European Journal of Medicinal Plants
4(1): 45-51, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Screening for Antimicrobial Activity of *Cissampelos pareira* L. Methanol Root Extract

Njeru S. Ngoci[1*], Musa Ramadhan[2], Mwaniki S. Ngari[3,5] and Oduor P. Leonard[4]

[1]Department of Medicine, Kisii University, Faculty of Health Sciences, PO Box 408, Kisii, Kenya.
[2]Department of Pathology, Rift Valley Provincial General Hospital, PO Box 71, Nakuru, Kenya.
[3]Department of Chemistry, Kisii University, Faculty of Science, PO Box 408, Kisii, Kenya.
[4]Department of Biological Sciences, Egerton University, PO Box 536, Egerton, Kenya.
[5]Department of Chemistry, Egerton University, PO Box 536, Egerton, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. All the authors have cordially supported the work and preparation of the manuscript. Author NSN designed and supervised the study and prepared the first draft of the manuscript. Authors MR and OPL managed the protocol and analyses of the study. Author MSN advised and guided the final draft of the manuscript. All the authors have read and approved the final manuscript.

Research Article

Received 21st June 2013
Accepted 10th September 2013
Published 9th October 2013

ABSTRACT

Aims: To screen for the antibacterial activity of *Cissampelos pareira* L. using six bacteria (Two Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and four Gram negative bacteria (*Escherichia coli, Pseudomonas aeroginosa, Klebsiella pneumoniae and Proteus vulgaris*). The phytochemicals that are responsible for the bioactivity were also screened.
Study Design: An *In vitro* antibacterial assay was done using disc diffusion.
Place and Duration of Study: Samples were collected from Mbeere community, Embu county-Kenya. Authentication of botanical identity was done at the department of biological sciences while extraction and phytochemical analysis was undertaken in the department of Chemistry, Egerton University-Kenya. Antimicrobial bioassay was carried out at Department of Microbiology, Rift Valley Provincial General Hospital.

**Corresponding author: Email: hicogn@gmail.com;*

Methodology: Disc diffusion test was used to determine antimicrobial activity to plant extracts. Chemical tests were used to determine the group of phytochemicals present in the sample extract.
Results: *Cissampelos pareira* L. methanol root extract demonstrated antibacterial activity to four of the six tested bacteria. The highest inhibition was demonstrated toward *S. aureus* (20 mm), *S. typhimurium* (17 mm), *K. pneumoniae* (14 mm) and *E. coli* at (9 mm). *P. vulgaris* and *S. pneumoniae* were not sensitive to the extract at all. The phytochemical screening demonstrated the presence of all phytochemicals tested (alkaloids, flavonoids, tannins, terpenoids and steroids).
Conclusion: This study reveals that *Cissampelos pareira* L. has antibacterial activity to both Gram positive and Gram negative bacteria. This antibacterial activity is associated with the variety of phytochemicals found in this plant. Therefore, the plant has potential to be harnessed for further study in drug discovery.

Keywords: Antibacterial activity; phytochemicals; root extracts; Cissampelos pareira L.; herb.

1. BACKGROUND INFORMATION

Infectious diseases are becoming a crisis as a major cause of human and animal mortality and morbidity. This is further aggravated by the rapid development of multi-drug resistance, limited anti-bacterial spectrum and adverse effects of available anti-microbial agents [1]. This has consequently increased the attention and demand given to antimicrobials derived from the plants since synthetic antibiotics have shown ineffectiveness against several pathogenic organisms as a result of increasing drug resistance [2].

Plants have been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses of plants from as early as 3000 BC. Indigenous cultures such as African and Native American tended to use herbs in their healing rituals, while others developed traditional medical systems such as Ayurveda and Traditional Chinese Medicine in which herbal therapies were used [3].

Plants have played an important role in the discovery of novel and useful drugs used in modern medicine. Currently, there are a number of drugs of plant origin which are useful, with life saving capacity and providing immediate therapeutic benefit [4]. Drugs derived from unmodified natural products or semi-synthetic drugs obtained from natural sources accounted for 78 % of the new drugs approved by the United States Food and Drug Administration (FDA) between 1983 and 1994 [5]. Approximately 28 % of new chemical entities between 1981 and 2002 were natural products or natural product-derived [6].

