African Journal of Pharmacology and Therapeutics Vol. 3 No. 3 Pages 74-78, 2014
Open Access to full text available at http://journals.uonbi.ac.ke/ajpt

Research Article

Phytochemical Evaluation and *In Vitro* Antibacterial Activity of Methanolic-Aqua Extract of *Indigofera arrecta* Leaves against selected Pathogenic Microorganisms

Chrispus M. Ngule [a,*], and Hellen M. Ndiku [b]

[a] *Department of Chemistry, University of Eastern Africa, Baraton, Kenya*
[b] *Department of Family and Consumer Sciences, University of Eastern Africa, Baraton, Kenya*

* **Corresponding author:** Department of Chemistry, University of Eastern Africa, Baraton, P.O. Box 2500-30100, Eldoret, Kenya; **Tel**: +254-71-2356292; **Email**: chrismutuku73@yahoo.com

Background: The continued emergence of drug resistant microorganisms has always been a concern to scientists and pharmaceutical companies. Plants have shown great potential in the fight against this challenge, therefore creating the need for scientist to turn in to plants to get new antimicrobial agents.

Objective: The objective of the current study was to qualitatively analyze the phytochemical composition and the antibacterial activity of aqueous methanolic extracts of *Indigofera arrecta* leaves.

Methodology: The plant samples were extracted using methanol-water solvent system. The phytochemical analysis was done using standard procedures while the bioassay study was done using well diffusion method.

Results*:* The plant extract was found to contain all tested phytochemicals. The plant extract inhibited the growth of all the microorganisms tested. *Bacillus cereus* had the highest zone of inhibition while *Escherichia coli* was the least inhibited.

Discussion*:* The plant extract inhibited the growth of all the bacteria tested, suggesting the presence of antibacterial compounds in the extract. However further research needs to be done to isolate these active compounds and perform *in vivo* studies to investigate their mode of action, safety and dosage.

Key words: phytochemical, antibacterial, plant extract, *Indigofera arrecta*

Received: May, 2014
Published: August, 2014

1. Introduction

Plants have been used since time immemorial to treat various diseases affecting human beings all over the world. Before the invention of synthetic drugs traditional medicine dominated the world. Studies by WHO have shown that a large number of individuals use medicinal plants for treatment even today (WHO, 1988). It is estimated that 80% of the population in developing world use traditional medicines which mainly consists of plants for their basic health care (Prabakaran, 2011). This could be attributed to poverty in these countries that make it hard for many people to access modern hospitals and purchase allopathic drugs for treatment. It is due to this reason that many people turn to plants which are believed to be non-toxic, readily available and affordable to the local population (Ngule, 2013).

The continued emergence of drug-resistant microorganisms has always been a concern to scientists and pharmaceutical companies. Drug-resistant microorganisms have also been an economic concern with impacts of them being felt by pharmaceutical

 ISSN 2303-9841

companies, patients, medical practitioners and the public (Gowan, 2001). However, plants provide an alternative source of active compounds which can be developed as drugs. The invention of active antibiotic compounds from plants has increased the interest on the study of plants as a source of new antibiotics (Cowan, 1999 and Charindy, 1999). According to Panda (2012), quercetin and rutin isolated from *Tragia involucrata* were found to have antibacterial activity against both gram positive and gram negative bacteria.

The genus Indigofera contains 700 species of plants which belong to the family Fabaceae. The plants in this genus are mainly found in the tropical and sub-tropical regions all over the world (Prabakaran, 2011). Various species of the genus Indigofera are used for the production of the dye indigo, treatment against epilepsy, liver disease, bronchitis, psychiatric illness, anticancer therapy and anti-inflammatory activity (Anand, 1979; Veira, 2007; Rajkapoor, 2005 and Tamara, 2011).

