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Antifungal Activity of Eight Selected Medicinal Herbs Used for the Treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya

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Abstract: A diploid fungus, *Candida albicans,* is a form of yeast that is a casual agent of opportunistic oral and genital infections in humans and is traditionally treated using herbs. Amongst the indigenous herbs used for the purpose in Kisii region, southwest Kenya are: *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis Peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia sciatica.* A study was carried out on these herbal plants in the year 2011and 2012. The objective was to determine the antifungal activity of these herbs that are also used for the treatment of diabetes, malaria and pneumonia. In the study, leaf samples of these plants were obtained from Kisii region, washed, air-dried and milled. The samples were extracted with four solvents namely hexane, dichloromethane, ethyl acetate and ethanol. Portions of the crude extracts were screened against *Candida albicans,* by the well diffusion method. Results showed that the standard antibiotics namely chloramphenicol, minocycline, erythromycin and cotrimoazol had diameters of the inhibition zones measuring (mm), 33, 32, 31 and 25, respectively which indicated inhibition of microbial growth. However, the extracts of hexane and solvents had no antifungal activity against the *Candida albicans* as they had diameters of the inhibition zones of 12 mm. The dichloromethane extracts of *Leonotis nepetifolia and Bidens pilosa* showed antifungal activity of diameters of the inhibition zones measuring 19 mm and 16 mm respectively. The ethyl acetate extracts of *Leonotis nepetifolia, Bidens Pilosa, Senna didymobotrya, Toddalia asiatica* and *Physalis Peruviana* recorded antifungal activity with diameters of the inhibition zones measuring (mm) 24, 18, 18, 17 and 15 respectively. The ethanol extracts of *Leonotis nepetifolia* and *Physalis Peruviana* displayed antifungal activity with diameters of the inhibition zones 27 mm and 19 mm, respectively. The dichloromethane, ethyl acetate and ethanol extracts of *Leonotis nepetifolia* recorded maximum antifungal activity against *Candida albicans*. The findings suggest that the herbal extracts of *Leonotis nepetifolia, Bidens Pilosa, Senna didymobotrya, Toddalia asiatica* and *Physalis Peruviana* have a potential to control *Candida albicans* as they have diameter zone of inhibition above 12 mm.

Key words: Medicinal Herbs · Antifungal Activity · Candida Albicans

INTRODUCTION

An antimicrobial is a compound that kills or inhibits the growth of microbes such as bacteria (antimicrobial activity), fungi (antifungal activity), viruses (antiviral activity) or parasites (antiparasitic activity) [1]. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids and glycosides etc, which have been found to have antimicrobial properties [2, 3]. Plants have been used as traditional medicine since time immemorial to control bacterial, viral and fungal diseases [4- 8]. Medicinal plants represent a rich source of antimicrobial agents [9- 12]. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [13]. The different parts of herbs used include leaves for treatment of ailments [13].

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In the Kisii region, the leave decoction of *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica*, are used for the treatment of diabetes, malaria and pneumonia [14]. Herbal medicines have been known to man for centuries [15]. Similar elaborate and rich pharmacopoeia systems have also been documented for other communities in Kenya such as the Gusii, Maasai, Luo, Abaluyia and the Kikuyu people [15].For most tribes of Kenya, knowledge and use of ethno-medicine was passed down orally from generation to generation presumably to trustworthy persons (usually first-born sons) that would continue the tradition and practice. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care [16]. Many of the herbs used today have been valued for their antimicrobial effects and medicinal powers in addition to their flavour and fragrance qualities [17, 18].

The objective of this study was to determine the antifungal activity of extracts from the eight selected medicinal herbs used for the treatment of diabetes, malaria and pneumonia in Kisii region, Southwest Kenya against *Candida albicans.*

MATERIAL AND METHODS

Plant Collection: In this study the leaves of the *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica* were collected from Kisii region, southwest Kenya. The verification of the herbal species was done by the Botanist; Egerton University. The leaves of the authenticated herbal plants were then collected from their site in Kisii region and air-dried for twelve weeks to obtain constant weight. The dried sample was cut into smaller pieces and then ground into fine particles with a grinder at the Department of Food Science and Technology, Faculty of Science, Jomo Kenyatta University of Agriculture and Technology. The powdered sample was bagged in black plastic bags and stored in an air-tight container for further work.