Among the estimated 250,000-500,000 plant species, only a small number has been screened phytochemically and their fraction submitted for biological or pharmacological screening. Over 2000 plant species are found to have medicinal value and are used for medicinal purpose in different forms [7].

Plants have been serving mankind for long by acting as rich sources of useful drugs, food, additives, flavouring agents, colourants, binders and lubricants. The medicinal value of plants is associated with some chemical substances also known as phytochemicals that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants origin are alkaloids, glycosides, essential oil, saponins, tannins, steroids, terpenoids, resins, flavonoids, proteins and others [7, 8].

Cissampelos pareira L. (*Menispermaceae*), locally in Mbeere community of Embu County in Kenya called '*Karigi – kanonongwe'* [9] is a woody, climbing ground creeper vine. It produces inedible, dark, grape-sized drupes of about 4 – 6 mm. It is also distributed throughout warm parts of Asia, East Africa, and America. The roots are used as a diuretic and febrifuge, as a remedy for heart trouble, dysentery and sores. The roots of this plant are mainly incorporated into many traditional Ayurvedic formulation prescribed for diseases like rheumatism, ulcers, fevers. *C. pareira* is believed to kill bacteria, prevents convulsions, ulcers, indigestion, skin irritations, manage cough, fever, intestinal worms, purgative stomachic, wounds, snake bite and in being an antiperiodic [8-10].

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Sample

The plant roots studied were collected from Kathuri Village, Mbeere community, Embu County in Kenya in April 2012. The plant was identified through ethnobotanical approach by consulting herbalists in the village. The taxonomical authentication of identity was undertaken by the departmental botanist at the Department of Biological Sciences of Egerton University, Kenya where sample voucher specimen NSN. 2 was deposited.

2.2 Preparation of Extract

The plant roots were air dried at room temperature before grinding to powder with a mechanical grinder. The powder (150 g) was macerated in Methanol (300 ml) for four days with intermittent shaking, the mixture was then filtered (Whatmann filter paper No. 1). The filtrate was concentrated and evaporated to dryness by a rotary vacuum evaporator (BÜCHI ROTAVAPOR R-205 V805, Flawil, Switzerland) at 40ºC and allowed to air dry.

2.3 Collection of Test Micro-Organisms

A total of six clinical microbial isolate strains were used. These micro-organisms were obtained and maintained in agar slants at Rift Valley Provincial General Hospital laboratory (department of bacteriology). The bacteria that were used included: Two Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and four Gram negative bacteria (*Escherichia coli, Pseudomonas aeroginosa, Klebsiella pneumoniae and Proteus vulgaris*).

2.4 Anti-Microbial Assays

This was done to evaluate *in vitro* activity of the anti-microbial agents. The results were reported quantitatively in terms of diameter of zones of inhibition [11]. Nutrient agar was used for sub culturing of the test micro-organisms at 37ºC for 24 hours and Mueller Hinton agar was used for sensitivity assay. The Media was reconstituted according to the Manufacturer's instruction, sterilized by autoclaving at 121ºC for 15 minutes and then dispensed aseptically into Petri dishes (9 cm diameter) in a volume of 22 milliliters molten agar and left to solidify and then stored in the refrigerator at 4ºC. Whatmann filter (No. 1) discs of 6 mm diameter were made by punching the paper and the blank discs were sterilized in hot air oven at 160ºC for one hour. They were then impregnated with 10 µl of the varying concentration of the crude extract solution. The impregnated discs were then

evaporated at 50ºC till they dried. Chloramphenicol at 50 µg/disc (Oxoid) was used as a positive control due to its broad spectrum property.

2.5 Disc Diffusion Test

The anti-microbial activity was assayed by disc diffusion method according to Ayo et al., [12] and Mbaveng et al. [13]. The bacterial strains were activated by growing them in Nutrient agar at 37ºC for 18 – 24 hours. A fresh inoculum was developed by suspending activated colonies in physiological saline solution (0.85% NaCl). The cell suspension was then standardized using 0.5 McFarland turbidity standard. The suspension was used to aseptically inoculate by swabbing the surface of Mueller Hinton agar plates. The impregnated discs with extracts were then planted at equidistant points on top of the inoculated agar medium by sterile forceps. The positive control Chloramphenicol disc was also planted. The inoculated plates were then incubated at 4ºC for 2 hours to allow pre-diffusion of extracts into the media then incubated at 37ºC for 24 hours after which they were inspected for zones of inhibition. Anti microbial activity was then evaluated by measuring the diameter of the inhibition zones.

