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Original Article

Antidiarrheal activity of *Tetradenia riparia* and *Wubergia ugandensis* ethnobotanical plants in Kenya

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ABSTRACT

Diarrhea continues to be a major problem especially in developing countries, affecting majorly young children under the age of 5 years. Plants have been used since time immemorial in the treatment against diarrhea. Due to increased drug resistant microorganisms the search for new antibiotics remains to be inevitable. The study was done to analyze the antibacterial potential of the selected two plants against diarrhea causing microorganisms. The plant samples were collected, identified, voucher specimen prepared and allowed to dry under room temperature. The dried samples were powdered and extracted using hydromethanolic solvent system. The bioassay was done using well diffusion method. From the results*Tetradenia riparia* had the highest zones of inhibition as compared to *Wubergia ugandensis (Table 1)*. *Tetradenia riparia* extract highly inhibited the growth of *Escherichia coli* with an inhibition zone of 13.33 ±0.333 and *Salmonella typhi* an inhibition zone of 12.00 ±0.000 (fig. 1). *Wubergia ugandensis* inhibited *Escherichia coli* the most with an inhibition zone of 11.00 ±0.577 and *Salmonella typhi* an inhibition zone of 10.00 ±0.000.The two plants showed great potential in the fight against diarrhea causing microorganisms.

Key words: Wubergia, Tetradenia, Diarrhea, Plants, Antibacterial, Phytochemical

INTRODUCTION

The use of plants in the fight against diarrhea dates back to the origin of man, however due to the introduction of synthetic drugs the old systems of traditional drugs use has decreased creating the need for scientific justification on the use of ethnobotanical drugs which are believed to be effective against various infectious microbes including diarrhea causing microorganisms. Diarrhea is a disease characterized by frequent excretion of watery stool due to the interruption of the gastrointestinal tract [1]. The condition continues to be a major health problem especially in developing countries affecting majorly young children under the age of 5 years [2]. The disease causes death to approximately 5-8 million people worldwide with young children under the age of five years constituting a high percentage of these deaths [3 & 4]. To combat this tread WHO set up a programme to study on the medicinal properties of plants in the fight against diarrhea. The organization recommended plants as the best source for a variety of active compounds in the struggle against diarrhea [5&6]. Plants have been used since time immemorial to maintain the human health especially in developing countries. The knowledge on the use of medicinal plants has been passed from generation to generation through grandparents to their grandchildren and also parents to their children which has led to accumulation of this information for many years. The quick civilization and western education, however has become a threat to this process of knowledge transfer, therefore creating the need for documentation of information on ethnobotany. The emergence of drug resistant microorganisms has also increased, therefore creating the need for continued search for new antibiotics [7].

According to Gislene [8], WHO recommends medicinal plants as the best source to obtain a variety of drugs. Despite the great achievements made in the search of new antibiotics, infectious diseasesstill remains to be a major threat to human health [9]. There is renewed interest in the use of plants as therapeutic agents due to the belief that green medicine is save, cheap and dependable as

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compared to allopathic drugs [10]. Plants have been used for their chemotherapeutic effects and as template molecules for synthetic or allopathic drugs synthesis [11]. The medicinal value of plants is associated with the presence of important pharmacological compounds commonly known as phytochemicals which have been found to have little purpose in the biological activities and also nutritional value of plants but research has proved them to have great medicinal importance.

The production of these compounds by plants is as a result of protection response of the plant against pathogens [12&13]. It is estimated that about 50,000 to 70, 000 plant species have medicinal values [14]. Globally millions of people from developing and developed countries use medicinal plants as a source of basic medical health care. It is estimated that about 80% of people leaving in developing countries and 40% of those leaving in developed countries use plants as a source of medicine [15&16]. The current study was done to analyse the antibacterial activity of *Wubergia ugandensis* and *Tetradenia riparia.* The plants were selected based on their ethnobotanical uses.

MATERIALS AND METHODOLOGY

Sample collection and preparation: The plant samples were randomly harvested in the month of July from the natural forest around University of Eastern Africa, Baraton. The samples were identified by a taxonomist in the Department of Biological Sciences, University of Eastern Africa, Baraton. Voucher specimens were prepared and stored in the Department of Biological Sciences herbarium. The samples were thoroughly mixed and spread to dry at room temperature in the chemistry laboratory for about three weeks and then ground into fine powder. The powdered samples were stored in transparent polythene bags.

