done to identify the mode of action of the active compounds in the plant.

Key words: *Tithonia diversifolia*; Antibacterial; Medicinal herbs; Leaves; Ethanol; Aqua.

Introduction

In continuation with our interest in the study on medicinal plants (Anthoney *et al.,* 2013; 2014; Anthoney *et al.,* 2015; Obey *et al.,* 2014; Obey *et al.,* 2015), we take up on antibacterial activity of ethanolic-aqua extract of *Tithonia diversifolia* against selected microorganisms.

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). 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.

Tithonia diversifolia is significant in phyto-medicine, and it is commonly known as Mexican sunflower. It is a shrub belonging to the family Asteraceae, Order *Asterales,* genus *Tithonia*, and species *diversifolia* with binominal name as *Tithonia diversifolia* (Hemsl) A. Gray. It is asucculent (scandent) shrub 1, 2-3 meters tall, with leaves opposite or alternate. Each mature stem may bear several flowers at the top of the branches. The specific name "diversifolia" means "separated leaves" from the Latin "diversus" (divergent) and folium.

Tithonia originated from Mexico, and it is now widely distributed throughout the humid and sub-humid tropics in Central and South America, Asia and Africa (Sonke, 1997), and it is common in indigenous fallow systems in Southeast Asia (M. Cairns, personal communication). Tithonia was probably introduced into Africa as an ornamental. It has been reported in Kenya (Niang *et al.,* 1996), Malawi (Ganunga *et al.,* 1998), Nigeria (Ayeni *et al.,* 1997), Rwanda (Drechsel and Reck, 1998) and Zimbabwe (Jiri and Waddington, 1998). It is found in Kenya on road-sides, crop fields and waste areas.

The tradition of plant collection and plant-based medication has been handed down from generation to generation, usually by words of mouth among many cultures (Gurib-Fakim, 1996). *T. diversifolia* has been used in traditional medicine for the treatment of various ailments (Obafemi, 2006). The

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hot water extract of aerial parts of this plant is used for the treatment of malaria in Nigeria and other places (Calzada, 1978). An oral decoction of the leaves and stem is used to cure hepatitis in Taiwan and gastro-intestinal disorders in Kenya and Thailand (Johns, 1995) Also, the infusion of the leaves is used for the treatment of measles in Cameroon (Kamden, 1986). A decoction of the flowers is used for the treatment of skin eczema (Gurib-Fakim, 1996). Extracts of the various parts of the plant have been reported to exhibit anti-malaria (Madureira, 2002), anti-inflammatory (Rungeler, 1998), anti-proliferation (Victor, 2004) insecticidal (Mata-Greenwood, 2002) and anti-bacterial (Hongsbhanich, 1999) activities. Some researchers (Bork, 1996) reported its cancer growth inhibitory property. It has been reported also that *T. diversifolia* improves glucose metabolism by reducing insulin resistance and so may be useful for the treatment of Type II diabetes (Miura, 2005). It remains a fact that much work has been done with the extract from *T. diversifolia* and a lot of its medicinal importance has been over emphasized. In the race to meet the millennium development goals, combat increasing drug resistance, tackle emergence of new diseases and high cost of orthodox medicine, traditional medicine is successfully making a rapid scientific come back globally.

The present study was carried out to evaluate the antibacterial activity of ethanolic extract of *Tithonia diversifolia* leaves against selected pathogenic organisms.

Materials and Methods

Extraction procedures

The fresh leaves of *Tithonia diversifolia* were air-dried at room temperature and reduced to powdery form using a laboratory mortar and pestle, a modification of Victor *et al (*Miura, 2002) technique. One hundred and eighty grams (180 g) of the powdery form was extracted using ethanol and water in the ratio 9:1. The macerated mixture was carefully filtered through What man filter paper size 0.1 micrometer. The filtrate was evaporated in well regulated water bath maintained at 45^0C, and the dilutions of the extract yielded a green solid extract weighing 9.82 g. The ethanol extract was stored in a refrigerator at 4^0C, and the dilutions of the extract were made with distilled water.

