Research Article [Anthoney *et al.*, 5(2): Feb., 2014:3289-3294]
CODEN (USA): IJPLCP **ISSN: 0976-7126**

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

In Vitro Antibacterial activity of Methanolic-aqua extract of *Tragia brevipes* Leaves

Anthoney SwamyT[1*], Mutuku Chrispus Ngule [2] and Jackie Obey K[3]
[1*&2]Department of Chemistry, University of Eastern Africa Baraton, P.O. Box 2500, Eldoret -30100, Kenya
[3]Department of Medical Laboratory Sciences, University of Eastern Africa, Baraton, P.O Box 2500, Eldoret, Kenya

Abstract

The plant *Tragia brevipes* is used traditionally as purgative and in the treatment of stomach problems, in the lower eastern part of Kenya. The aim of this research was to evaluate the antibacterial activities of the crude extract of *Tragia brevipes leaves*. The crude was extracted using methanol and water in the ratio of 9:1. Antibacterial activity was done using well diffusion methods and the data analysed using SPSS software to compare the means and also check whether there was significance between the zones of inhibitions caused by the plant extract and also those caused by the positive control. *Tragia brevipes* was found to inhibit the growth of *Escherichia coli* (12.00 ± 0.577), *Salmonella sp.* (10.33 ± 0.333), *Enterobacter aerogenes* (9.33 ± 0.333), *Bacillus cereus* (23.67 ± 0.882), *Serratia liquefaciens* (5.00 ± 2.646) and *Proteus vulgaris* (8.67 ± 0.333). The results obtained scientifically justify the traditional use of the plant to treat against enteric microbes such as *Salmonella typhi* and *Enterobacter aerogenes*. *Tragia brevipes* can be used as an antibiotic against all the microorganisms tested.

Key-Words: Antibacterial activity, Tragia brevipes, Medicinal herbs, Well diffusion method

Introduction

Tragia brevipes is armed with needle like elongations on the leaves which the plant uses for protection against its enemies. The plant roots are used as purgative and also given to expectant mothers during labor pain to increase uterus contraction rate. The leaves of the plant are used to treat against rheumatism by rubbing them on the knees and other joints [1]. The leaves are also used traditionally to treat against stomach problems.

Since plants cannot avoid the enormous number of potential enemies in their surrounding by simply moving away they must develop other ways to do so. Plants produce a group of compounds known as secondary metabolites to protect themselves against herbivores and pathogenic microbes. Almost every ecosystem contains a wide variety of viruses, fungi, bacteria, mites, nematodes, insects, mammals and other herbivores; hence the need for the plants to develop some defense mechanisms [2].

*** Corresponding Author**
E.mail: drtanthony2011@yahoo.com

Secondary metabolites have no direct roles in the processes of photosynthesis, protein synthesis, translocation, nutrient assimilation, differentiation, carbohydrates formation, proteins and lipids, they differ from primary metabolites by the way they are distributed in the plant kingdom. The secondary metabolites unlike primary metabolites which are found throughout the plant kingdom, the secondary metabolites are found in specific species or related plant species only. Secondary metabolites can be grouped into three main categories' viz. terpenoids, alkaloids and phenolic.

Those chemicals produced by the plant and have effect on the growth, health, or behaviors of other organisms are called allelochemics. They can be divided into two, one those that appear to benefit the producer such as insect repellents, growth suppressants on competing species and poisons that protect against feeding organisms and two, those that seem to benefit the recipients [3]. The secondary metabolites are also referred to as phytochemicals. The difference in the type of these important compounds in the plants kingdom is the root for the difference in characteristics in plants of different species. Phytochemicals are non-nutritive plant chemicals that can protect the plant and

also prevent it against diseases [4]. Recent researches have shown that these secondary metabolites can protect humans and animals against pathogens [5].