Extraction procedure: Using electric analytical beam balance 100g of the powdered samples were placed in 500 ml conical flask, methanol and water were then added in the ratio of 9:1 respectively until the samples were completely submerged in the solvent. The mixture was then agitated for thorough mixing and kept for 24 hours with frequent shaking 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 residue was obtained and stored at 4^{0}C.

BIOASSAY STUDY

Bacteria source and media preparation: The bacteria used in the study were commercial pure cultures from Carolina biological supply company (USA). The colonies for use in the study were obtained from the pure cultures and then transferred in to blood agar plates. The plates were then incubated at 37^{0}C for 24 hours. The blood agar media was prepared according to the manufacturer's instructions. The plates were sterilized by the use of an autoclave at 121^{0}C. Approximately 20ml of the prepared media was transferred in to the sterilized plates and the surface of the media was flamed using a Bunsen burner flame to remove air bubbles. The Mueller Hinton broth was prepared according to the manufacturer's instructions. About 5ml of the broth was transferred into sterile test tubes. The transfer of the media to the plates and test tubes was done under sterile germicidal wood.

Preparation of the Bacterial Suspension: The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard [17&18]. 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). This was mixed with 99.5 ml of 1% sulphuric acid solution. Three – five identical colonies of each bacterium were taken from a blood agar plate (Himedia) culture using a sterile swab in to Mueller Hinton broth (Himedia). The broth culture was incubated at 37^{0}C 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.

Preparation of the Extract Concentrations and Antibiotic: Extracts stoke solutions were prepared by dissolving 500 mg in 1 ml of dimethylsulfoxide (DMSO). An antibiotic control was made by dissolving 500 mg of penicillin in 1 ml of sterile distilled water. DMSO (100%) served as a negative control.

Determination of the Bioactivity of the Extract: Mueller Hinton agar plates were prepared as per the manufacturer's instructions. The media and the plates were sterilized in an autoclave at 121^{0}C for 15 minutes. The media was poured on the plates. The plates were flamed on the surface using a non-luminous flame to remove air bubble. The cork borer was sterilized using a non-luminous flame. The plates and all the equipment's to be used for the experiment were then transferred in to a germicidal wood. The germicidal lamp was put on

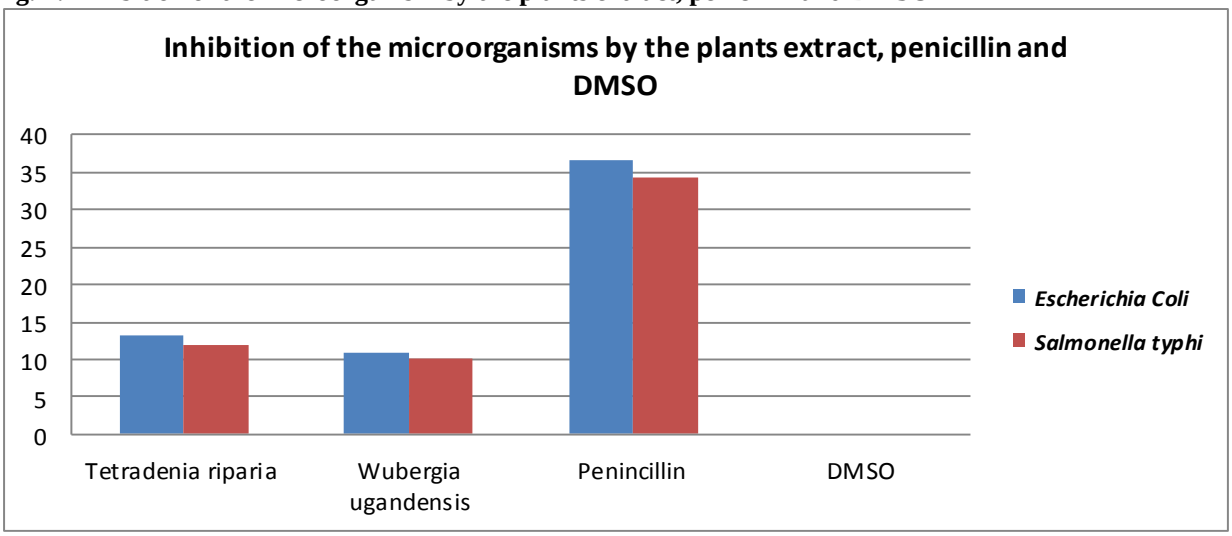
for 30minutes to sterilize the surface of the plates and other equipments. The bacterial suspension was smeared on the media and six wells with a diameter of 6cm each were drilled in each agar plate using a cork borer. Four of the wells were filled with 0.1ml of the 500mg/ml of the extract. The other wells were filled with 0.1ml 0f 500mg/ml of penicillin and 0.1ml of 100% DMSO positive and negative controls respectively. Three plates were made for each bacterial organism and extract giving a triplicate reading for each microorganism and extract. The plates were labeled on the underside and incubated at 37^0C for between 24 to 48 hours and the zones of inhibition measured in millimeters with the aid of a ruler.

