African Journal of Pharmacology and Therapeutics Vol. 3 No. 1 Pages 14-18, 2014
Open Access to full text available at http://journals.uonbi.ac.ke/ajpt

Research Article

Antibacterial and Antifungal Activity of *Dombeya torrida* (J.F. Gmel) and *Hydnora abyssinica* (A. Braun)

Stanley N. Ndwigah [a,*], Beatrice K. Amugune [a], Grace N. Thoithi [a], Julius W. Mwangi [b], Hannington N. Mugo [a], and Isaac O. Kibwage [a]

[a] *Department of Pharmaceutical Chemistry, School of Pharmacy, University of Nairobi, Kenya*
[b] *Department of Pharmacology & Pharmacognosy, School of Pharmacy, University of Nairobi, Kenya*

* **Corresponding author:** Department of Pharmaceutical Chemistry, School of Pharmacy, University of Nairobi, P.O. Box 19676-00202 KNH, Nairobi, Kenya; **Tel**: +254-20-716962; **Email**: snndwigah@uonbi.ac.ke

Background: The decoction of *Dombeya torrida* bark is used to treat indigestion while its roots are used for treatment of chest pains and colds. *Hydnora abyssinica* decoction is used as a cure for throat complaints, as an astringent in dysentery, for treatment of typhoid, anthrax, and East Coast Fever.

Objectives: The present study was aimed at investigating the antibacterial and antifungal activities of *D. torrida and H. abyssinica*.

Methodology: The *D. torrida* stem-bark and leaves and *H. abyssinica* whole plant were collected from Kiambu County and Embu County, respectively. Extraction of the plants was carried out using chloroform, methanol and water. The extracts were screened for activity against *Staphylococcus aureus, Staphylococcus epididermis, Bacillus pumilus, Escherichia coli, Saccromyces cerevisiae* and *Candida albicans* using agar diffusion assay and autobioassay.

Results: *Dombeya torrida* bark decoction had the highest activity against *S. aureus* with an inhibition zone diameter of 16.91 mm. *Hydnora abyssinica* macerate had least activity against *S. aureus* with a zone diameter of 8.86 mm. *Dombeya torrida* bark decoction had the highest activity against *S. Epididermis* with a diameter of 17.05 mm with *Hydnora abyssinica* macerate having the least activity. Activity against *E. coli* was highest for *D. torrida* bark decoction with zone diameter of 16.56 mm. *Hydnora abyssinica* chloroform extract had the highest activity against *B. pumilus* with a zone diameter of 17.04 mm. The highest activity observed against *S. cerevisiae* was with *D. torrida* chloroform extract with a zone diameter of 17.69 mm with *H. abyssinica* macerate having the least activity (7.70 mm). *Dombeya torrida* chloroform extract was the most active extract against *C. albicans* with a zone diameter of 20.09 mm.

Conclusion: The plants under study, *D. torrida* and *H. abyssinica* were chosen on the basis of folklore. Above results support the folklore that *H. abyssinica* is used as a cure for throat complaints, as an astringent in dysentery, treatment of diarrhoea and amoebic dysentery. Results of D. *torrida* extracts also supports its folklore use to treat chest pains and colds as many of these conditions are usually caused by bacterial infections.

Keywords: antibacterial, antifungal, *Dombeya torrida*, *Hydnora abyssinica*, autobioassay

Received: February, 2014
Published: March, 2014

1. Introduction

Traditional medicines play an essential role in healthcare with the World Health Organisation (WHO) estimates of 80 % of world inhabitants relying on traditional medicine for their primary healthcare. About 85 % of the traditional medicines involve use of plant extracts with natural products being the skeletal frameworks of about 60 % of modern drugs that are available today (Cragg and Newman, 2001).