According to Anthoney [6], the use of medicinal plants is greatly increasing even in the western world with 40% of the population in the developed countries using herbal medicine; however the number of the population using herbal medicine in the developing countries is still high with approximately 90% of the population using herbal medicine for treatment. The high number in the developing countries could be attributed to the lack of enough financial capability to acquire the commercially synthesized drugs which are expensive. There is growing interest in the use of herbal medicine which can be attributed to the believe that green medicine has no side effects and also dependable as compared to expensive synthetic drugs which have been associated with adverse side effects [7].

Secondary metabolites have medicinal value due to the presence of chemical compounds which produce definite physiological actions in the body of humans [8 & 9]. Researches done on plants have shown plants to possess great pharmacological values viz. antifungal activity, antibacterial activity, anticancer activity, antioxidant activity, anti-diabetic activity, hepatoprotective activity, anti – inflammatory activity, haemolytic activity, larvicidal activity, central nervous system activity, pupicidal activity, anthelmintic activity and pain relief activity [10, 11 & 12]. Due to multiple compositions in plants they have been known to possess multiple medicinal values hence making a good source of raw materials for the pharmaceutical industry [13]

The defense mechanisms of plants and the compounds produced to protect themselves from the environment have been studied in depth in the past and studies are still on. These compounds which plants produce have been analysed and found to have great medicinal value.

Researches on species of Tragia have shown diverse pharmacological activity against various ailments affecting human beings today. According to Joshi [14], *Tragia involucrata* showed potent cytotoxicity with methanol and hexane showing the highest activity on MCF-7 and KB cell lines. The cytotoxicity of the plant was attributed to the presence of flavonoids in methanol and terpenoids in hexane extracts. These results show that *Tragia involucrata* can be used to synthesis anticancer drugs. *Tragia involucrata* showed significant anti-fertility activity with extracts from the plant reducing the number of the litters born [15]. According to Hosahally [16], *Tragia cannabina* showed significant anti-inflammatory activity with the methanolic and chloroform extracts of the plant showing the highest significance as compared to standard ibuprofen. *Tragia plukenetii* a species in the same genus with *Tragia brevipes* was found to have antipyretic, antiasthmatics, antiplasmodic, diuretic and analgesic activity [17]. According to Farook [18], the plant *Tragia involucrata* was also found to have antihyperglycemic and hypolipidemic effect. To the best of our knowledge very little data is found on literature on the medicinal value of the plant *Tragia brevipes.* This study was done to analyse the antibacterial activity *Tragia brevipes* leaves against the selected bacteria.

Material and Methods

Sample Collection and Preparation

The herb was randomly collected from the natural forest around University of Eastern Africa, Baraton. The plant samples were identified by a taxonomist from Baraton University. The samples were thoroughly mixed and spread to dry at room temperature in the chemistry laboratory for about three weeks. They were then ground into fine powder and put in transparent polythene bags.

Extraction procedure

Using electric analytical beam balance 50 grams of the powdered leaves of *Tragia brevipes* was placed in 1000 ml conical flask, methanol and water were then added in the ratio of 9:1 respectively until the leaves were completely submerged in the solvent. The mixture was then agitated for thorough mixing and kept for 24 hours on a shaker for effective extraction of the plant components. The extract was filtered using Butchner funnel; Whatman no.1 filter paper and a vacuum and pressure pump. The filtrate was re-filtered again using the same apparatus. The solvent was evaporated using rotary vacuum evaporator (R-11) with a water bath at 40^{o}C. The extract was brought to dryness using vacuum and pressure pump at room temperature. The residue was then obtained and analysed for antibacterial activity.

Bioassay Study

Preparation of the Extract Concentrations and Antibiotic

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

Preparation of the Bacterial Suspension

The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard, a

procedure similar to that used by *Biruhalem* [19] and Donay *et al.*, [20] with slight modifications. The McFarland standard was prepared by dissolving 0.05 g of $BaCl_2$ in 50 ml of water to obtain a 1% solution of Barium chloride (w/v). This was mixed with 99.5 ml of 1% sulphuric acid solution. Three – five identical colonies of each bacterium was taken from a blood agar plate (Himedia) culture and dropped in Mueller Hinton broth (Himedia). The broth culture was incubated at 37^0C for 2 - 6 hours until it achieved turbidity similar to the 0.5 McFarland standards. The culture that exceeded the 0.5 McFarland standard were each adjusted with the aid of a UV spectrophotometer to $0.132A^0$ at a wavelength of 600 nm in order to obtain an approximate cell density of $1x10^8$ CFU/ml.

