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IN VITRO ANTIBACTERIAL EVALUATION OF ETHANOLIC EXTRACT OF *THUNBERGIA ALATA* LEAVES EXTRACT AGAINST SELECTED MICROORGANISMS

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Abstract: The aim of this study was to analyse the antibacterial activity of ethanolic extract of *Thunbergia alata* leaves against laboratory strains of selected microorganisms. The ethanolic extract of *Thunbergia alata* leaves are being used in the treatment of aphrodisiac, skin problems, back and joint pains, eye inflammation, piles and rectal cancer. The mean zones of inhibition of the extract against microorganisms were 29.000 ± 0.577 mm for *Staphylococcus epidermidis,* 21.750 ± 0.629 mm for *Enterobacter aerogenes,* 19.000 ± 0.577 mm for *Bacillus cereus and* 14.250 ± 0.478 mm for *Streptococcus a- hemolytic.* The microorganism such as *E. coli, Serratia marcescens and Streptococcus -γ-hemolytic* did not show any zone of inhibitions. The penicillin positive control showed large zones of inhibition except against *Serratia marcescens* and the dimethyl sulfoxide negative control did not show any zone of inhibition. This report suggests that the extract was active against all the other organisms except *E. coli, Serratia marcescens* and *S-γ-hemolytic.* Analysis of variance showed that the zones of inhibition of the extract and antibiotic control against the microorganisms were significantly different ($p < 0.0001$). The Tukey's honestly test further showed both significant and non-significant comparisons between the extract and controls for various bacterial organisms. This study has shown that controlling the growth of microorganisms *in vitro* can be achieved by the ethanolic extract of *Thunbergia alata.*

Key words: Thunbergia alata; Leaves; Antibacterial; Ethanol; Extract

INTRODUCTION

In continuation of our interest in biological activities of natural medicinal plants extracts (Anthoney *et al.,* 2013; Anthoney *et al.,* 2014; Anthoney *et al.,* 2015 and Obey *et al.,* 2014), we report here the antibacterial activities of ethanolic extract of *Thunbergia alata* leaves are being used in the treatment of aphrodisiac, skin problems, back and joint pains, eye inflammation, piles and rectal cancer.

The plant *Thunbergia alata* is native to tropical areas of East Africa; black-eyed Susan vine is a tropical evergreen twining vine that eventually climbs to 20' in frost free areas. Blooms in summer to fall, but best bloom is often in late summer and fall after the hot summer temperatures moderate. A number of different cultivars are available with white, creamy white, light yellow and sulphur yellow flowers (missouribotanicalgarden, a552).

T. alata is an herbaceous vine, often cultivated as an ornamental, which has escaped and naturalized mostly in disturbed areas in tropical, subtropical and warmer temperate regions of the world (Cabi, 2014 ; Starr *et al.*, 2003; Meyer and Lavergne, 2004; Queensland Department of Primary Industries and Fisheries, 2011). It is a fast-growing vine with the capability of reproducing sexually by seeds and vegetatively by cuttings, fragments of stems and roots (Starr *et al.*, 2003; Vibrans, 2009). Once established, it completely smothers native vegetation by killing host-trees, out-competing understory plants, and negatively affecting the germination and establishment of seedlings of native species (Starr *et al.*, 2003; Meyer and Lavergne, 2004). *T. alata* is included in the Global Compendium of Weeds (Randall, 2012) and it is also considered an aggressive invasive plant in Australia, Japan, Singapore, Costa Rica, Cuba, Puerto Rico, Brazil, Colombia, Paraguay, and numerous islands in the Pacific including Hawaii and French Polynesia.

Leaves of *T. alata* herb are crushed and added to water. The liquid is given as a drink to children who have pains in the mouth and tongue. Leaves and buds are pounded and mixed with ghee, then used as an embrocation for the treatment of backache, hydrocele, and pain in joints. Whole plant decoction drunk by expectant mothers for treatment of the placenta and hardening the fœtus head (Kokwaro, 1993).