The plant *Indigofera arrecta* is used to relieve ulcer pain. It is also used traditionally to treat stomach problems in many communities in Kenya such as the Kamba and the Nandi communities. The plant is a chief source of blue dye. *Indigoferra arrecta* is an erect, woody, large, shrub that grows to a height of 3m. The plant is majorly found in open deciduous forest, forest margins and uncultivated lands. It is natively found in east, central, south and western parts of Africa. It is also found in Swaziland, Laos, Phillippines, and Vietnum. The plant grows well in hot and moist climate of an average rainfall of 1750mm/year (Orwa, 2009). Antibacterial activity of the plant against bacteria causing diarrhea and pneumonia has been reported; however, there is paucity on literature on the plant's use against a wide range of pathogenic bacterial microorganisms (Balungile, 2013 and Tomani, 2008). The current study was done to determine the antibacterial activity of the methanolic-aqua extract of *Indigoferra arrecta* leaves against a wide range disease causing bacteria.

2. Materials and Methods

2.1 Sample Collection and Preparation

The leaves of the plant were randomly harvested from the natural forest around University of Eastern Africa, Baraton. The samples were identified by a taxonomist in the Department of Biology, University of Eastern Africa, Baraton. A voucher specimen was deposited in the Baraton University Biology Department herbarium.

The samples were thoroughly mixed and spread to dry at room temperature in the shade for about three weeks and then ground into fine powder. The powdered samples were stored in transparent polythene bags.

2.2 Extraction procedure

One hundred grams of the powdered samples were placed in 500 ml conical flask; methanol and water (9:1) were then added until the samples were completely submerged in the solvent (Chatha et al, 2006). 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 and the solvent was evaporated under reduced pressure at 40 °C. The extract thus obtained was used in the experiment.

2.3 Qualitative phytochemical analysis

The phytochemical analysis of the extract for tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids, steroids and phenols was done using standard procedures with minor adjustments (Trease, 1989; Harborne, 1973 and Sofowara, 1993).

2.4 Media preparation and primary cultures

The blood agar media (Himedia, India) was prepared according to the manufacturer's instructions. The plates were sterilized by the use of an autoclave at 121 °C. Approximately 20 ml of the prepared media was poured in to the sterilized 90 mm plates. The surface of the media was flamed using a Bunsen burner flame to remove air bubbles. The Mueller Hinton broth (Himedia, India) was prepared according to the manufacturer's instructions. About 5ml of the broth was transferred in to sterile test tubes.

The bacteria used in the study were commercial pure cultures from Carolina Biological Supply Company (USA). The colonies used in the study were obtained from the pure cultures and then transferred to the plates and test tubes using a sterile cotton swab. The plates and test tubes were then incubated at 37 °C for 24 hrs.

2.5 Preparation of the Bacterial Suspension

The turbidity of each of the bacterial suspension was prepared to match 0.5 McFarland standard (Biruhalem, 2001; Donay, 2007). 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 to five identical colonies of each bacterium were taken from the blood agar plate culture and dropped in Mueller Hinton broth. The broth culture was incubated at 37 °C for 2 - 6 hrs until it achieved turbidity similar to the 0.5 McFarland standard. 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.

2.6 Preparation of the Extract and Antibiotic working solutions

Extracts stock 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, while 100% DMSO served as a negative control.

2.7 Antibacterial assay

Mueller Hinton agar plates were prepared by the manufacturer's instructions. The media and the plates were sterilized in an autoclave at 121 °C for 15 min. The cork borer was sterilized using a non luminous flame. The plates and all the apparatus to be used in the experiment were then transferred in to a germicidal hood for 30 min. The bacterial suspension was smeared on the media and 5 wells with a diameter of 6 mm each were drilled in each agar plate using a sterile cork borer. Three of the wells were filled with 0.1 ml of the 500 mg/ml of the extract and the other wells were filled with 0.1 ml 0f 500 mg/ml of penicillin and 0.1 ml of 100% DMSO positive and negative controls, respectively. Three plates were made for each bacterial organism and each extract, thereby giving triplicate readings for each microorganism and extract. The plates were labeled on the underside and incubated at 37 °C for 24 to 48 hrs and the zones of inhibition measured in millimeters with the aid of a ruler.

3. Results and Discussion

The extract obtained was crystalline in nature with a dark brown color. The percentage yield of the extract was 29.8%, indicating a high proportion of compounds soluble in the methanolic-water mixture.