Determination of bioactivity of the Extract

Mueller Hinton agar plates were prepared by the manufacturer's instruction. 0.1 ml of each of the prepared bacterial suspension for the test was transferred to 3 plates for each organism to give a triplicate for each concentration and organism. Five wells were drilled in each agar plate. Three of the wells were filled with the extract dilution and the other wells were filled with Augmentin and DMSO control respectively. Three plates were made for each bacterial organism and extract giving a triplicate reading for each microorganism and extract. The wells were labeled on the underside of the plate. The plates were incubated at 37^0C for between 24 to 48 hours and the zones of inhibition were measured in millimeters with the aid of a ruler.

Results and Discussion

Table 1: Zones of Inhibition (mean± S.E.) of 500 mg/ml of *Tragia brevipes* Against Selected Microorganisms

Microorganism	Mean ± S.E.	Penicillin	DMSO
Escherichia coli	12.00±0.577	51.00±0.00	0.00±0.00
Salmonella sp.	10.33±0.333	30.00±0.00	0.00±0.00
S. liquefaciens	5.00±2.646	45.00±0.00	0.00±0.00
Enterobacter aerogenes	9.33±0.333	33.00±0.00	0.00±0.00
Bacillus cereus	23.67±0.882	30.00±0.00	0.00±0.00
Proteus vulgaris	8.67±0.333	31.00±0.00	0.00±0.00

Table 2: Tukey's Honestly Significant Difference Among Microorganisms Using 500mg/ml of *Tragia brevipes* Extract

Comparison	P. value	Significance
E. coli vs. Salmonella	0.911	NS
E. coli vs. S. liquefaciens	0.013	S
E. coli vs. Enterobacter aerogenes	0.620	NS
E. coli vs. Bacillus cereus	0.000	S
E. coli vs. Proteus vulgaris	0.402	NS
Salmonella sp. Vs S. liquefaciens	0.067	NS
Salmonella sp. Vs Enterobacter aerogenes	0.989	NS
Salmonella sp. vs. Bacillus cereus	0.000	S
Salmonella sp vs. P. vulgaris	0.911	NS
S. liquefaciens vs. E. aerogenes	0.175	NS
S. liquefaciens vs. B cereus	0.000	S
S. liquefaciens vs. P. vulgaris	0.998	NS
E. aerogenes vs. B. cereus	0.000	S
E. aerogenes vs. P. vulgaris	0.998	NS
B. cereus vs. P. vulgaris	0.000	S

KEY: S=Significant; NS= Not Significant

From the study (Table 1) the plant extract was found to have antibacterial activity against all of the six bacteria it was tested against. *Tragia brevipes* was found to inhibit the growth of *Escherichia coli* (12.00 ± 0.577), *Salmonella sp.* (10.33 ± 0.333), *Enterobacter aerogenes* (9.33 ± 0.333), *Bacillus cereus* (23.67 ± 0.882), *Serratia liquefaciens* (5.00 ± 2.646) and *Proteus vulgaris* (8.67 ± 0.333).

Analysis of variance the mean zones of inhibition of 500 mg/ml of *Tragia brevipes* (Table 2) showed that there was significant difference in the zones of inhibition among the bacterial organisms ($p<0.001$). Further comparisons showed that the zones of inhibition of *E. coli* were significantly bigger than that

of *S. liquefaciens* ($p<0.05$), *Bacillus cereus* significantly bigger than *E. coli* ($p<0.001$), *B. cereus* significantly bigger than Salmonella sp., and *B. cereus* significantly bigger than the rest of the organisms ($p<0.001$). All the other comparisons for the remaining pairs were not significantly different in their zones of inhibition. For *Tragia brevipes*, the minimum inhibitory concentrations determined were 250 mg/l for E. coli (8mm) and 250 mg/ml for *Bacillus cereus* (15mm). The results obtained in this work are in conformity with the data recorded by Panda [21], were a similar species *Tragia involucrata* demonstrated antibacterial activity against *Staphylococcus aureus* a gram positive bacteria and *Escherichia coli* a gram negative bacterial. The plant also showed antibacterial activity against all the gram positive and gram negative bacteria used in the study by Panda [21].