Thunbergia alata is also used in traditional medicine. The country like India, the fresh root extract of *A. alata* is used as a health tonic and an aphrodisiac (Kar *et al.*, 2013; Prota, 2014). And in East Africa, *it* is used as a vegetable or animal feed. It is mainly used for skin problems, back and joint pains, eye inflammation, piles and rectal cancer. Gall sickness and some ear problems in cattle are also treated with this plant. Fruits and seeds are used in souvenirs (Smithies, 2007).

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

MATERIALS AND METHODS

Sample collection and Extraction procedure

The leaves of the *Thunbergia alata* were collected around Baraton University campus. The samples were identified by a taxonomist in the University of Eastern Africa, Baraton. The fresh leaves of the *Thunbergia alata* leaves were air – dried for three weeks; the dried leaves were ground into powder. Forty grams (40 g) of the powdered leaves were mixed with 400 ml of ethanol – water (70:30).

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The mixture was kept for 24 hours on a shaker for effective extraction of the plant components. The extract was filtered and the solvent was evaporated to dryness at a temperature of 40°C using rotary vacuum evaporator. The extract was brought to dryness using vacuum and pressure pump. The yield was kept at 4°C prior to use.

Preparation of the Bacterial Suspension

The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard. The McFarland standard was prepared by dissolving 0.5 g of $BaCl_2$ in 50 ml of water to obtain a 1% solution of Barium chloride (w/v). Sulphuric acid (1%) was prepared in a 100-ml volumetric flask. To prepare the 0.5 McFarland Standard, 0.5 ml of the 1% $BaCl_2$ solution was mixed with 99.5 ml of H_2SO_4 solution. Measure the turbidity of the 0.5 McFarland Standards with the aid of a spectrophotometer at a wavelength of 625 nm to read an optical density of between 0.08 to 1.0. At this absorbance, the McFarland Standard represents a bacterial cell density of approximately 1.5 x 10^8 CFU/ml (1.0 x10^8 – 2.0 x 10^8 CFU/ml. It was then transferred to a screw-capped bottle and sealed with parafilm to prevent evaporation due to exposure to air. The bacterial suspensions were then tested against the McFarland standards until they reached the absorbance of the McFarland standard and then they were ready for use.

Preparation of Extract Concentrations and Antibiotics

Stock solutions for the extract were prepared by dissolving 500 mg in 1 ml of dimethyl sulfoxide (DMSO). An antibiotic control was made by dissolving 1µg of penicillin in 1 ml of sterile distilled water. DMSO served as a negative control.

Screening for the antibacterial potential of the plant extract

The agar well diffusion procedure used in the experiment was similar to that used by Taye *et al*., (2011) and Jeyachandran and Mahesh (2007). The microorganisms used for this study were laboratory strains of *Bacillus cereus, Staphylococcus epidermidis, Escherichia coli, Enterobacter aerogenes, Serratia marcescens, Streptococcus a- hemolytic* and *Streptococcus γ-hemolytic*. A single colony for each of the organisms was picked from agar plate and dissolved in 5 ml of Mueller Hinton broth. The broth was incubated overnight at 37°C. 5 ml of plain Mueller Hinton broth was incubated alongside the organisms to ensure that the medium was not contaminated. The spectrophotometer was set to 625 nm wavelength and each of the microbial cultures was pipetted into cuvettes to measure the absorbance. A cuvette of plain Mueller Hinton broth was used a blank at 0.000 absorbance. The absorbance of the microorganisms were measured. The bacterial organisms exceeding 0.1absorbance were adjusted by adding bacterial suspension until the absorbance fell between 0.08 to 0.10, matching the McFarland Standard. The organisms falling below 0.08 absorbance were also adjusted until the McFarland standard absorbance was achieved. All the organisms, therefore, reached a cell density of 1 x 10^8 cfu/ml (Ngeny *et al.,* 2013). 100 µl of each of the organisms were then inoculated onto agar plates for the bioassay (Agyare *et. al.,* 2013). Three 6 mm wells were made into each agar plate using a sterile metal cork borer. 100 µl of the standard drug penicillin was placed in one well, the extract in another well and dimethyl sulfoxide (DMSO) was placed in the third well on each plate. The experiment was run in triplicate for each extract and each organism tested. The plates were incubated for 24 to 48 hours and the zones of inhibition were measured in millimetres with the aid of a meter rule.