Extraction: A sample of the powder weighing 30 g was extracted with 160 ml of hexane, dichloromethane, ethyl acetate and 96% ethanol. The extraction was carried out in a ¼ L flask. Four extractions were done in each solvent used and the extracts were concentrated to about one-sixth of the original volume at 60°C under reduced pressure using a rotary evaporator. The extracts were air-dried for three weeks to a constant weight and kept in air-tight containers for further work.

Antimicrobial Activity Test: The crude extracts were tested against 24 hour broth cultures of *Candida albicans*. The tests were performed at the Department of Food and Science Technology, Microbiology laboratory of the Faculty of Science, in Jomo Kenyatta University of Agriculture and Technology (JKUAT).

Determination of Antifungal Activity

Making up Extract Solution: Approximately 0.02g of dried hexane, dichloromethane, ethyl acetate and ethanol crude extract of each of the eight herbs was weighed and transferred to a 10 ml volumetric flask. The respective solvent was added to make up the 10 ml solution (0.02g in 0.01L).

Microorganisms: Micro-organisms namely *Candida albicans* were obtained from the Department of Food and Science Technology, Microbiology Laboratory and stored in a refrigerator of the same laboratory, in JKUAT.

Nutrient Agar: Nutrient agar was purchased from the Pharmacy Association in Nairobi. About 7g of nutrient agar was suspended in 250ml of distilled water in a 1L flask, stirred, boiled to dissolve and then autoclaved for 15 minutes at 121°C. The pH range for the nutrient agar was between 7.0 and 8.0.

Reference and Control: The references were antibiotic in nature. Erythromycin (15µg), Minocycline (5µg), Chloramphenicol (30µg) and Cotrimoazol (25µg) was chosen as the reference for the fungus species used: *Candida albicans*. The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion.

Well Diffusion Method: Bioassay tests were performed on the herbal crude extracts to ascertain their activity against *Candida albicans*. In the test tube, 20ml nutrient agar was melted at 100°C and stabilized at 45°C for about 15 minutes. About 0.1ml inoculums were added from culture tubes to the agar in the test tube by the use of a loop. The test tube containing the agar and the inoculums was then rolled in between the palms gently to mix the inoculums thoroughly with the agar. The loop was flamed before it was used each time. The content of the test tube was poured into a Petri dish and allowed to set. The Petri

dishes were then labelled with the respective organism (inoculums) and date. By means of a 6 mm cork borer, four cups were bored, well separated and equidistant from each other in the agar. The cups were labelled with the four crude extracts. Each cup was filled with its corresponding 0.02mg/ml extract to about three-quarters full. They were kept on a bench at room temperature for about 60 minutes (for the extracts to diffuse into the agar). The plates were then incubated aerobically at 37°C and examined for any zone of inhibition after 48 hours. The same procedure was repeated with the references using the chosen antibiotics and control using the pure solvent hexane, dichloromethane, ethyl acetate and ethanol. The reading was done against a dark background under reflected light. The diameters of the zones of growth of inhibition were measured with the help of Hi Antibiotic zone scale (range 1cm-35 cm or 10mm-400mm) from the underside of the covered plates for spots with inhibitions. The average of the diameters was taken. The actual zones were calculated by subtracting the diameter of the cups (6 mm) from the total zone of growth.

Data Collection: The antifungal activity of the selected eight herbs against fungus *Candida albicans* were obtained by measuring the diameters of the inhibition zones and compared them with that of the control drug erythromycin, chloramphenicol, minocyline and cotrimoazol. Antifungal activity was expressed as the mean zone of inhibition diameters (mm) produced by the herb extracts.