The plant *Tragia brevipes* methanol aqua extract can be a potent antibiotic against all the ailments caused by the bacteria tested against. The plant can be used to treat against *Salmonella typhi* which causes typhoid fever and also *Salmonella paratyphi* A, B and C which causes paratyphoid fever. The isolation, purification and the use of the active compounds from the plant can be a great breakthrough in the treatment against this dangerous disease in the world. Salmonellosis can cause relapse, death or cause serious complications that may include gastrointestinal bleeding, typhoid encephalopathy and intestinal perforation to approximately 10% of all the infected patients [22 & 23]. The compounds can also be used as sanitary agents in food handling and in the veterinary industry to treat domestic animals, which are carriers of these pathogens hence preventing infection to human at the human-animal interphase [24].

The extract from the plant *Tragia brevipes* also was found to inhibit the growth of *Bacillus cereus.* From these results it is therefore worthy to mention that the plant can be used to treat against all the infections caused by *Bacillus cereus* and even other species of Bacillus viz self-limited gastroenteritis, posttraumatic wounds, surgical wounds infections, burns, ocular infections such as endophthalmitis, corneal abscess and panophthalmitis [25& 26]. The plant compounds can also be used to treat immunologically compromised patients including AIDS and malignant disease victims [27 & 28]. The extract of the plant was found also to inhibit the growth of *Enterobacter aerogenes,* hence showing high potency of the plant to treat against this important hospital pathogen which is responsible for nosocomial respiratory tract infections [29 & 30]. The plants capability to inhibit the growth of *E. coli* is a scientific proves that the plant can be used to great extent to treat against enteric infections caused by the bacteria. The plants extract can treat against gastro-intestinal diseases, ear infections, urinary tract infections and wounds infections caused by *Proteus vulgaris* [31 & 32].

Conclusion

The antibacterial activity could be attributed to the phytochemicals present in the plant which according to Anthoney [33], plant leaves were found to contain tannins, saponins, terpenoids, flavonoids, and phenols. According to Ngule [34], the plants infused stem was also found to contain tannins, saponins, phenols, and alkaloids. Similar phytochemicals found in the plant have shown great pharmacological activity in other studies and therefore their presence in the plant greatly gives it its high potency in pharmacology [35 & 36]. The data obtained and recorded in this study is a scientific justification of the plants traditional use. The traditional practice is therefore recommended for the use of the plant in the treatment of all the ailments caused by the bacteria used in this study. More research needs to be done to isolate the plants active compounds, there medicinal value in comparison with other commercial drugs, analyse there effect in the in vivo environment and there mode of action against various microorganisms.

Acknowledgement

We are grateful to the Almighty God for the wonderful care and good health He gave to all authors of this work during the period of this research. The authors of this paper are very much thankful to the Department of Chemistry and Department of Medical Laboratory Sciences, University of Eastern Africa, Baraton for their permission and support in conducting this research. Authors also thank taxonomist Mr. Joel Ochieng Ondiek, University of Eastern Africa, Baraton for his great assistance in plant identification.

References

1. Kokwaro OJ. *Medicinal plants of East Africa*, 3rd ed. University of Nairobi press. Nairobi, Kenya, 2009.
2. Taiz L and Zeiger E. *Plant physiology* 4th ed. Sinaver Association, Inc. Sunderland, U.S.A, 2006.
3. Ting IP. *Plant physiology*. Addison-Wesley publishing company, Inc. Philippines.

