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IN VITRO ANTIBACTERIAL ACTIVITY OF ETHANOLIC – AQUA EXTRACT OF TEPHROSIA VOGELII BARK AGAINST LABORATORY STRAINS OF SELECTED MICROORGANISMS

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Abstract

The study was conducted to analyze the antibacterial activity of the bark of ***Tephrosia vogelii***. The plant sample was extracted using ethanol and water in the ratio 7:3. Antibacterial activity was done using the agar well diffusion methods and the data was analysed using SPSS software to compare the means and also check whether there was significant difference between the zones of inhibitions caused by the plant extract and also those caused by the positive control. A study was carried out to investigate the bioactivity of the plant by using microorganism such as *Bacillus cereus, Escherichia coli, Salmonella typhi, Streptococcus pyogenes, Serratia marcescens, Serratia liquefaciens, Enterobacter aerogenes* and *Staphylococcus epidermidis*. From the study the plant *Tephrosia vogelii* was found to inhibit the growth of *B.cereus* with a zone of inhibition of 15.667 ± 0.333, *S. pyogenes* zone 16.666 ± 0.333 and *S. epidermidis* 17.667 ± 0.333. The bacteria which had a zone of inhibition of less than 8 mm were termed to be unsusceptible. The data collected and documented in this paper is a scientific justification that ethanol-water extract of bark of *T. vogelii* exhibits notable inhibitory activity against pathogenic organisms such as *B. cereus, S. pyogenes* and *S. epidermidis.*

Key words: *Tephrosia vogelii,* Antibacterial, Medicinal herbs, Bark, Ethanol - aqua.

Introduction

Research on medicinal plants is of great importance taking into account the old and new problems emerging day by day. Most information about natural healing has been passed on from generation to generation. With growing knowledge on technology and civilization this information transfer is no longer taken seriously in the society, hence, endangering the knowledge on traditional methods of treatment with one of them being the use of medicinal plants. This calls for a great need to have the knowledge on medicinal plants reserved and kept for future reference (Prajapati & Purohit, 2003).

In continuation with our interest in the study on medicinal plants (Anthoney, Ngule, & Obey, 2013, 2014; Obey & Anthoney, 2014), we take up on *Tephrosia vogelii. Tephrosia vogelii*, native to Africa, a tall, shrubby plant. Plants reach a height of 2 to 3 meters and usually behave in their native habitats as short-lived perennials. They become rather woody and shrub- like in appearance. With ample space, considerable branching occurs, but plants tend to be taller and less branched in dense stands. The conspicuous flowers are about 2.5 cm. across and may be purple, red, or white. There may be as many as 20 to 30 flowers on each compact raceme and up to 200 flowers per plant. The plants bloom for 3 to 6 weeks (Gaskins, White, Martin, Delfel, Elfel, & Barnes, 1972).

Tephrosia vogelii is a known nitrogen-fixing species, cultivated as green manure in Indonesia and many other parts of Africa. It is also planted as a windbreak and as a temporary shade crop. Crude extract from leaves of *Tephrosia vogelii* is potentially used to control ticks and worms in the Ugandan animal production systems. It has also been used to control larval stages of mosquitoes and is effective against soft bodied insects and mites including aphids and red spider mites. Dried leaves have the potential to protect stored legume seeds from damage by the bruchids as used by farmers in Southern Africa. For control of weevils (less effective) and grain borer, mix 100-250 g powdered dry leaves to 100 kg of beans or cowpea. Before eating beans, wash thoroughly. *Tephrosia vogelii* plant extracts were once used as fish poison but now many countries term this as illegal. To make the insecticide, mix dry powdered leaves 10% w/v in water containing 1% liquid soap for 24 hours.

Dilute 5-10 times to give 1-2% concentration and spray in the early evening to reduce exposure (Mwaura, Stevenson, Ofori, Anjarwalla, Jamnadass, & Smith, n.d.).