Source of test organisms

The test organisms include *Bacillus cereus, Escherichia coli, Staphylococcus epidermidis, Enterobactoraerogens, Streptococcus alpha-hemolytic* and *Streptococcus gamma-hemolytic*. These organisms were obtained from the stock culture of the Medical Laboratory Department, University of Eastern Africa, Baraton.

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). About 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 × 108 CFU/mL.

Preparation of the extract concentration and antibiotic

Stock solutions of the extracts were prepared by dissolving 500mg in 1ml of dimethylsulfoxide (DMSO). A serial double dilution was prepared for each extract to obtain 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml,31.25mg/ml, 15.63mg/ml, 7.81mg/ml, 3.91mg/ml, and 1.95mg/ml respectively. An antibiotic control was made by dissolving 1µg of positive control in 1ml of sterile distilled water. DMSO served as a negative control.

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 107-108 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°C in an incubator for 18-24 hours 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.

Results and discussion

Table 1: Zone of inhibition (mm ± S.E.) of Ethanolic extract of *Tithonia diversifolia* leaves

Microorganism	Zone of Inhibition (mm ± S.E.)	Positive Control (mm ± S.E.)	DMSO control (mm ± S.E.)
Bacillus cereus	26.200 ± 0.489	31.200 ± 0.400	0.00 ± 0.000
Staphylococcus epidermidis	36.800 ± 0.489	47.400 ± 0.400	0.00 ± 0.000
Escherichia coli	10.400 ± 0.509	24.400 ± 0.510	0.00 ± 0.000
Enterobacter aerogenes	31.200 ± 0.489	36.600 ± 0.600	0.00 ± 0.000
Streptococcus a- hemolytic	29.600 ± 0.748	33.600 ± 0.400	0.00 ± 0.000
Streptococcus γ- hemolytic	0.000 ± 0.00	16.000 ± 0.707	0.00 ± 0.000

Key: S.E. = standard error; DMSO = dimethylsulfoxide

Table 2: Tukey's honestly significant differences between microorganisms treated with the Ethanolic extract of *Tithonia diversifolia* leaves and antibiotic control.

Comparison	P-value	Significance
Bacillus cereus vs S. epidermidis	0.000	S
Bacillus cereus vs Escherichia coli	0.000	S
Bacillus cereus vs Enterobacter aerogenes	0.000	S
B. cereus vs Streptococcus a- hemolytic	0.000	S
B. cereus vs Streptococcus γ- hemolytic	0.000	S
Bacillus cereus vs Bacillus cereus control	0.000	S
S. epidermidis vs Escherichia coli	0.000	S
S. epidermidis vs Enterobacter aerogenes	0.000	S
S. epidermidis vs S. a- hemolytic	0.000	S
S. epidermidis vs S. γ- hemolytic	0.000	S
S. epidermidis vs S. epidermidis control	0.000	S
E.coli vs Enterobacter aerogenes	0.000	S
E.coli vs S. a- hemolytic	0.000	S
E.coli vs S. γ- hemolytic	0.000	S
E.coli vs E.coli control	0.000	S
E. aerogenes vs S. a- hemolytic	0.000	S
E. aerogenes vs S. γ- hemolytic	0.000	S
E. aerogenes vs E. aerogenes control	0.000	S
S. a- hemolytic vs S. γ- hemolytic	0.000	S
S. a- hemolytic vs S. a- hemolytic control	0.000	S
S. γ- hemolytic vs S. γ- hemolytic control	0.000	S

Key: **NS** = Not Significant; **S**= Significant

The ethanolic extract of *Tithonia diversifolia* leaf extract showed the highest average zone of inhibition against *Staphilococcus epidermidis* (36.80mm) followed by *Enterobacter aerogenes* (31.20mm), *Streptococcus a-hemolytic* (29.60mm), *Bacillus cereus*(26.2mm), *Escherichia coli* (10.40 mm) and *Streptococcus γ-hemolytic* had no effect on the zone of inhibition (Table 1). A zone of inhibition greater than 8mm was considered as active. A one-way analysis of variance (ANOVA) showed that there was a significant difference in the zone of among the positive control and the extracts for some of the microorganisms($P<0.001$).