Phytochemical analysis showed the presence of all the phytochemicals tested. Some of these classes of compounds have been shown to possess antimicrobial and anti-parasitic activities.

The presence of tannins in the plant may confer anti-parasitic effects properties to the extract which may make it useful in treating parasitic infections (Akiyama, 2001). Furthermore, the growth of many fungi, yeast, bacteria and viruses has been proven to be inhibited by tannins (Chung, 1998).

Terpenoids have shown great potency against infectious microorganisms. According to Andrews et al. (1980), terpenoids have shown *in vivo* inhibition of the growth of various pathogenic bacteria. They have also shown potency against *Plasmodium falciparum*, the causative agent of malaria (Morata, 2008). Terpenoids have also been found to inhibit the growth of fungi *Candida albicans* (Morales, 2002).

Alkaloids on the other hand have been found to exhibit anti-malarial activity among other activities (Banzouzi, 2004; Boye, 1983 and Karou, 2006).

Antibacterial activity

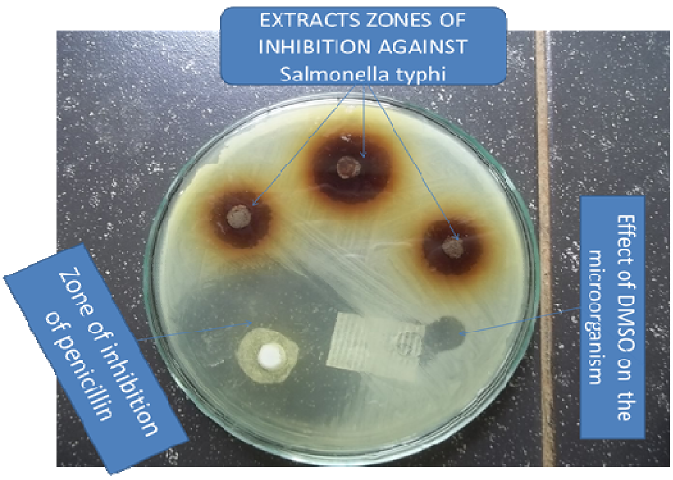
The methanol – water extract inhibited the growth of all the organisms it was tested against (**Table 1**). *Bacillus cereus* had the largest zone of inhibition, followed by *Serratia liqeufaciens*, *Salmonella typhi*, *Proteus vulgaris*, and *Enterobacter aerogenes. Escherichia coli* was the least sensitive bacteria and had the smallest zone of inhibition. **Figure 1** shows the zones of inhibition observed for *Salmonella typhi*.

Table 1: Antimicrobial activity of *Indigofera arrecta* methanolic-aqua extract against selected pathogenic bacteria

Microorganisms	Extract mean ±S.E (mm)	Penicillin mean ± S.E (mm)	DMSO Mean ± S.E (mm)
Serratia liquefaciens	18.67±0.882	39.00±0.577	0.00±0.000
Salmonella typhi	16.00±0.577	40.33±0.333	0.00±0.000
Proteus vulgaris	16.00±1.000	35.00±0.577	0.00±0.000
Escherichia coli	11.67±0.333	41.00±0.577	0.00±0.000
Enterobacter aerogenes	14.00±1.154	35.00±0.577	0.00±0.000
Bacillus cereus	22.67±1.453	39.00±0.577	0.00±0.000

S.E. = Standard error

Figure 1: Zones of inhibition of the plant extract, positive and the negative control against *Salmonella typhi*



ISSN 2303-9841

A multiple comparison of the zones of inhibition (results not shown) showed that the zones of inhibition of *Serratia liquefaciens* were significantly larger than those of *Escherichia coli* (p>0.001) and *Enterobacter aerogenes* (p< 0.05). However, there was no significance difference in the zones of inhibition *of Serratia liqeufaciens* as compared to those of *Salmonella typhi, Proteus vulgaris* and *Bacillus cereus* (p>0.05). Interestingly, the zones of inhibition of *Salmonella typhi* were significantly larger than those of *Escherichia coli.* However, the zones of inhibition of *Salmonella typhi* were significantly lower than those of *Bacillus cereus*, but were not significantly different from those of *Proteus vulgaris* and *Enterobacter aerogenes* (p>0.05). It is notable that the zones of inhibition caused by penicillin against all the organisms were significantly higher than those caused by the plant extracts against all the organisms used.