4. Ngbebe J, Yakubu RA and Nyam DA. Phytochemical screening for active compounds in Canarium scheweinforthii (Atile) leaves from Jos North, Plateau states, Nigeria. *Research Journal of Biological Sciences*, 2008; 3(9): 1076-1078.
5. Kubmarawa D, Khan ME and Punah AM. Phytochemical screening and antibacterial activity of extracts from Parkia clappertoniana key against human pathogenic bacteria. *Journal of Medical Plant Research*, 2008; 2(12): 352-355.
6. Anthoney ST, Jackie O, Ngule CM. *In Vitro* control of selected pathogenic organisms by *Vernonia adoensis* roots, *International Journal of Pharmacy and Life Sciences*, 2013; 4(8): 2855-2859.
7. Bandaranayake M, Wickramasinghe AL, Aqil F, and Owlish M. Modern phytomedicine turning medicinal plants to drugs: Quality control screening toxicity and regulation of herbal drugs, WILEY-VCH Versa GmbH Co.KGaA, weinnheim, year; 1: 25-57.
8. Anpin Raja RD, Prakask JW and Jeeva S. Antibacterial activity of some medicinal plants used by Kani tribe, southern western Ghats, Tamilnadu and India in ; P.C. *Trivedi, editor. Ethnic tribes and medicinal plants Jaipur; Pointer publishers*, 2010:28-45.
9. Jeeva S, Kiroba S, Mishra BP, Venugopal N, Das SSM, Sukumaran S. Weeds of Kanyakumari district and their value in rural life. *Indian J. Tradit Knowledge*, 2006; 5: 501-509.
10. Mir AM, Sawhney SS and Jassal MMS. Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. *Woodpecker Journal of Pharmacy and Pharmacology*, 2013; 2(1): 01-05.
11. Sukirtha K and Growther I. Antibacterial analysis of selected medicinal plants. *Journal of natural product and plant resource*, 2012; 2(6): 644-648.
12. Ngule CM, Anthoney ST and Obey JK. Phytochemical and bioactivity evaluation of *Senna didymobotrya* fresen irwin used by the Nandi community in Kenya. *Int. J. Bioassays*, 2013, 2 (07), 1037-1043.
13. Anthoney ST, Ngule CM, Jackie OK. Qualitative assessment of phytochemicals of infused *Senna didymobotrya* leaves. *International Journal of Pharmacy*, 2013; 4(1): 40-45.
14. Joshi CG, Gopal M and Byregowda SM. Cytotoxic activity of *Tragia involuctara* linn. Extracts. American-Eurasian *Journal of Toxicological Sciences*, 2011; 3(2):67-69.
15. Joshi GC and Gopal M. Antifertility activity of hexane and ethyl acetate extracts of aerial parts of *Tragia involucrata*. *Journal of Pharmacology and toxicology*, 2011:1-6.
16. Hosahally RV, Seru G, Sutar PS, Joshi VG, Sutar KP and Karigar AA. Phytochemical and pharmacological evaluation of *Tragia cannabina* for anti-inflammatory activity. *International Current Pharmacological Journal*, 2012; 1(8): 213-216.
17. Kalaivanan M and Jesudass LL. Pharmacological studies on ethanol extract of *Tragia plukenetii*. *IOSR Journal of Pharmacy*, 2012; 2(6): 01-07.
18. Farook SM and Atlee WC. Antidiabetic and hypolipidemic potential of *Tragia involucrata* Linn.IN Streptozotocin-nicotinamide induced Type 11 diabetic rats. *International Journal of Pharmaceutical sciences*, 2011; 3: 103-109.
19. Biruhalem T, Giday M, Animut A, Seid J. Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. *Asian Pacific Journal of Tropical Biomedicine*, 2011; 370-375.
20. Donay JL, Fernandes P, Lagrange PH, Herrmann JL. Evaluation of the inoculation procedure using a 0.25 McFarland Standard for the BD Phoenix Automated Microbiology System. *Journal of Clinical Microbiology*, 2007; 45 (12), 4088-4089.
21. Panda D, Kumar S, and Dash GK. Phytochemical examination and antimicrobila activity of various solvent extracts and the selected isolated compounds from roots of *Tragia involucrata* linn., International *Journal of Pharmaceutical Sciences and Drug Research*, 2012; 4(1): 44-48.
22. Scherer CA and Miller SI. *Molecular pathogenesis of Salmonellae. In Groisman.E.A.(Ed.). Principles of bacterial pathogenesis*, United States of America: Academic press, 2001: P. 265-316.
23. Parry CM. Epidemiological and clinical aspects of human typoid fever. In Matroieni, P. and Maskell D.(Eds.). *Salmonella infections: Clinical immunological and molecular aspects,*