 ISSN 2303-9841

Throughout human history, natural products have been the foundation for the discovery and development of therapeutics used to treat diseases ranging from infectious disease to cancer. The advent of HIV/AIDS and multidrug resistant infectious diseases has led to increased investigation of natural products in search for their cure. Despite the investigation of terrestrial flora, it is estimated that only 5-15 % of approximately 250,000 species of higher plants have been systematically investigated, chemically and pharmacologically. The potential of large areas of tropical rain forests remains virtually untapped. Thus the interest in nature as a source of potential chemotherapeutic agents continues (Cragg and Newman, 2001). In Kenya, many plants are used in management of various disease conditions (Gachathi, 1989; Musila et al., 2004; Kokwaro, 2009) but have hardly been studied. These plants include *Dombeya torrida* (J. F. Gmel) and *Hydnora abyssinica* (A. Braun).

Dombeya torrida is a shrub (or tree) and grows within an altitude of 1850 to 2700 m. In Kenya *D. torrida* is found in highlands forests throughout Kenya and is splendid when in flower (Blundell, 1992). In East Africa a decoction of the flowers and bark is taken to treat indigestion (Brink, 2007; Kokwaro, 2009). Its roots are used for treatment of chest pains and colds (Gachathi, 1989). *Hydnora abyssinica* is parasitic and grows underground on acacia roots in dry bushland and is only seen when the tip of the flowers breaks the surface of the ground. The hosts for *H. abyssinica* are species of *Acacia* (Gachathi, 1989; Agnew and Shirley, 1994). The pseudo-rhizome is normally boiled and the infusion drunk as a cure for throat complaints, as an astringent in dysentery, for treatment of stomach trouble and for removing the placenta if it does not come out in time (Kokwaro, 2009). It is also used in treatment of diarrhoea and amoebic dysentery (Musila, et al., 2004) and to treat typhoid, anthrax, cancer and East Coast Fever (Mwangi et al., 2001).

These medicinal uses given indicate that the plants have ability of inhibiting growth of microbes. The objective of this study was to determine the antifungal and antibacterial activity of *Dombeya torrida* and *Hydnora abyssinica* extracts.

2. Methods

2.1 Plant collection

The *D. torrida* stem-bark, leaves and flowers were collected at Kinale forest in Kiambu County, Kenya. A voucher specimen number **2006/002** is deposited at the School of Biological Sciences herbarium, University of Nairobi. *H. abyssinica* whole plant was collected at Kimunyi Village in Embu County, Kenya. A voucher specimen number **2006/001** is deposited at School of Biological Sciences herbarium, University of Nairobi. The *D. torrida* stem-bark, leaves and flowers and *H. abyssinica* pseudo-rhizomes were air-dried at room temperature, finely ground and the powder stored at room temperature in a dry place.

2.2 Plant extraction and sample preparation

About 1 kg of the plant powder was weighed and extracted with chloroform for 48 hr in a Soxhlet apparatus. The material extracted with chloroform was dried at room temperature and re-extracted with methanol for 48 hr. *Dombeya torrida* leaf powder was extracted by percolation using dichloromethane: methanol (50:50) mixture in an open column. The extracts were filtered through filter paper, reduced *in vacuo* to dryness and then stored at 2-8 °C until further processing. About 402 g of plant material was weighed into a conical flask and subjected to cold maceration with 1,000 ml of water for 7 hr with occasional stirring. Another 405 g of plant material was added to 2,000 ml distilled water. The mixture was heated to boiling point using a hot plate and allowed to boil for 5 min then allowed to cool, with continuous stirring to yield a decoction. The extracts were filtered through filter paper and reduced *in vacuo* to about 100 ml, freeze-dried and then stored at 2-8 °C.

2.3 Microorganisms

Gram-positive and gram negative bacteria and fungi were used in the screening for antimicrobial activity. The gram positive were *Staphylococcus aureus* and *Staphylococcus epididermis* with strain code of NC 07447and NC1336, respectively.

Gram-negative bacteria were *Bacillus pumilus* (NC 08241) and *Escherichia coli*(ATTC 25922). The fungi were *Saccromyces cerevisiae* and *Candida albicans* and both yeasts were standard strains. These microorganisms were sourced from Drug Analysis and Research Unit (DARU), Department of Pharmaceutical Chemistry, University of Nairobi.

