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ANTIBACTERIAL ACTIVITY OF *TETRADENIA RIPARIA* PRE-PURIFIED FRACTIONS AGAINST DIARRHEA CAUSING MICROORGANIMS

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ABSTRACT

Diarrhea is a major public health problem especially in developing countries affecting majorly young children under the age of 5 years. The disease causes death to approximately 6 million people worldwide with young children constituting a great percentage of these deaths. Plants have long heritage in their use to treat diarrhea. The current study was undertaken to ascertain the potential of *Tetradenia riparia* pre-purified fractions in the treatment against diarrhea causing microorganisms. The fractions tested viz: hexane, ethyl acetate and water inhibited the growth of *Escherichia coli* and *Salmonella typhi* the two diarrhea causing microorganisms used. Ethyl acetate fraction gave the best zones of inhibitions. The inhibition of ethyl acetate fraction was significantly high compared to the conventional drug used, penicillin. The plant *Tetradenia riparia* is used traditionally by the Meru people in Kenya in the treatment of bloody diarrhea. Therefore, the inhibition of the plant against the selected diarrheal microorganisms is a major contribution towards provision of a scientific prove of its use in this course. Further studies needs to be done to isolate the active compounds, elucidate their structures and their mode of action. Formulation of the plant's extracts in to aseptic solutions is a prudent idea in the fight against diarrhea causing microorganisms.

KEYWORDS: Tetradenia, Antibacterial, Diarrhea, plants, ethnomedicine.

INTRODUCTION

Diarrhea is a disease characterized by frequent excretion of watery stool due to interruption of the gastrointestinal tract.[1] Diarrhea is a major public health problem especially in developing countries affecting majorly young children under the age of 5 years.[2] The disease causes death to approximately 6 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 the WHO set up a programme to study on the medicinal properties of plants in the fight against diarrhea. The organization has recommended plants as the best source for a variety of active compounds in struggle against diarrhea.[5,6] Plants have been used for a long period of time to maintain human health especially in developing countries. The knowledge on the use of medicinal plants has been passed from generation to generation by the old grandmothers and grandfathers to their grandchildren and also parents to their children this has led to accumulation of this information for thousands of years. The quick civilizations 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 diseases still remains to be a major threat in the 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 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 medical 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 countries use medicinal plants as a source of basic medical health care. It is also 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 *Tetradenia riparia* pre-purified fractions with special attention diarrhea causing microorganisms.

MATERIALS AND METHODS

Sample collection and preparation

The plant leaves were collected in the natural forests around University of Eastern Africa, Baraton, Nandi County, Kenya. The samples were air dried in shade at room temperature and grounded in to fine powder using an electric laboratory mill.

Organic solvent extraction and fractionation

Using analytical beam balance 300g of the sample was placed into a 250ml conical flask, 80% methanol was added until the sample was completely submerged. The mixture was agitated for thorough mixing. The extraction process was allowed to continue for 24-48 hrs with frequent shaking for effective extraction of the plant components. The mixture was vacuum filtered using Butcher funnel, whatman no. 1 filter paper with the help of a vacuum pump. The filtrate was re-filtered using the same apparatus. The solvent was then removed using rotary vacuum evaporator with a water bath at 40^0C.[17] The crude extract for was fractionated using a separating funnel in different solvents in order of their increasing polarity viz, hexane, ethyl acetate and water respectively and the solvent removed using a rotar vapor machine at 40 ^{o}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 37^0C 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 poured 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 in to 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.[18,19] 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 37^0C 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.

Preparation of the Extract Concentrations and Antibiotic

Extracts stoke solutions were prepared by dissolving 100 mg in 1 ml of dimethylsulfoxide (DMSO). An antibiotic control was made by dissolving 100 mg of penicillin in 1 ml of sterile distilled water. DMSO 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 121^0C 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 bubbles. 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 100mg/ml of the extract. The other wells were filled with 0.1ml 0f 100mg/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: Antibacterial activity of *Tetradenia riparia* ethyl acetate fraction *against* selected pathogenic microorganisms.