RESULTS AND DISCUSSION

Table 1: The antibacterial activity of *Tetradenia riparia*, *Wubergia ugandensis* and Penicillin against selected microorganisms

BACTERIA	*Tetradeniariparia*	*Wubergiaugandensis*	Penincillin	DMSO
Escherichia Coli	13.33 ±0.333	11.00 ±0.577	36.67 ±0.667	0.00 ±0.000
Salmonella typhi	12.00 ±0.000	10.00 ±0.000	34.33 ±0.577	0.00 ±0.000

Fig. 1: Inhibition of the microorganism by the plants extract, penicillin and DMSO



The results obtained shows that *Tetradenia riparia* had the highest zones of inhibition as compared to *Wubergia ugandensis (Table 1)*. *Tetradenia riparia* extract highly inhibited the growth of *Escherichia coli* with an inhibition zone of 13.33 ±0.333 and *Salmonella typhi* an inhibition zone of 12.00 ±0.000 (fig. 1). *Wubergia ugandensis* inhibited *Escherichia coli* the most, with an inhibition zone of 11.00 ±0.577 and *Salmonella typhi* an inhibition zone of 10.00 ±0.000. Penicillin which was used as the positive control inhibited both *Escherichia coli* and *Salmonella typhi*. Dimethyl sulfoxide which was used as the negative control did not inhibit the growth of any of the microorganisms. The analysis of variance showed that there was significant difference in the zones of inhibition among the microorganisms. The Tukey's pair wise comparison showed that the zones of inhibition caused by *Tetradenia riparia* hydromethanolic extract against *Escherichia coli* were insignificant as compared to those of *Salmonella typhi* ($p>.05$). However the zones of inhibition caused by the penicillin against the organisms were significantly higher as compared to those caused by the plant extract ($p<.001$). The zones of inhibition cause by the *Wubergia ugadensis* extract against *Escherichia coli* were not significant as compared to those of *Salmonella typhi* ($p>.05$). However the zones of inhibition caused penicillin against the *E. coli* and *S. typhi* were found to be significantly higher as compared to those caused by the plant extract against them ($p<.001$). The current study is inconformity with previous studies in which the plant *Tetradenia riparia* was found to have antibacterial activity against various microorganisms [19]. This study is also consistent with the study done by Mbwambo et al [20] in which the plant *Wubergia ugandensis* bark was found to have antimicrobial activity, however, in this study the leaves of the plant were used. The study is also different since it employs the use of a solvent system to extract the active ingredients from the plants. The zones of inhibition caused by the plant *Tetradenia riparia* methanol extract in the previous studies were lower as compared to those obtained in the current study. This could be

attributed to the difference in the type of solvents used or the geographical location from which the plants were collected. The study demonstrates that the two plants have great antibacterial activity against diarrhea causing microorganisms. This study can partly be a scientific justification of the plants ethnobotanical use. The inhibition of the two plants against *Escherichia coli* and *Salmonella typhi* is noteworthy since the two microorganisms have been known to be causative agents of diarrhea. *Escherichia coli* has been associated with most acute and chronic diarrheal out breaks in the past. The bacterium has also been found to cause travelers' diarrhea to people travelling in most developed countries [21]. *Salmonella typhi* causes both acute and chronic diarrhea to humans all over the world [22]. The bacteriaum also causes travelers' diarrhea [23].