T. vogelii's well known properties are ichthyotoxic, insecticidal, food parasiticidal, antifeedant, antibacterial and pest crops control (Ibrahim, M'batchi, Mounzeo, Bourobou, & Passo 2000; Wanga, Akenga, Imbuga, Gitonga, Olubayo, & Namungu, 2000). *Tephrosia vogelii* is used on a small scale as a fish poison, insecticide, shade for coffee seedlings, and for other purposes. *T. vogelii* is a potential source of rotenone and related rotenoids for insecticidal and piscicidal uses. The United States annually imports more than 1,600 tons of Derris and Lonchocarpus roots, the main sources of rotenone, for processing. *Tephrosia vogelii* is a promising alternative source with adaptability in the Southeastern U.S and Puerto Rico. Most of the rotenoids in *Tephrosia vogelii* are concentrated in the leaves (Gaskins et al., 1972).

Medicinal plants have been used since ancient times to treat many illnesses which affect humankind. Herbal medicine is still a matter of argument in the current world with many still doubting its efficiency. This has been due to greedy practitioners who want to become wealthy by pretending to know much about the diseases which their clients claim to have, hence, leading to the application of wrong treatment and administration of wrong drugs which do not cure the patient and therefore leading to the worsening of the situation or even death of the victim (Kokwaro, 2009).

Studies on several plants have been done all over the world and plants have shown great potential in the treatment of diseases affecting both humans and animals. Study reports on Potentilla fulgens have shown the plant to have anti-hyperglycemic, hypoglycemic, anti-hyperlipidemic, antitumor, antioxidant, anti-inflammatory and anti-ulcerogenic properties (Koul, Jaitak, & Kaul 2011). The use of medicinal plants is as old as man (Anthoney et al., 2013). In the past few decades medicinal plants have been tested extensively and found to have several pharmacological uses such as, antibacterial activity, antifungal activity, anti-diabetic activity, anticancer activity, antioxidant activity, hepatoprotective activity, haemolytic activity, anti-inflammatory activity, larvicidal activity, anthelmintic activity, central nervous system activity and pain relief activity (Mir, Sawhney, & Jassal, 2013; Sukirtha & Growther, 2012; Anthoney et al., 2013).

This study was carried out to investigate the antibacterial activity of ethanolic – aqua extract of bark of *Tephrosia vogelii* against selected microorganisms.

Material and Methods

Sample Collection and Extraction Procedure

The bark of the *Tephrosia vogelii* was collected around the University of Eastern Africa, Baraton. The samples were identified by a taxonomist at the University of Eastern Africa, Baraton. The fresh bark of the *Tephrosia vogelii* was air – dried for three weeks; the dried barks were ground into powder. Forty grams (40 g) of the powdered bark were mixed with 400 ml of ethanol – water (70:30). 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 40oC using rotary vacuum evaporator. The extract was brought to dryness using vacuum and pressure pump. The yield was kept at 4oC prior to use.

Bioassay Study

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 BaCl2 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% BaCl2 solution was mixed with 99.5 ml of H2SO4 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 108 CFU/ml (1.0 x 108 – 2.0 x 108 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 the extract concentrations and antibiotic. Stock solutions for the extract were prepared by dissolving 500 mg in 1 ml of dimethylsulfoxide (DMSO). An antibiotic control (positive control) was prepared by dissolving 1mg of penicil-

lin 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 was used in the experiment (Taye, Giday, Animut, & Seid, 2011; Jeyachandran & Mahesh, 2007). The microorganisms used for this study were laboratory strains of *Bacillus cereus, Escherichia coli, Salmonella typhi, Streptococcus pyogenes, Serratia marcescens, Serratia liquefaciens, Enterobacter aerogenes* and *Staphylococcus epidermidis*. A single colony for each of the organisms was picked from Mueller Hinton agar plate and dissolved in 5 ml of Mueller Hinton broth. The broth was incubated overnight at 370C. Five millilitres (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 cuvette to measure the absorbance. A cuvette of plain Mueller Hinton broth was used a blank at 0.000 absorbance. The absorbance of the microorganisms was measured. The bacterial organisms exceeding 0.1 absorbance were adjusted by adding bacterial suspension until the absorbance fell between 0.08 to 0.10, matching the 0.5 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 108 cfu/ml (Ngeny, Magiri, Mutai, Mwikwabe, & Bii, 2013). One hundred (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. One hundred micro litres (100 µl) of the standard drug penicillin was placed in one well, the extract in another well and dimethylsulfoxide (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 & 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 pair-wise comparisons between average zones of inhibition among the bacterial organisms by SPSS version 21.0.