2.4 Culture media preparation

The culture media were prepared according to the British pharmacopeia (2008) specifications and the manufacturer's instructions. Tryptone Soy Agar (TSA) was used to culture bacteria. Around 4 g of TSA was suspended in 100 ml distilled water for each strain of the bacteria. The agar was heated to boiling to dissolve completely and then sterilised by autoclaving at 121 °C for 15 min. the agar was then allowed to cool to about 50 °C. A loop was sterilized using a foot operated burner and used to scoop samples from the master cultures which were suspended in distilled water. The agar was inoculated with 1 ml of bacterial culture innoculum [approximately 10^6 colony forming units (cfu) per ml]. It was swirled and 20 ml poured per plate (in 4 plates) and allowed to cool and set for about 20 min at 40 °C. Six wells were made in the plate using a cork borer.

Sabouraud Dextrose Agar (SDA) was used to culture *C. albicans* and *S. cerevisiae*. About 13 g of SDA was suspended in 200 ml distilled water and heated to boiling to dissolve completely. The agar was sterilised by autoclaving at 121 °C for 15 min and allowed to cool to about 50 °C. A loop was sterilized using a foot operated burner and used to scoop samples from the master cultures which were suspended in distilled water. The agar was inoculated with 1 ml of fungal culture inoculum approximately 10^6 cfu per ml. The agar was swirled and 20 ml poured per plate (in 2 plates) and allowed to cool and set for about 20 min at 40 °C. Six wells were made in the plate using a cork borer.

 ISSN 2303-9841

2.5 Antimicrobial assay

This was carried out using agar diffusion method. About 100 mg of the plant extracts was dissolved in 1 ml distilled water to avail 100 mg/ml samples. Fifty micro litres of samples containing 100 mg/ml were introduced per well.About 50 µl erythromycin and nystatin solutions containing 0.3 mg/ml were spotted as standards for antibacterial and for antifungal effects, respectively. Distilled water and dimethylsulfoxide (DMSO) were the negative controls. These standards were sourced from DARU. The plates with the samples were left at room temperature in the laminar flow equipment for about 1 h for the extract to diffuse. The plates were then incubated for 18 hr in an incubator at 37 °C for bacteria and 35 °C for fungi after which zones of inhibitions were read. All screenings were done in duplicate.

2.6 Autobioassay

Glass plates (20 x 20 cm) were cleaned with distilled water and dried. Silica gel GF_{254} (normal phase) was used to make a slurry in a ratio of 1:2 of silica with distilled water. The glass plates were put in a special applicator and the slurry poured onto them. A spreader was passed over them at a uniform speed to receive a uniform coating of adsorbent layer of 0.75 mm. The slurry was allowed to dry. After 30 min, the plates were activated by heating in an oven at 105 °C for 1 hr. After cooling down, the plates were spotted with *D. torrida* methanol, chloroform and dichloromethane/methanol extracts; and *H. abyssinica* methanol, chloroform and dichloromethane/methanol extracts 2.5 cm apart. About 100 µl of samples containing 100 mg/ml were spotted. The amounts spotted were high since this was for preparative work. The plates were then developed in a saturated tank using chloroform-methanol (90:10) mixture as the mobile phase. This allowed for separation of the components of each extract depending on its polarity. The developed TLC plate was removed from the developing tank and allowed to dry at room temperature for 30 min. About 50 µl erythromycin and nystatin solutions containing 0.3 mg/ml were spotted as standards for antibacterial and for antifungal effects, respectively. These standards were sourced from DARU. The TLC plate was put on a glass plate. Sabouraud Dextrose Agar and TSA already inoculated with individual microbial culture inoculum were swirled and 50 ml poured on to the TLC plate to form an even layer covering the developed plate. It was allowed to cool and set for about 20 min. The plates were then incubated in an oven at 37 °C for bacteria and 35 °C for fungi for 18 h after which zones of inhibitions were read. The auto bioassay was done in duplicate.