Microorganisms	Extract mean ± mean S.E (mm)	Penicillin mean ± S.E (mm)	DMSO mean ± S.E (mm)
Salmonella typhi	32.33±0.577	22.67±0.667	0.00±0.000
Escherichia coli	30.33±0.882	31.33±0.333	0.00±0.000

S.E = Standard Error

Table 2: Tukey's honestly significant difference among microorganisms using 100mg/l of *Tetradenia riparia* ethyl acetate fraction.

Comparison	P- value	Significance
S. typhi vs *E. coli*	0.589	NS
S. typhi vs *S. typhi* control	0.000	S
E. coli vs *E. coli* control	0.996	NS

Key: S = Significant, NS= Not Significant

Table 3: Antibacterial activity of *Tetradenia riparia hexane fraction against* selected pathogenic microorganisms.

Microorganisms	Extract mean ± mean S.E (mm)	Penicillin mean ± S.E (mm)	DMSO mean ± S.E (mm)
Salmonella typhi	16.67±0.577	22.67±0.577	0.00±0.000
Escherichia coli	16.00±0.882	31.33±0.333	0.00±0.000

Table 4: Tukeys honestly significant difference among microorganisms using 100mg/l of *Tetradenia riparia hexane fraction*.

Comparison	P- value	Significance
S. typhi vs *E. coli*	0.998	NS
S. typhi vs *S. typhi* control	0.000	S
E. coli vs *E. coli* control	0.000	S

Key: S = Significant, NS= Not Significant

Table 5: Antibacterial activity of *Tetradenia riparia* aqua fraction against selected pathogenic microorganisms.

Microorganisms	Extract mean ± mean S.E (mm)	Penicillin mean ± S.E (mm)	DMSO mean ± S.E (mm)
Salmonella typhi	16.33±0.667	22.67±0.667	0.00±0.000
Escherichia coli	18.33±0.333	31.33±0.333	0.00±0.000

Table 6: Tukeys honestly significant difference among microorganisms using 100mg/l of *Tetradenia riparia* fraction.

Comparison	P- value	Significance
S. typhi vs *E. coli*	0.177	NS
S. typhi vs *S. typhi* control	0.000	S
E. coli vs *E. coli* control	0.000	S

Key: S = Significant, NS= Not Significant

The ethyl acetate fraction inhibited the growth of both *S. typhi* and *E. coli* with zones of inhibition of 32.33±0.557 and 30.33±0.882 respectively. The zones of inhibition caused by ethyl acetate fraction against *S. typhi* were significantly high compared to those caused by the conventional drug (Positive control). In all tests DMSO was used as the negative control with no zones of inhibition observed. The hexane fraction inhibited the growth of both organisms. However, the zones of inhibition observed were slightly lower compared to those of ethyl acetate fraction but similar to those of the water fraction (Table 1, 2, 3). These results signify that most of the active compounds are found in the mid polar ethyl acetate solvent fraction or more concentrated in this fraction. This should start in a new paragraph with previous studies in which the plant inhibited the growth of *E. coli* and *S. typhi*.[20, 21] In this study the fractionation of the crude extract showed increased activity especially in ethyl acetate fraction compared to those observed in the crude extracts of the previous studies.[22] The inhibition of *Escherichia coli* and *Salmonella typhi* is noteworthy since the two microorganisms have been known to be common agents in cases 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 travellers diarrhea to people travelling from developing countries.[23] *Salmonella typhi* causes both acute and chronic diarrhea to human all over the world.[24] The bacterium also causes travelers' diarrhea.[25] This study demonstrates that the plant has great antibacterial activity against these diarrhea causing microorganisms, which can partly be a scientific justification of the plants ethnobotanical use in the treatment against diarrhea.

The plant *Tetradenia riparia* is used traditionally by the Meru people in Kenya in the treatment of bloody diarrhea.[20] Therefore the inhibition of the plant against the selected diarrheal microorganisms is a major contribution towards provision of a scientific prove of its use in this course. The high zones of inhibition observed in ethyl acetate are recommendable,

paving way for further studies on the use of *Tetradenia riparia* bioactive compounds as leads in the fight against diarrhea causing microorgansims.