Result and Discussion

Ethanolic extract of *T. vogelii* was active against *B. cereus, S. pyogenes and S. epidermidis* (Table 1).

Table 1

Zone of Inhibition (mm ± S.E.) of Ethanolic – Aqua Extract of Tephrosia Vogelii Bark

Microorganism	Zone of Inhibition (mm ± S.E.)	Penicillin Control	DMSO control
Bacillus cereus	15.667 ± 0.333	30.000 ± 0.000	0.00 ± 0.000
Escherichia coli	0.000 ± 0.000	28.333 ± 0.333	0.00 ± 0.000
Salmonella typhi	0.000 ± 0.000	29.333 ± 0.667	0.00 ± 0.000
Streptococcus pyogenes	16.667 ± 0.333	30.667 ± 0.333	0.00 ± 0.000
Serratia marcescens	0.000 ± 0.000	29.667 ± 0.333	0.00 ± 0.000
Serratia liquefaciens	0.000 ± 0.000	32.000 ± 0.000	0.00 ± 0.000
Enterobacter aerogenes	0.000 ± 0.000	30.667 ± 0.333	0.00 ± 0.000
Staphylococcus epidermidis	17.667 ± 0.333	34.000 ± 0.000	0.00 ± 0.000

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

The extract was not active against the gram negative bacteria tested. The zones of inhibitions were 15.667 ± 0.333 for *B. cereus*, 16.666 ± 0.333 for *S. pyogenes* and 17.667 ± 0.333 for *S. Epidermidis*. All the other organisms had zone of inhibition 0.000 ± 0.000. All the extracts were significantly smaller in zones of inhibition than the positive control. ANOVA results showed that all the organisms were significantly different from each other and their controls ($P < 0.001$).

Tukey's test has shown that the zones of inhibition for *B.cereus* is significantly bigger than those of *E.coli, S. typhi, S. Marcescens, S. liquefaciens* and *E. aerogenes* (Table 2). The zone of inhibition of *B. cereus* was not significantly different from that of *S. pyogenes* ($P > 0.05$), but was significantly smaller than that of *S. epidermidis* ($P < 0.05$). All the bacteria had zones of inhibition significantly smaller than their control ($P< 0.001$).

The zones of inhibition of *E. coli* was not significantly different from those of organism such as *S. marcescens, S. liquefaciens, E. aerogenes and S. typhi* ($P > 0.05$). The zones of inhibition were significantly smaller than those of S. *pyogenes, S. epidermidis* and its antibiotic control ($P < 0.05$).

The zone of inhibition of *S. typhi* was similar to *S. marcescens, S. liquefaciens and E. aerogenes* ($P > 0.05$), but significantly smaller than those of *S. epidermidis* and its antibiotic control. The zones of inhibitions of *S. pyogenes* were significantly larger than those of *S. marcescens, S. liquefaciens, and E. aerogenes* but significantly smaller than its control. The

Table 2

Tukey's Honestly Significant Difference Test for the Zone of Inhibition of Ethanolic-aqua Extract of Tephrosia vogelii Bark