The study shows that the *Tithonia diversifolia* leaves can inhibit the growth of all the microorganisms tested against except *Streptococcus γ-hemolytic.* On further comparison with the Tukey's pair wise comparison test, it was shown that the zones of inhibition the positive control was significantly higher than the extract concentrations ($P<0.001$). Zones of inhibition ranging from 25 to 30mm is considered strongly active, 10 to 20 moderately active and below 8mm low active, when the crude extractions are examined for antibacterial activity. All the microorganisms show significance except *Streptococcus γ-hemolytic.*

Turkey's test shown that the zone of inhibitions for *Staphylococcus epidermidis* was significantly bigger than all the organisms tested against ($P < 0.05$) but significantly smaller that its positive control (table 2). The zone of inhibitions *E. aerogenes* was significantly bigger than the organisms tested except S. *epidermidis* and its positive control ($P < 0.05$). The zone of inhibitions of *Streptococcus a-hemolytic*was significantly bigger than those of *B. cereus* and *E. coli* but significantly smaller than those of *S. epidermidis, E. aerogenes* and its control.

The zone of inhibitions of *B. cereus* was significantly bigger than *E. coli* but was significantly smaller than those of *Staphylococcus epidermidis, Enterobacter aerogenes, Streptococcus a- hemolytic* and its control. The zone of inhibitions of *E. coli* was significantly smaller than those of all the other organisms tested and its control. However, there was no zone of inhibitions produced by negative control DMSO against any of the organisms.

Tithonia diversifolia soap exhibited the highest activity against the microorganism *E. coli (*Karerue*t al.,* 2010). *T. diversifolia* leaf extract has shown antifungal and antibacterial activities Linthoingambi (2013). According to Ogunfolakan (2010), *Tithonia diversifolia* leaf extract has a great antimicrobial effect on bacterial isolates used with MZD > 10 mm implying that each of the extract can be used as a broad spectrum antibacterial agent.

According to Linthoingambi (2013), *Tithonia diversifolia* leaves were tested against nine plant pathogenic fungal species *(Alternaria alternata, Alternaria solani, Aspergillus flavus, Aspergillus niger, Cuvularia lunata, Drechslera oryzae, Fusarium oxysporum, Penicillium expansum* and *Penicilliumitalicum),* one antagonist fungus *(Trichoderma viride)* and four human pathogenic bacteria *(Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus).* Among the extracts, petroleum extract showed the highest antifungal activity followed by methanol and chloroform extracts. All the three extracts showed inhibitory effect against all the tested bacteria.

Conclusion

From the data provided it was clear that *Tithonia diversifolia* leaves has a great potential in limiting the spread of *Staphylococcus epidermidis* and *Enterobacter aerogenes* and also to some extent to other microorganisms in which the plant was tested against. Several plants are currently being investigated to know their antimicrobial and medicinal properties. The present study reveals that leaf 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. Further studies need to be done to identify the mode of action of the active compounds in the plant.

Acknowledgement

The authors of this paper are very much thankful to the Department of Chemistry and Laboratory Sciences, University of Eastern Africa, Baraton.

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Cite this article as:
Anthoney Swamy T, Jackie Obey K, Miyogo Edwin and Lasiti T. Timothy. Bioassay screening of the Ethanolic extract of *Tithonia diversifolia* leaves on selected microorganisms. *International Journal of Bioassays* 5.2 (2016): 4794-4798.

Source of support: Nil
Conflict of interest: None Declared