New York: Cambrige University Press, 2006: p. 1- 18.
24. Bouchrif B, Paglietti B, Murgia M, Piana A, Cohen N, Ennaji M, Rubino S and Timinouni M. Prevalence and antibiotic-resistance of Salmonella isolated from food in morocco. *Journal of Infection in Developing Countries*, 2009; 28(3): 35-40.
25. Sankararaman S and Velayuthan S. *Bacillus cereus*.Peditrics in Review, 2013; 34: 196.
26. Garcia-Arribas ML, Plaza CJ, De La Rose MC and Mosso MA. Characterisation of *Bacillus cereus* strains isolated from drugs and evaluation of their toxins. *J.Appl. Bacterial*, 1988; 64: 257-264.
27. Cotton DJ, Gill VJ, Marshall DJ, Gress M, Thaler M and Pizzo P. Clinical fetures and therapeutic interventions in 17 cases of bacillus bacteremia in an immunosuprressed patient population .J. Clin. Microbiol, 1987; 25: 672-674.
28. Tuazon CU, Murray HW, Levy C, Solny MN, Curtin JA and Shegren JN. Serious infections from Bacillus species. *JAMA*, 1979; 241:1137-1140.
29. Wenzel RP, Sahm DF, Thornsbery C, Draghi DC, Jones ME and Karlowsky JA. In Vitro susceptibilities of gram-negative bacteria isolated from hospitalized patients in four European countries, Canada and the United States in 2000-2001 to expanded –spectrum cephalosporins and comparator antimicrobials: implications for theraphy . *Antimicrob. Agents Chemother*, 2003; 47: 3089-3098.
30. Thiolas A, Bollet C, Scola B, Raoult D and Page's JM. successive emergence of *Enterobacter aerogenes* strains resistant to imipenem and colistin in apatient. *Antimicrobial Agents and Chemotherapy*, 2005; 49(4): 1354-1358.
31. Goodwin CS, Kliger BN and Drewett SE. Colistin-sensitive Proteus organisms: Including indole-negative *Proteus vulgaris*, non-swarming on first isolation. *Br. J. exp. Path.*, 1971, 52: 138-141.
32. Neter RE and Farrar HR. *Proteus vulgaris* and *Proteus morganii* in diarrhea disease of infants. *The American Journal of Digestive Diseases,* 1943; 10(9): 344-347.
33. Anthoney ST, Ngule CM, Ngule EM and Francis R. Qualitative analysis of phytoconstituents in *Tragia brevipes* plant. *International Journal of Pharmaceutical Research and analysis*, 2013; 3(2): 93-98.
34. Ngule CM, Anthoney ST. Phytochemistry of infused *Tragia brevipes* stem. *International Journal of Medical and Pharmaceutical Sciences Research and Review,* 2013; 1(3): 01-14.
35. Anthoney ST, Obey J and Ngule CM. *In Vitro* control of selected pathogenic organisms by *Vernonia adoensis* roots. *International Journal of Pharmacy and Life Sciences,* 2013; 4(8): 2013.
36. Ngule CM, Anthoney ST and Obey J. *In Vitro* control of selected pathogenic microorganisms by *Vernonia adoensis* leaves. *International Journal of Bioassays,* 2013; 02(08): 1113-1117.

How to cite this article
Anthoney S.T, Ngule C.M. and Jackie O.K. (2014). *In Vitro* antibacterial activity of methanolic-aqua extract of *Tragia brevipes* leaves. *Int. J. Pharm. Life Sci.*, 5(2):3289-3294.
Source of Support: Nil; Conflict of Interest: None declared

Received: 23.12.13; Revised: 30.12.13; Accepted:07.01.14