3. Results

3.1 Antibacterial activity

Clear inhibitions zones were seen with all extracts although they were less than in the positive control but bigger than those caused by distilled water (blank). The inhibition zones are as shown in **Table 1** below. *D. torrida* bark decoction had the highest activity against *S. aureus* with an inhibition zone diameter of 16.91 mm while *H. abyssinica* macerate had least activity against *S. aureus* with a zone diameter of 8.86 mm. *D. torrida* bark decoction had the highest activity against *S. epididermis* with a diameter of 17.05 mm while *H. abyssinica* macerate had least activity against *S. epididermis* with a zone diameter of 8.66 mm. Activity against *E. coli* was highest for *D. torrida* bark decoction with zone diameter of 16.56 mm followed by *H. abyssinica* chloroform extract at 15.84 mm. *D. torrida* leaf decoction had least activity at 9.20 mm zone diameter. *H. abyssinica* chloroform extract had the highest activity against *B. pumilus* with a zone diameter of 17.04 mm while itsmacerate had least activity against *B. pumilus* at 8.51 mm.

Table 1: Antibacterial activity (zones of inhibition) of various *Dombeya torrida* and *Hydnora abyssinica* extracts

Plant extract	Zones of inhibition (mm)			
	Staph. aureus	***Staph. epididermis***	***E. coli***	***B. pumilus***
D. torrida bark macerate	16.73	16.29	15.82	12.31
D. torrida bark decoction	16.91	17.05	16.56	12.71
D. torrida leaf decoction	9.15	8.92	8.62	9.08
D. torrida leave macerate	9.81	9.60	9.20	9.54
D. torrida bark methanol	16.00	15.69	15.82	13.66
D. torrida $CHCl_3$*	12.07	11.30	11.89	12.07
H. abyssinica decoction	10.48	12.52	12.91	8.58
H. abyssinica macerate	8.86	8.66	9.28	8.51
H. abyssinica $CHCl_3$*	15.83	15.75	15.84	17.04
H. abyssinica methanol	11.87	11.37	12.21	11.66
Water blank	7.84	7.87	7.30	7.92
DMSO blank	8.02	8.02	8.02	8.02
Gentamicin sulphate (0.32 mg/ml)	24.20	22.70	23.73	23.66

*: *Chloroform extracts were solubilized using DMSO*

 ISSN 2303-9841

Table 2: Antifungal activity (zones of inhibition) of various *Dombeya torrida* and *Hydnora abyssinica* extracts

Plant extracts	Zones of inhibition (mm)	
	S. cerevisiae	***C. albicans***
D. torrida bark macerate	7.70	10.55
D. torrida bark decoction	7.70	11.31
D. torrida bark methanol	16.75	11.04
D. torrida $CHCl_3$*	17.69	20.09
H. abyssinica decoction	11.13	11.71
H. abyssinica macerate	7.70	-
H. abyssinica $CHCl_3$*	17.38	-
H. abyssinica methanol	15.88	9.41
Water blank	7.70	7.68
DMSO blank	7.70	7.70
Nystatin (0.3 mg/ml)	15.95	18.15

*: *Chloroform extracts were solubilized using DMSO*
(-) *indicate zones for* C. albicans *were not very clear*

Table 3: Results for autobioassay of *Dombeya torrida* and *Hydnora abyssinica* extracts

Extract	*B. pumilus*		*Staph. aureus*		*C. albicans*	
	Active spots	**R_f values of spots**	**Active spots**	**R_f values of spots**	**Active spots**	**R_f values of spots**
D. torrida bark methanol	1	0	1	0	1	0
D. torrida bark chloroform	2	0.3, 0.7	2	0.4, 0.6	band	0.7 to 0.9
D. torrida leaves dichloromethane: methanol	1	0	2	0, 0.3	1	0
H. abyssinica methanol	1	0	1	0	1	0 to 0.2
H. abyssinica chloroform	2	0.1, 0.6	3	0, 0.2, 0.8	band	0.7 to 0.9
H. abyssinica dichloromethane: methanol	5	0, 0.1, 0.2, 0.3, 0.8	3	0, 0.1, 0.3	band	0 to 0.3
Erythromycin (Standard)	a	a	a	a	-	-
Nystatin (standard)	-	-	-		b	b

a: Clear inhibition zones 3 cm in diameter.
b: Clear inhibition zones 1.2 cm in diameter

3.2 Antifungal activity

None of the negative controls exhibited antifungal activity while the positive control (nystatin) showed inhibition against both fungi showing that the nutrient media conditions supported fungal growth and inhibition in test extracts was due to the presence of inhibitory constituents in the plant extracts. The inhibition zones are as shown in **Table 2**.