The current study is in conformity with the previous studies since similar species of the genus Indigofera have also shown significant antibacterial activity. For example, *Indigofera tinctoria* methanol extract demonstrated a antibacterial activity against methicillin sensitive *Staphylococcus aureus,* methicillin resistant *S. aureus,* Enterococcus species, Streptococcus species and *Moraxella cataruhalis* (Magesh, 2012). According to Ngocu, et al (2012), the roots of the plant *Indigofera lupatana* showed great activity against *Bacillus subtilis*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella typhi*.

The current study is also in conformity with previous studies in which the plant *Indigoferra arrecta*, aqueous and ethanolic extracts were found to have antibacterial activity against *Escherichia coli* and *Salmonella typhi.* However the current study goes further to demonstrate the antibacterial activity of the plant against a wider range of pathogenic microorganisms.

4. Conclusion

The current study shows that the plant *Indigofera arrecta* exhibits notable inhibitory activity against of all the pathogenic test bacteria. The plants antibacterial activity could be attributed to the presence of these important phytochemicals such as alkaloids and flavonoids which have previously demonstrated significant antibacterial activity. The current study partially supports the plant's traditional use in the treatment against various diseases. However further research needs to be done to isolate the active compound(s) and perform additional bioassays, including *in vivo* studies, to investigate their mode of action, safety and dosage.

Conflict of Interest declaration

The authors declare no conflict of interest

Acknowledgements

The authors of this paper are thankful to the Department of Chemistry, Department of Biology and Department of Medical Laboratory Science, University of Eastern Africa, Baraton for cooperation and support. The authors also thank the taxonomist Mr. Joel Ochieng' Ondiek for invaluable assistance in the identification of the plant.

References

Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K (2001). Antibacterial action of several tannins against *Staphylococcus aureus, J. Antimicrob. Chemother.* **48**: 487-491.

Anand KK, Chand D, Ghatak BJR (1979). Protective effect of alcoholic extract of *Indigofera tinctoria* Linn. in experimental liver injury. *Indian J. Exp. Biol.* **17**: 685-687.

Andrews RE, Parks LW, Spence KD (1980). Some effects of Douglas triterpenes on certain microorganisms. *App. Environ. Microbiol.* **40**:301-304.

Argal A and Pathak AK (2006). CNS activity of *Calotropis gigantean* roots. *J. Ethnopharmacol.* **19**: 425-428.

Balungile M, Ashwell RN, Jeffrey FF, and Johannes VS *(*2013). *In Vitro* Antimicrobial Activity of Extracts from Plants Used Traditionally in South Africa to Treat Tuberculosis and Related Symptoms. *Evid. Based Complement. Alternat. Med.* http://dx.doi.org/10.1155/2013/840719 .

Banzouzi JT, Prado R, Menan H, Valentin A, Roumestan C, Mallie MPY, and Blanche Y (2004). Studies on medicinal plants of Ivory Coast: Investigation of an active constituent. *Phytomed.* **11**: 338-341.

Biruhalem T, Giday M, Animut A, Seid J. (2001). Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. *Asian Pac. J. Trop. Biomed.* **1**: 370-375.

Boye GI and Ampufo O (1983). Proceedings' on the First International Seminar On Cryptolepic (ends BoakyeYiadom k Bamgbose, S.O.A) (University of Kumasi, Ghana).

Charindy CM, Seaforth CE, Phelps RH, Pollard GV, Khambay BP. (1999). Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. *J. Ethnopharmacol.*, **64**: 265-270.

Chung KT, Wong YT, Wei CI, Huang YW and Lin Y (1998).Tannins and human health. *Crit. Rev. Food Sci. Nutr.* **38**: 421-464.

Cowan MM (1999). Plant Products as Antimicrobial Agents. *Microbiol. Rev.* **12**: 564 - 582.