Statistical Analysis

A random sampling procedure was done for the entire test and the experiment was conducted in triplicate assays on Mueller Hinton agar plates (Jeyachandran and Mahesh, 2007). The mean values and standard error were calculated for the zones of inhibition. Analysis of variance was used to determine if there was significant difference among the average zones of inhibition of the bacterial organisms by the extract and controls. The Tukey's honestly significant difference test was used to determine pairwise comparisons between average zones of inhibition among the bacterial organisms by SPSS version 21.0.

RESULTS AND DISCUSSION

The ethanolic extract of *Thunbergia alata* leaves was active against *B. cereus, S. epidermidis, E. aerogene* and *Streptococcus a- hemolytic* (Table 1). *The zone of inhibitions were* 19.000 ± 0.577 for *B. cereus*, 29.000 ± 0.577 for *S. epidermidis*, 21.750 ± 0.629 for *E. aerogenes* and 14.250 ± 0.478 for *Streptococcus a- hemolytic and* other organisms used had zone of inhibition 0.000 ± 0.000. However, the entire microorganism used had zone of inhibition significantly smaller than the positive control penicillin. But negative control DMSO did not show any zone of inhibition. ANOVA results showed that all the organism were significantly different from each other and their control ($P < 0.001$).

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

Microorganism	Zone of Inhibition (mm ± S.E.)	Penicillin Control	DMSO control
Bacillus cereus	19.000 ± 0.577	32.000 ± 0.000	0.00 ± 0.000
Staphylococcus epidermidis	29.000 ± 0.577	48.750 ± 0.478	0.00 ± 0.000
Escherichia coli	0.000 ± 0.000	24.000 ± 0408	0.00 ± 0.000
Enterobacter aerogene	21.750 ± 0.629	39.000 ± 0.913	0.00 ± 0.000
Serratia marcescens	0.000 ± 0.000	0.000 ± 0.000	0.00 ± 0.000
Streptococcus a- hemolytic	14.250 ± 0.478	32.250 ± 0.250	0.00 ± 0.000
Streptococcus γ- hemolytic	0.000 ± 0.000	14.750 ± 0.478	0.00 ± 0.000

Key: S.E. = Standard error; DMSO = Dimethyl sulfoxide

Tukey's multiple comparison test (Table 2) showed that the zone of inhibition of *B. cereus* is significantly bigger than those of *E. coli, S. marcescens, S- γ -hemolytic* and *S- a -hemolytic* and was significantly smaller than those of microorganism such as *S-epidermidis, E-aerogenes*, and its antibiotic control ($P < 0.05$).

The zones of inhibition of *S. epidermidis* were bigger than all the other microorganism tested and however, significantly smaller than its positive control. The zone of inhibitions of *E.coli* was smaller than *S. epidermidis, E. aerogenes, B. cereus, S-a-hemolytic* and its control but was not significantly differ from *S. marcescens* and *S-a-hemolytic.*

The zones of inhibitions of *E-aerogenes* was smaller than *S. epidermidis* and its control but significantly larger than those of microorganisms such as *E.coli, S. marcescens, S-γ-hemolytic, S-a-hemolytic* and *B. cereus*. The zones of *S. marcescens* were significantly smaller than *S. epidermidis, E. aerogenes, Bacillus cereus* and *S-a-hemolytic*. The zones of inhibition of *S. marcescens* was not significantly different from *E. coli, S-γ-hemolytic* and its control.