CONCLUSION

Further study needs to be done to isolate the active compounds, elucidate their structure and determine their mode of action. Formulation of the plant's extracts in to aseptic solutions is a prudent idea in the fight against diarrhea causing microorganisms.

REFERENCES

1. Guerrant R.L., Van Gilder T., Steiner T.S., Theilman M.N., Slutsker L., Tauxe R.V. Practice guidelines for the management of infectious diarrhea. *Clin. Infect. Dis.*, 2001; 32: 331-35.
2. Fauci A.S., Bravnwold E., Isselpacker K., Wilson J.D., Kasper D.L., Hauser S.L., Longo D.L., editors. *Harrison's Principles of Internal Medicine*. 13th ed. New York: McGraw Hill Company; 1993: 236-42.
3. Akuodor GC, Muazzam M. Usman-Idris, U. A. Megwas, J. L. Akpan, K. C. Chilaka D. O. Okoroafor, U. A. Osunkwo. Evaluation of the Antidiarrheal Activity of Methanol Leaf Extract of *Bombax Buonopozense* in Rats. *Ibnosina. J. Med. B.S.*, 2011; 3(1): 15-20.
4. WHO. *Country Health System Fact Sheet*. 2006.
5. Mukherjee J,, Das R., Balasubramania K., Saha M., Pal B.P. Anti-diarrheal evaluation of Nelumbo nucifera rhizome extract, *Indian Journal of pharmacology*, 1995; 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 M.L. epidemiology of drug resistance: Implications for a post-antimicrobial era. *Science*, 1992; 257: 1050-1055.
8. Gislene G.F., Locatelli J., Freitas C.P., Silva G.L. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. *Brazilian Journal of Microbiology*, 2000; 31: 247-256.
9. Akyuz M., Onganer A.N., Erecevit P. and Kirbag S. 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 G.B., Ramamurthy T., Bhattacharya S.K., Dutta B., Takeda Y. & Sack D.A. Global dissemination of Vibrio parahaemolyticus serotype O3: K6 and its serovariants. *Clin Microbiol Rev*., 2007; 20: 39–48.
11. Ameyaw Y., Duker – Eshun G. 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 C.M., Swamy A. and Obey J.K. Phytochemical and bioactivity evaluation of *Senna didymobotrya* 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., Cenkci S., Erliyaoglu N., Konuk M., Kok M.S., Bagci Y. An ethnobotanical survey of inner west Anatolia, Turkey. *Hum. Ecol.,* 2008; 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. 17-21 November. 1994; p. 316.
16. Adongo S.O., Murungi J., Wanjau R. and Ndengwa F. Analysis of selected essential elements of medicinal plants used in Chuka community, Tharaka Nithi county, Kenya. *The Scientific Journal of Science and Technology*. Special issue.
17. Ngule C.M. and Ndiku H.M. Phytochemical Evaluation and In Vitro Antibacterial Activity of Methanolic-Aqua Extract of *Indigofera arrecta* Leaves against selected Pathogenic Microorganisms. *African Journal of Pharmacology and Therapeutics*, 2014; 3(3):74-78.
18. Biruhalem T., Giday M., Animut A. and 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.
19. Donay J.L., Fernandes P., Lagrange P.H. and Herrmann J.L. 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.
20. Njau E.F.A., Alcorn J.M., Buza J., Chirino-Trejo M. and Ndakidemi P. Antimicrobial activity of *Tetradenia riparia* (HOCHST.) lamiaceae, a medicinal plant from Tanzania. *European Journal of Medicinal Plants,* 2014; 4(12): 1462-1478.

21. Ndiku M.N. and Ngule M.N. Antibacterial activity and Preliminary phytochemical screening of hot- aqua extract of Tetradenia riparia leaves. *Int. J. Pharm. Life Sci.*, 2014; 5(8): 3780-3786.
22. Anthoney, S.T. and Ngule C.M. *Herbal immunoenhancers and indigenous herbs, plants and fruits and its traditional implications in therapy including alternative remedies.* Science and Education Development Institute, Nigeria, (2014).