Donay JL, Fernandes P, Lagrange PH, Herrmann JL (2007). Evaluation of the inoculation procedure using a 0.25 McFarland Standard for the BD Phoenix Automated Microbiology System. *J. Clin. Microbiol.* **45**: 4088-4089.

Gowan JE (2001). Economic impact of antimicrobial resistance. *Emer. Infect. Dis.* **7**: 286-292.

Harborne JB (1973). Phytochemical methods. Chapman and Hall Ltd, London. pp 49-188

Karou D, Savadogo A, Canini A, Yameogo S, Montesano C, Simpore J, Colizzi V and Traore AS (2006). Antibacterial

 ISSN 2303-9841

activity of alkaloids from *Sida acuta*. *Afr, J. Biotechnol*. **5**: 195-200.

Magesh V, Kunjumman J, Yuvaraj G (2012). Antibacterial activity and mutagenicity of leaves of *Indigofera tinctoria* Linn. *J. Exp. Integr. Med.* **2**:263-269.

Morales G, Sierra P, Mancilla A, Paredes A, Loyola LA, Gallardo O, Borquez J. (2002). Secondary metabolites from four medicinal plants from northern Chile: Antimicrobial activity and biotoxicity against *Artemia salina*. *J. Chil. Chem. Soc*. **48**:13-18.

Murata T, Miyase T, Muregi FW, Naoshima-Ishibashi Y, Umehara K, Warashina T, Kanou S, Mkoji GM, Terada M, and Ishih A (2008) Antiplasmodial triterpenoid from *Ekebergia capensis*. *J. Plant Nat. Prod*. **71**:167-74.

Ngocu SN, Mwaniki GC, Mwendia CM, and Matasyoh JCS (2012). Screening of *Indigofera lupatana* Baker F. root extracts for antibacterial activities. *Afr. J. Pharmacol. Ther.***1**: 71-77.

Ngule CM, Anthoney ST and Jackie O (2013). Phytochemical and bioactivity evaluation of *Senna didymobotrya* Fresen Irwin used by the Nandi community in Kenya. *Int. J. Bioassays.* **2**(07): 1037-1043.

Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S (2009). *Agroforestree Database*: A tree reference and selection guide version 4.0. *World Agroforestry Centre*, Kenya.

Panda D, Dash SK, Dash GK. (2012). Phytochemical examination and antimicrobial activity of various solvent extracts and the selected isolated compounds from roots of *Tragia involucrate* linn. *Int. J. Pharm. Sci. Drug Res.* **4**: 44-48.

Prabakaran M, Chandrakala N and Panneerselvam A (2011). Antimicrobial activity of *Indigofera glandulosa* (wild). *Asian J. Plant Sci. Res.* **1**:18-25.

Rajkapoor B, Murugesh N, Chodon D, Sakthisekaran D (2005). Chemoprevention of N-Nitrosodiethylamine Induced Phenobarbitol Promoted Liver Tumors in Rat by Extract of *Indigofera aspalathoides*. *Biol. Pharm. Bull.* **28**: 364- 366.

Sofowara A (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan Nigeria, pp 191-289.

Tamara RC, C´assia RPC, Adriana CS, Lourdes CS, IlceMara SC, Wagner V, Eliana AV (2011). Mutagenic Activity of *Indigofera truxillensis* and *I. suffruticosa* Aerial Parts. *Evid. Based Complement. Alternat. Med.* doi: 10.1093/ecam/ nep123.

Trease GE & Evans WC (1989). *Pharmacognosy,* 11th Ed, Brailliere Tindall, London, pp 45-50.

Vieira JRC, De Souza IA, Do Nascimento SC, and Leite SP (2007). *Indigofera suffruticosa*: an alternative anticancer therapy. *Evid. Based Complement. Alternat. Med.* **4**: 355–359.

WHO/IUCN/WWF International Consultation on the Conservation of Medicinal Plants (Chiang Mai, Thailand, 1988).

Chatha SAS, Anwar F, Manzoor M, Bajwa JR (**2006**). Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays. *Grasas Aceites Sevilla*, **57**: 328-335.

 ISSN 2303-9841