Table 2: Tukey's honestly significant differences between microorganisms treated with the Ethanolic extract of *Thunbergia alata* 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	0.006	S
Bacillus cereus vs Serratia marcescens	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 Serratia marcescens	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 Serratia marcescens	1.000	NS
E.coli vs S. a- hemolytic	0.000	S
E.coli vs S. γ- hemolytic	1.000	NS
E.coli vs E.coli control	0.000	S
E. aerogenes vs Serratia marcescens	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
Serratia marcescens vs S. a- hemolytic	0.000	S
Serratia marcescens vs S. γ- hemolytic	1.000	NS
Serratia marcescens vs Serratia marcescens control	1.000	NS
S. a- hemolytic vs S. γ- hemolytic	0.000	S
S. a- hemolytic vs S. a- hemolytic contol	0.000	S
S. γ- hemolytic vs S. γ- hemolytic control	0.000	S

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

The zones of inhibition of *S-a-hemolytic* was significantly larger than *S-γ-hemolytic, E. coli* and smaller than other microorganism used and its control. And the zones of inhibition of *S-γ-hemolytic* was significantly smaller than *S. epidermidis, E. aerogenes, B. cereus, S-γ-hemolytic* and its control but was not significantly differ from *E.coli* and *S. marcescens.*

According to Henry (2001), Strains of methicillin-resistant *Staphylococcus aureus* (MRSA), which had been largely confined to hospitals and long-term care facilities, are emerging in the community. The changing epidemiology of MRSA bears striking similarity to the emergence of penicillinase-mediated resistance in *S. aureus* decades ago. Even though the origin (hospital or the community) of the emerging MRSA strains is not known, the prevalence of these strains in the community seems likely to increase substantially.

According to Sanford (1994), Humans are a natural reservoir for *S. aureus*, and asymptomatic colonization is far more common than infection. Colonization of the nasopharynx, perineum, or skin, particularly if the cutaneous barrier has been disrupted or damaged, may occur shortly after birth and may recur anytime thereafter (Payne *et al*., 1966). Family members of a colonized infant may also become colonized. Transmission occurs by direct contact to a colonized carrier. Carriage rates are 25% to 50%; higher rates than in the general population are observed in injection drug users, persons with insulin-dependent diabetes, patients with dermatologic conditions, patients with long-term indwelling intravascular catheters, and health-care workers (Wadlvogel, 2000). Young children tend to have higher colonization rates, probably because of their frequent contact with respiratory secretions (Ross *et al.,* 1974 and Adcock *et al.,* 1998). Colonization may be transient or persistent and can last for years (Sanford *et al.,* 1994 and Henry, 2001).

Enterohaemorrhagic E. coli (EHEC) causes bloody diarrhoea (haemorrhagic colitis), non-bloody diarrhea and haemolytic uremic syndrome (HUS). The principal reservoir of EHEC is the bovine intestinal tract and initial outbreaks were associated with consumption of undercooked hamburgers. Subsequently, a wide variety of food items have been associated with disease, including sausages, unpasteurized milk, lettuce, cantaloupe melon, apple juice and radish sprouts (James et al., 2004).

Enterotoxigenic E. coli (ETEC) causes watery diarrhea, which can range from mild, self-limiting disease to severe purging disease. The organism is an important cause of childhood diarrhoea in the developing world and is the main cause of diarrhoea in travellers to developing countries (Nataro, 1998).

Enteroaggregative E. coli (EAEC), are increasingly recognized as a cause of often persistent diarrhea in children and adults in both developing and developed countries, and have been identified as the cause of several outbreaks worldwide (James *et al.,* 2004).

The evolution of pathogenic *E. coli* that has resulted in formation of distinct path types capable of colonizing the gastrointestinal tract, urinary tract or meninges illustrates how key genetic elements can adapt a strain to distinct host environments. Using *E. coli* K-12 as a base-model', several features can be added (PAIs, plasmids, transposons or phage) or subtracted (black holes or pseudogenes) to modify the base model to adapt to specific environments and to enable these modified strains to cause disease in an immunocompetent human or animal host (James *et al.,* 2004).