2.6 Phytochemical Tests

Determination of presence of alkaloids, flavonoids, tannins, terpenoids and steroids was done qualitatively using the protocols according to Ngoci et al. [5].

3. RESULTS

Cissampelos pareira L. methanol root extract demonstrated antibacterial activity to four of the tested bacteria. Highest inhibition was demonstrated toward *S. aureus* (20 mm), *S. typhimurium* (17 mm), *K. pneumoniae* (14 mm) and *E. coli* at (9 mm). *P. vulgaris* and *S. pneumoniae* were not sensitive to the extract (Table 1).

Table 1. Anti-bacterial activity result for the methanol root extract

Microorganism	Inhibition zones diameter in mm					STD
	Extract concentration (µg x 10^2)					
Gram negative	**50**	**25**	**12.50**	**6.25**	**3.125**	**50 µg**
S. typhimurium	17± 0.7	13±0.6	10±1.2	6.3±0.3	0	27± 0.6
K. pneumoniae	14± 0.6	10±0.6	7±0.6	0	0	22± 0.5
E. coli	9± 0.6	6± 0.0	0	0	0	44± 0.5
P. vulgaris	0	0	0	0	0	35± 0.6
Gram positive						
S. aureus	20± 0.6	17± 0.6	14±0.6	8±0.6	0	35± 0.6
S. pneumoniae	0	0	0	0	0	25± 0.5

STD – Represents positive control (Chloramphenicol); Values of inhibition zones are in mm (mean±SEM, n=3).

Phytochemical screening demonstrated the presence of all phytochemicals tested (alkaloids, flavonoids, tannins, terpenoids and steroids (Table 2).

Table 2. Phytochemical results

Phytochemicals	Methanol extract result
Alkaloids	Positive
Flavonoids	Positive
Tannins	Positive
Terpenoids	Positive
Steroids	Positive

4. DISCUSSION

Methanol root extract of *Cissampelos pareira* L. had a broad spectrum activity by inhibiting both Gram positive and Gram negative bacteria. This is in agreement with work in Nepal by Maharjan et al. [14] where they demonstrated activity of *Cissampelos pareira* L root extract toward *K. pneumoniae, E. coli* and *S. aureus*, although the activity demonstrated in this study was higher. However a study by Basha et al. [8] in India showed that the *Cissampelos pareira* L root extract had no activity against *K. pneumoniae.* This difference can be associated with diversity of plants bioactive compounds which is influenced by genetic characteristics, environmental factors such as climate, altitude and soil type; the period in plants life history when collection took place, the treatment after collection and existence of a distinct phenotype of a particular species (also known as chemical races) [15]. This diversity can either be in regard to presence and absence of certain phytochemicals or be in the levels of concentration of a certain phytochemical in a plant sample. This can explain why in the studies by Maharjan et al. [14], they demonstrated absence of alkaloids while in this particular study, alkaloids where shown to be present.

The activity toward bacteria can only be attributed to the bioactive compounds that tested positive in the extract sample. Alkaloids which were present in the extract usually have antimicrobial activity where they function by potentiating the role of immune cells as well as by interfering with the microbial DNA and cell wall [16]. Flavonoids and tannins which tested positive in the extract have also been shown to have antibacterial activity where they act by complexing microbial proteins thus interfering with bacterial adhesion and inactivating bacterial enzymes [5,17-19]. They also act by disrupting microbial membranes as it is with terpenoids and phytosteroids [17-19]. The extract fraction inhibited some bacteria that are associated with gastrointestinal infections and respiratory tract infections. This is supportive of the traditional usage of the plant in management of such conditions.

5. CONCLUSION AND RECOMMENDATION

This study supports that *Cissampelos pareira* L. has antimicrobial activity to different bacteria. This antibacterial activity is associated with the variety of phytochemicals found in this plant. The plant has potential to be harnessed for further study in drug discovery.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENTS

Authors highly acknowledge the support and facilities provided by the Departments of Chemistry and Biological Sciences, Egerton University and Department of Microbiology, Rift Valley Provincial General Hospital.