CONCLUSION

The inhibition of the plants against the tested microorganisms is significantly important, since the selected microorganism have been found to be major causative agents of diarrhea. It is worthy to mention that the antibacterial activity of the plants could be attributed to the presence of important pharmaceutical compounds in the plants [19]. However, further research needs to be done to fractionate the methanol water extracts in an attempt to purify them further and formulate aseptic and antibiotic agents to fight against diarrhea.The active compounds needs to be isolated, determination of their structural makeup done and their mode of action determined.

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REFERENCES

1. Guerrant RLet al. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis 2001; 32: 331-35.
2. Fauci AS et al. *Harrison's Principles of Internal Medicine*. 13th ed. New York: McGraw Hill Company 1993; 236-42.
3. Akuodor GC et al. Evaluation of the Antidiarrheal Activity of Methanol Leaf Extract of *BombaxBuonopozense* in Rats. Ibnosina J Med BS 2011, 3(1):15-20.
4. WHO, *Country Health System Fact Sheet*. 2006.
5. Mukherjee J et al. Anti-diarrheal evaluation of Nelumbonucifera rhizome extract, Indian Journal of pharmacology1995; 22:2624.
6. Eisner T. Chemical prospecting. *A call for action*. In: Borman FH, Kelleet SR, editors. Ecology, Economic and Ethics: The Broken Circle. New York: Yale University press; 1990.
7. Cohen ML. Epidemiology of drug resistance: Implications for a post-antimicrobial era. Science1992; 257: 1050-1055.
8. Gislene GF et al. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian Journal of Microbiology 2000, 31: 247-256.
9. Akyuz Met la. Entimicrobial activity of some edible maushrooms in the eastern and souther Anatolia region of Turkey. Gazi University Journal of Science 2010; 23(2): 125-130.
10. Nair GB et al. Global dissemination of Vibrio parahaemolyticus serotype O3:K6 and its serovariants. ClinMicrobiol Rev2007, 20: 39–48.
11. Ameyaw Y et al. The alkaloid contents of the ethno-plant organs of species in the eastern region of Ghana. Int. J. Chem. Sci 2009; 7(1): 48-58.
12. Ngule CM et al. Phytochemical and bioactivity evaluation of *Sennadidymobotrya*fresen Irwin used by the Nandi community in Kenya. International Journal of Bioassays 2013, 2(7): 1037-1043.
13. Ghasemzadeh A,Ghasemzadeh N. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. Journal of Medicinal Plants Research 2011; 5(31): 6697-6703.
14. Kargroglu M et al. An ethnobotanical survey of inner west Anatolia, Turkey. Hum. Ecol2008; 36: 763-777.
15. Singh V. Ethnobotanical observations on Dards tribe of Gurez valley in Kashmir Himalaya. Ethnobiology in Human Welfere: abstracts of the fourth international congress of ethnobotany, Lucknow, Utter Pradesh, India. 1994, 17-21: 316.
16. Adongo SO et al. Analysis of selected essential elements of medicinal plants used in Chuka community, TharakaNithicounty, Kenya. The Scientific Journal of Science and Technology 2012; Special issue.
17. Biruhalem T et al. Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. Asian Pacific Journal of Tropical Biomedicine2011; 370-375.
18. Donay JL et al. 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.
19. Njau EFA et al. Antimicrobial activity of Tetradeniariparia (HOCHST.) lamiaceae, a medicinal plant from Tanzania. European Journal of Medicinal Plants 2014; 4(12): 1462-1478.
20. Mbwambo ZH et al. Antibacterial and antifungal activities of extracts of Zanthoxylumchalybeum and Warburgiaugandensis, Ugandan medicinal plants. Tanzania Journal of Health Research 2009; 11(2):75-78.
21. David JC et al. Enteroaggregative Escherichia coli: A Review of Trends, Diagnosis, and Treatment. Infections in Medicine 2007; 100-108.
22. Mcgavin DM et al.*Thompson's Special Veterinary*, 3rd ed. Mosby Philadephia, USA, 2001; 43-46.
23. Hallstrom K, McCormick BA. Salmonella Interaction with and Passage through the Intestinal Mucosa: Through the Lens of the Organism. Front Microbiol 2011; 2:88. doi:10.3389/ fmicb.2011. 00088.