B. cereus in association with food poisoning and eye infection, recognition and appreciation for the multitude of other serious infections such as fulminant sepsis and devastating central nervous system infections are lacking. The suspicion of the association of *B. cereus* with these mounting infectious complications moves with a fatal lethargy in its recognition as a bona fide human pathogen. Clinicians and clinical microbiologists must both give serious consideration to the significance of a *B. cereus* isolate from a clinical specimen, especially if the patient is immunosuppressed (Edward, 2010).

Enterobacter aerogenes is a Gram – negative, catalase positive, indole negative, rod shaped bacterium (Sanders, 1997). *E. aerogenes* is a nosocomial and pathogenic bacterium that causes opportunistic infections including most types of infections. The majority are sensitive to most antibiotics designed for this bacteria class, but this is complicated by their inducible resistance mechanisms, particularly lactamase which means that they quickly become resistant to standard antibiotics during treatment, requiring change in antibiotic to avoid worsening of the sepsis.

According to Gabriel, some of the infections caused by *E. aerogenes* result from specific antibiotic treatments, venous catheter insertions, and /or surgical procedures. *E. aerogenes* is generally found in the human gastrointestinal tract and does not generally cause disease in healthy individuals. It has been found to live in various wastes, hygienic chemicals, and soil.

Enterobacter aerogenes causes disease in humans through inadvertent bacteria transfer in hospital settings. A selection of enteric bacteria like *E. aerogenes* are opportunistic and only infect those who already have suppressed host immunity defences. Infants, the elderly, and those who are in the terminal stages of other disease or are immunosuppressed are prime candidates for such infections (Janda, 2006).

The genus *Enterobacter* is more specifically a nosocomial opportunistic pathogen and is sought out to be one of the many key causes for extra intestinal infections next to *E. coli*. Infections commonly attributed to *E. aerogenes* are respiratory, gastrointestinal, and urinary tract infections, specifically cystitis, in addition to wound, bloodstream, and central nervous system infections (Brooks, 2007; Lederberg, 2000 and Sankaran, 2000). Furthermore, *E. cloacea* and *E. aerogenes* are the species most commonly associated with adult cases of meningitis. Colonies of *Enterobacter* strains may be slightly mucoid.

In the clinical setting, *Enterobacter aerogenes* and *Enterobacter cloacae* are the most frequently isolated in samples of infected hospitalized patients. The majority of the infections are etiologically due to inadvertent transfer of bacteria during surgery or prolonged treatment in hospitals in patients who use venous or urethral catheters. Enterobacteriaceae may account for 80% of clinically significant isolates of gram-negative bacilli and for 50% of clinically significant bacteria in clinical microbiology laboratories. Additionally, they account for nearly 50% of septicaemia cases and more than 70% of urinary and intestinal tract infections. The severity of these infections thus create an importance to target, isolate, identify and test for susceptibility for the causes of these nosocomial infections (Sankaran, 2000).

From the data obtained in this study it is therefore worthy to mention that the plant can be used to treat against all the infections caused by *S. epidermidis, E. aerogenes, B. cereus* and *S-γ-hemolytic*. These results are pertinent in addressing problems like drug resistance in treating diseases caused by the selected microorganisms using conventional antibiotics.

CONCLUSION

The ethanolic extract of *Thunbergia alata* leaves has shown antimicrobial activity against four of the seven organisms tested. These results are pertinent in addressing problems like drug resistance in treating diseases caused by the selected microorganisms using conventional antibiotics. Further analysis of the extract needs to be carried out using spectroscopic techniques for structural elucidation of the active ingredients found in the extract. The extract can also be tested against other bacterial and fungal organisms to determine if better results can be obtained for other organisms.

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