Data Analysis: The null hypothesis being tested is that there is no significant biological activity displayed by the Compounds present in the selected traditional herbs used in Kisii region to treat diabetes, malaria and pneumonia diseases. Results obtained in this study were expressed as mean inhibition zone (mm) ± S.D of three replicates. The mean and the S.D of each herbal extract were used to compute the calculated t-value. Differences between the critical t-value and calculated t-values of the diameter of the inhibition zones of the herbal extracts on *Candida albicans* were computed. For all the eight herbal species, the null hypothesis was retained because the calculated t-value was less than the critical t-value at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Zone of inhibitions of hexane extracts against *Candida albicans*: Results obtained show that the antifungal activity of the control pure solvent hexane and all eight herbal hexane leaf extracts recorded 12mm diameters of the inhibition zones, indicating no inhibition zone or antifungal activity [19]. However, the used standard antibiotics minocycline, chloramphenicol and cotrimoazol measured (mm) diameters of the inhibition zones of 30, 24 and 18 respectively (Table 1).

Zone of Inhibitions of Dichloromethane Extracts Against *Candida Albicans*: Results obtained show that the pure solvent dichloromethane recorded 12 mm, indicating no antifungal activity. However, the herb *Leonotis nepetifolia* dichloromethane leaf extract recorded antifungal activity of (19 mm) against *Candida albicans.* The dichloromethane leaf extract of *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa* and *Toddalia asiatica* recorded (12 mm) diameters of the inhibition zones, indicating no antifungal activity. The herbal dichloromethane leaf extract of *Leonotis nepetifolia* recorded the highest antifungal activity of (19mm) against *Candida albicans* while the rest of the tested herbs showed no antifungal activity [20]. However, the standard antibiotics minocycline, chloramphenicol and erythromycin diameters of inhibition zones, measured (mm), 32, 33 and 31 respectively recorded higher antifungal activity than leaf extract of *Leonotis nepetifolia* of 19mm (Table 1).

Zone of Inhibitions of Ethyl Acetate Extracts Against *Candida Albicans*: The results obtained show that the pure solvent ethyl acetate diameters of inhibition zones measured (12 mm), indicating no antifungal activity [21, 22]. The herbal ethyl acetate leaf extract of *Leonotis nepetifolia* recorded diameters of inhibition zones (24mm) indicating antifungal activity against *Candida abacas*. However, ethyl acetate leaf extract of *Senna didymobotrya and Bidens pilosa* recorded antifungal activity of (18 mm), followed by *Toddalia asiatica* (17 mm) and *Physalis peruviana* (15 mm). The ethyl acetate leaf extract of *Carissa spinarum, Urtica dioica* and *Warburgia ugandensis* recorded diameters of inhibition zones (12mm), indicating no antifungal activity. The herbal ethyl acetate leaf extract of *Leonotis nepetifolia* recorded the highest antifungal activity of (24 mm) while *Physalis peruviana* showed the lowest antifungal activity of *(*15 mm) against *Candida albicans.* However, the standard antibiotic cotrimoazol recorded the highest antifungal activity of (25mm), followed by

Table 1: Zone of inhibitions of hexane, dichloromethane, ethyl acetate and ethanol extracts of the eight selected medicinal herbs against *Candida albicans.*

		Diameter of zone of inhibition (mm)			
Selected medicinal herb species	Volume of extract (ml)	Hexane extract	Dichloromethane extract	Ethyl acetate extract	Ethanol extract
Carissa spinarum	0.2	12±0.0	12±0.0	12±0.0	12±0.0
Urtica dioica	0.2	12±0.0	12±0.0	12±0.0	12±0.0
Warburgia ugandensis	0.2	12±0.0	12±0.0	12±0.0	12±0.0
Senna didymobotrya	0.2	12±0.0	12±0.0	18±1.0	12±0.0
Physalis peruviana	0.2	12±0.0	12±0.0	15±1.0	19±1.0
Bidens pilosa	0.2	12±0.0	12±0.0	18±1.0	12±0.0
Leonotis nepetifolia	0.2	12±0.0	19±1.0	24±1.0	27±1.0
Toddalia asiatica	0.2	12±0.0	12±0.0	17±1.0	12±0.0
Control (Hexane)	0.2	12±0.0	12±0.0	12±0.0	12±0.0
References					
Minocycline	30µg	30±1.0	16±1.0	16±1.0	16±1.0
Chloramphenicol	30µg	24±1.0	12±0.0	12±0.0	25±1.0
Cotrimoazol	25µg	18±1.0	25±1.0	25±1.0	24±1.0