COMPETING INTERESTS

The entire work is in full consent of all the authors and all the authors have declared that there is no competing interest exists.

REFERENCES

1. Doughart JH, Okafor B. Anti-microbial activity of *Senna alata Linn*. East and Central Afr J. Pharm Sci. 2007;10: 17-21.
2. Akgul C, Saglikoglu G. Antibacterial activity of crude methanolic extract and its fractions of aerial parts of *Anthemis tinctoria*. Indian J. Biochem and Biophy. 2005;42:395-397.
3. Taylor L. Herbal Medicine versus the FDA. 2013. Accessed 15/6/2013. Available: http://www.rain- tree.com/news-01132012.htm#.UbwTxtiF7Mw.
4. Kannan M, Lija L, Francis Xavier T, Auxillia T. Antimicrobial activity of the medicinal plant *Senna obtusa roxb.* Inter. J Bio Pharm and Appl Sci. 2013;2(5):1135-1140.
5. Ngoci SN, Mwendia CM, Mwaniki CG. Phytochemical and cytotoxicity testing of *Indigofera lupatana* Baker F. J. Animal & Plant Sci. 2011;11(1):1364-1373.
6. Moustafa MFM, Alamri SA, Taha TH, Alrumman SA. *In vitro* antifungal activity of *Argemone ochroleuca* Sweet latex against some pathogenic fungi. . Afr J Biotech. 2013;12(10):1132-1137.
7. Amer WM, Abouwarda AM, El Garf IA, Dawoud GTM and Abdelmohsen G. Phytochemical composition of *Solanum elaeagnifolium* Cav. and its antibacterial activity. J Bio Pharm and Appl Sci. 2013;2(6):1282-1306.
8. Basha KS, Sudarsanam G, Hari Babu Rao D, Niaz Parveen. Evaluation of antibacterial activity of some medicinal plants used by Sugali tribe of Yerramalais forest of Andhra Pradesh, India. J. Res in Plant Sci. 2011;1:027-031.
9. Riley BW, Brokensha D. The Mbeere in Kenya; Botanical identity and use Vol (ii), University press of America, USA; 1998.
10. Chitravadivu C, Bhoopathi M, Balakrishnan V, Elavazhagan T, Jayakumar S. Antimicrobial Activity of Laehiums Prepared by Herbal Venders, South India. American-Eurasian J Sci Res. 2009;4(3):142-147.
11. Collins CH, Lyne PM, Grange JM. Microbiological methods. 7th ed. Butterworth-Heinemann, Great Britain; 1995.
12. Ayo RG, Amupitan JO, Zhao Y. Cytotoxicity and anti-microbial studies of 1,6,8-trihydroxy-3-methyl-anthraquinone (Emodin) isolated from the leaves of *Cassia nigricans Vah*l. Afri. J. of Biotech. 2007;6:1276-1279.
13. Mbaveng AT, Ngameni B, Kuete V, Simo IK, Ambassa P, Roy R, et al. Anti-microbial activity of the crude extracts and five flavonoids from the twigs of *Dorstenia barteri (Moraceae).* J. Ethnopharmac. 2008;116:483-489.
14. Maharjan BL, Mainali L, Baral B. Phytochemical screening and antimicrobial assay of some nepalese medicinal plants. Sci World. 2011;9(9):90-92.
15. Houghton PJ, Raman A. Laboratory Hand book for the Fractionation of Natural Extracts. 1st ed. Chapman and Hall, London; 1998.

16. Ngoci SN, Macharia E, Ng'ang'a E. Screening for anti-bacterial activity and phytochemicals of *Leonotis nepetifolialeaves* methanol extract. J Biotech Sci. 2013; 1(1):15-21.
17. Cowan MM. Plant products as anti-microbial agents. Clin Micro Rev. 1999;12**:**564-582.
18. Samy RP, Gopalakrishnakone P. Review: Therapeutic potential of plants as anti-microbials for drug discovery. J Evidence-Based Compl and Altern med. 2008; doi:10.1093/ecam/nen036.
19. Njeru SN, Matasyoh JC, Gathinji MC, Mwendia CM. Antibacterial activity of methanol root extract of *Indigofera lupatana* Baker F. East J of Med. 2012;17:11-16.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=281&id=13&aid=2227