COMPARISON	P-VALUE	SIGNIFICANCE
B. cerus vs E. coli	0.000	S
B. cerus vs S .typhi	0.000	S
B. cerus vs S. pyogenes	0.458	NS
B. cerus vs S. marcescens	0.000	S
B. cerus vs S. liquefaciens	0.000	S
B. cerus vs E. aerogenes	0.000	S
B. cerus vs S. epidermidis	0.001	S
B. cerus vs B. cerus control	0.000	S
E.coli vs S. typhi	1.000	NS
E.coli vs S .pyogenes	0.000	S
E.coli vs S. marcescens	1.000	NS
E.coli vs S. liqufaciens	1.000	NS
E.coli vs S. aerogenes	1.000	NS
E.coli vs S. epidermidis	0.000	S
E.coli vs E.coli control	0.000	S
S. typhi vs S. pyogenes	0.000	S
S. typhi vs S. marcescens	1.000	NS
S .typhi vs S. liquefaciens	1.000	NS
S. typhi vs E. aerogenes	1.000	NS
S. typhi vs S. epidermidis	0.000	S
S. typhi vs S. typhi control	0.000	S
S. pyogenes vs S. marcescens	0.000	S
S. pyogenes vs S. liquefaciens	0.000	S
S. pyogenes vs E. aerogenes	0.000	S
S. pyogenes vs S. epidermidis	0.458	NS
S. pyogenes vs S .pyogenes control	0.000	S
S. marcescens vs S. liquefaciens	1.000	NS
S. marcescens vs E. aerogenes	1.000	NS
S. marcescens vs S. epidermidis	0.000	S
S. marcescens vs S. marcescens control	0.000	S
S. liquefaciens vs E. aerogenes	1.000	NS
S. liquefaciens vs S. epidermidis	0.000	S
S. liquefaciens vs S. liquefaciens control	0.000	S
E. aerogenes vs S. epidermidis	0.000	S
E. aerogenes vs E. aerogenes control	0.000	S
S. epidermidis vs S. epidermidis control	0.000	S

S = Significant; NS = Not significant

zone of inhibition of *S. pyogenes* was not significantly different form *S. epidermidis* ($P > 0.05$).

The zones of inhibitions of *S. marcescens* were similar to those of organism such as *S. liquefaciens* and *E. aerogenes*, but significantly smaller than that of *S. epidermidis* and significantly smaller that its antibiotic control. The zone of inhibition of *S. liquefaciens* was similar to that of *E. aerogenes*, but smaller than those of S. epidermidis and its antibiotic control.

The zones of inhibition of *E. aerogenes* was significantly smaller than that of *S. epidermidis* and its antibiotic control. The zones of inhibition of *S. epidermidis* were significantly smaller than its antibiotic control. There was no zone of inhibition produced by negative control DMSO against any of the organisms.

According to Kabera (2014) and Dzenda (2007), the plant *Tephrosia vogelii* contain phytochemical such as alkaloids, tannins, flavonoids and terpenoids-steroids. The preliminary investigation showed the efficacy of *Tephrosia vogelii* against gastrointestinal nematodes in goats and therefore their potentials in assuring more the animal health care in Rwanda by treating the parasitic nematodes in goats by plant-based drugs instead of crude extracts (Kabera, 2014). The extracts (methanol and aqueous) of the three plants contained saponins, phenolic compound, tannins, anthocyanins, steroids, triterepenes, alkaloids, coumarins, anthroquinones, glycosides and essential oils. Glycosides and steroids were absent in the methanol and aqueous extracts of the three plants. Anthocyanins were present only in the methanol extracts of *T. vogelii* (Teugwa, Sonfack, Fokom, Penlap, & Amvam, 2013).

According to Makoshi (2011), the leaves extracts of *T. vogelii* contains saponins, cardiac glycosides and flavonoids. Among the six extracts tested and the standard for the in vitro antioxidant activity using the DPPH method, the methanolic extracts of C. macrostachyus and the aqueous extracts of *T. vogelii* showed the lowest and the highest antioxidant activity, with IC50 values of 0.30 and 0.11 mg/ml, respectively (Teugwa et al., 2013).

According to Teugwa et al. (2013), the antifungal activity of *M. laurentii, T. vogelii* and *C. macrostachyus* showed that the extracts of these plants possess antifungal properties and can be effective antibiotics since they inhibited the growth of fungal causative agents of skin diseases and their observation was in line with the work of Ajaiyeoba, Rahman, and Chondhary (1998).

The result obtained from the bioassay study has shown that it is possible to control the spread of pathogenic microorganisms such as *B.cereus, S. pyogenes and S. epidermidis* using ethanol-water extract of bark of *T. vogelii.*

Conclusion

The current study shows that ethanol-water extract of bark of *T. vogelii* exhibits notable inhibitory activity against the pathogenic organism such as *B. cereus, S. pyogenes and S. epidermidis*. More research needs to be done to identify the exact structures of the bioactive compounds and their effects in the in vivo environment. Further study needs to be done to identify the specific compounds which are acting against the microbes.

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