minocycline (16mm) against *Candida albicans*. The standard antibiotic chloramphenicol recorded diameters of inhibition zones (12mm), indicating no antifungal activity against *Candida albicans* (Table 1).

Zone of Inhibition of Ethanol Extracts Against *Candida Albicans*: Results obtained indicate that the herbs *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Bidens pilosa, Toddalia asiatica* and pure solvent ethanol, measured diameters of inhibition zones (12 mm), indicating no antifungal activity. The herbal ethanol leaf extract of *Leonotis nepetifolia* and *Physalis peruviana* measured antifungal activity (mm), 27 and 19 respectively against *Candida albicans* [23-25]. The ethanol herbal leaf extract of *Leonotis nepetifolia* recorded the highest antifungal activity of (27mm) while *Physalis peruviana* recorded the lowest antifungal activity of (19mm) *against Candida albicans*. However, standard antibiotic cotrimoazol, Chlorophenicol, minocycline recorded antifungal activity (mm), 25, 24 and 16 respectively (Table 1).

CONCLUSIONS

The hexane leaf extracts of the eight herbs studied showed no antifungal activity against *Candida albicans*. However, the dichloromethane leaf extracts of the *Leonotis nepetifolia* had antifungal activity against *Candida albicans*. Results also showed that the ethyl acetate leaf extracts of *Senna didymobotrya, Physalis Peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica,* recorded antifungal activity against *Candida albicans*. The highest antifungal activity against *Candida albicans* was noted in ethanol leaf extracts of *Leonotis nepetifolia* and lowest in ethanol leaf extracts of the *Physalis Peruviana*. Future work should target the isolation and purification of bioactive constituents of the dichloromethane, ethyl acetate and ethanol leaf extracts of the *Senna didymobotrya, Physalis Peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica* herbs to identify the active compounds associated with the antifungal activities.

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REFERENCES

1. Jagessar, R.C., A. Mars and G. Gomes, 2008. Selective Antimicrobial properties of *Phyllanthus acidus* leaf extract against *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* using Stokes Disc diffusion, Well diffusion, Streak plate and a dilution method. Nature and Science, 6(2): 24-38.

2. Lewis, K. and F.M. Ausubel, 2006. Prospects of plant derived antibacterial. Nature and Biotechnology, 24: 1504-1507.
3. Savithramma, N., M. Linga Rao and D. Suhrulatha, 2011. Screening of medicinal plants for secondary metabolites. Middle-East Journal of Scientific Research, 8: 579-584.
4. Balakumar, S., S. Rajan, T. Thirunalasundari and S. Jeeva, 2011. Antifungal activity of Aegle marmelos (L.) Correa (Rutaceae) leaf extract on dermatophytes. Asian Pac. Jo. Trop. Biomed. 1: 309-312.
5. Nisar, M., S Ali and M. Qaisar, 2011. Preliminary Phytochemical Screening of Flowers, Leaves, Bark, Stem and Roots of Rhododendron arboretum. Middle-East Journal of Scientific Research, 10(4): 472-476.
6. Anpin Raja, R.D., S. Jeeva, J.W. Prakash, M. Johnson and V. Irudayaraj, 2011. Antibacterial activity of selected ethnomedicinal plants from South India. Asian Pac. J. Trop. Med., 4: 375-378.
7. Sharma, A. R. Verma and P. Ramteke, 2009. Antibacterial activity of some medicinal plants used by tribals against Uti causing pathogens. World Applied Sciences Journal, 7(3): 332-339.
8. Tirupathi Rao, G., K. Suresh Babu, J. Ujwal Kumar and P. Sujana, 2011. Veerabhadr Rao, Sreedhar AS. Anti-microbial principles of selected remedial plants from southern India. Asian Pac. J. Trop. Biomed., 1: 298-305.
9. Parekh, J., N. Karathia and S. Chanda, 2006. Screening of some traditionally used medicinal plants for potential antibacterial activity. Ind. J. Pharma. Sci., pp: 832-834.
10. Kambizi, L. and A.J. Afolayan, 2008. Extracts from *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrhoea*. African J. Biotechnol., 7: 12-15.
11. Thatoi, H.N., S.K. Panda, S.K. Rath and S.K. Dutta, 2008. Antimicrobial Activity and Ethnomedicinal Uses of Some Medicinal Plants from Similipal Biosphere Reserve, Orissa. Asian Journal of Plant Sciences, 7: 260-267.
12. Afolayan, A.J., D.S. Grierson, L. Kambizi, I. Madamombe and P.J. Masika, 2002. *In vitro* antifungal activity of some South African medicinal plants. S. Afri. J. Bot., 68: 72-76.
13. Mahesh, B. and S. Satish, 2008. Antimicrobial activity of some important medicinal plant against plant and human Pathogens. World Journal of Agricultural Sciences, 4(S): 839-843.
14. Gisesa, W.N.O., 2004. An Ethnopharmacological Investigation of Plants used by Abagusii Traditional Medical Practitioners, PhD Thesis, School of Pure and Applied Sciences, Kenyatta University.
15. Karinge, J.W., 2006. A Survey of Traditional Health Remedies Used by the Maasai of Southern Kaijiado District, Kenya. Ethnobotany Research & Applications, 4: 061-073.
16. Calixto, B.J., 2000. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Brazilian Journal of Medical and Biological Research, 33(2): 179-189.
17. Ceylan, E. and D.Y. Fung, 2004. Antimicrobial activity of spices. Journal of Rapid Methods and Auto Microbiology, 12: 1-55.
18. Davidson, P.M., J.N. Sofos and A.L. Branen, 2005. Antimicrobials in food. 3 ed. CRC Press, Taylor and Francis Group. Boca Raton FL 33431, USA.
19. Chitravadivu, C., M. Bhoopathi, T. Elavazhagan, S. Jayakumar and V. Balakrishnan, 2009. Screening of antimicrobial activity of medicinal plant oils prepared by herbal venders, South India. Middle-East Journal of Scientific Research, 4(2): 115-117.
20. Hasan, M., F. Das, R. Khan, A. Hossain and Rahman, M. 2009. The determination of antibacterial and antifungal activities of *Polygonum hydropiper* (*L.*) roots extract. Advances in Biological Research, 3 (1-2): 53-56.
21. Khond, M., J.D. Bhosale, T. Arif, T.K. Mandal, M.M. Padhi and R. Dabur, 2009. Screening of some selected medicinal plants extracts for *In-vitro* antimicrobial activity. Middle-East Journal of Scientific Research, 4(4): 271-278.
22. Bansod, S. and M. Rai, 2008. Antifungal activity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus fumigatus* and A. niger. World Journal of Medical Sciences, 3 (2): 81-88.
23. Chitravadivu, C., M. Bhoopathi, V. Balakrishnan, T. Elavazhagan, and S. Jayakumar, 2009. Antimicrobial activity of Laehiums prepared by herbal venders, South India. American-Eurasian Journal of Scientific Research, 4(3): 142-147.
24. Ravikumar, S., G.P. Selvan and A.A. Gracelin, 2010. Antimicrobial activity of medicinal plants along Kanyakumari Coast, Tamil Nadu, India. African Journal of Basic & Applied Sciences, 2(5-6): 153-157.
25. Babu, D. and R.S. Subhasree, 2009. Antimicrobial activities of *Lawsonia inermis* - A Review. Academic Journal of Plant Sciences, 2(4): 231-232.