The highest activity observed against *S. Cerevisiae* was with *D. torrida* chloroform extract with *H. abyssinica* macerate having the least activity. The activity of the chloroform extracts against *S. cerevisiae* was higher than for standard. *D. torrida* chloroform extract was the most active extract against *C. albicans*.

3.3 Autobioassay

The extracts showed variable activity. **Table 3** above gives a summary of the results giving the number of active spots and their R_f values. Some extracts had a band of inhibition represented as a R_f range. The *D. torrida* bark methanol and leaves

dichloromethane/methanol extracts together with *H. abyssinica* methanol extract showed only one spot of inhibition against the 3 µg at the extract application point on the TLC plate. The *H. abyssinica* dichloromethane/methanol extract had most activity against *B. pumillus* with 5 spots of inhibition. This extract had comparable effects to the ones of *H. abyssinica* chloroform against *S. aureus*. The *D. torrida* chloroform extract and *H. abyssinica* chloroform and dichloromethane/methanol extracts had a marked inhibition effects against *C. albicans* with big bands showing presence of antifungal constituents in these extracts.

4. Discussion and Conclusion

The plants under study, *D. torrida* and *H. abyssinica,* were chosen on the basis of their folklore uses. *D. torrida* and *H. abyssinica* extracts were found to have antimicrobial activity against *S. aureus*, *S. epididermis, E. coli, B. pumilus*, *S. cerevisiae* and *C. albicans*. These results were in agreement with *in vitro* bioassay of *H. abyssinica* aqueous, methanol and chloroform extracts by Saadabi and Ayoub (2009) which showed ability to inhibit growth of 6 human pathogenic fungi. The above results support their folklore uses. The *D. torrida* decoction of the bark is taken by the Maasai to treat indigestion after a large meal of meat (Kokwaro, 2009) with its roots being used for treatment of chest pains and colds (Gachathi, 1989).These conditions are linked to bacterial and fungal infections as both bacteria and fungi lead to respiratory tract infections. Fungal infections *Aspergillosis, Histoplasmosis* and *Coccidiomycosis* affect the lungs causing chest pain and chills. Bacteria include *S. aureus*, and *Strep. pneumonia*. Stomach infections can also lead to indigestion. Thus the antimicrobial effects of *D. torrida* can give relief in these conditions.

H. abyssinica is in folklore used for treatment of diarrhoea, amoebic dysentery, typhoid, anthrax, cancer and East Coast Fever (Mwangi et al., 2001; Musila, et al., 2004). There are many causes of diarrhea, which include viruses, bacteria and parasites. Amoebic dysentery is caused by the *Entamoeba histolytica*, typhoid is caused by *Salmonella typhi* while anthrax is caused by *Bacillus anthracis* in cattle. East Coast fever (theileriosis) is a disease of cattle, sheep and goats caused by the protozoan parasite *Theileria parva*. The antimicrobial activity demonstrated with the plants under study gives an indication of probable ability of *D. torrida* and *H. abyssinica* to manage the conditions claimed.

The present work has shown that *D. torrida* and *H. abyssinica* extracts possess antimicrobial activity. Traditionally, the decoctions and the macerates are used medicinally. These should be investigated further to isolate compounds which may be responsible for the activity. The isolated compounds can then be used in making new drugs or be used as template for synthesis of new antimicrobial medicines.

Conflict of Interest declaration

The authors declare no conflict of interest

Acknowledgements

The authors are grateful to *Deutsch Academischer Auslader-Dienst* (DAAD) for funding the study and to Mr. Simon Mathenge who assisted in collection and identification of the plants.

References

Agnew A.D.Q. and Agnew S. (1994). Upland Kenya wild flowers; a flora of the ferns and herbaceous flowering plants of Upland Kenya.2nd edition. *East Africa Natural History Society*, Nairobi, Kenya. pp 47.

Blundell M. (1992). *Wild flowers of East Africa*. *Collins Photo Guide*. Harper Collins Publishers, London. 21, pp 301.

Brink M. (2007). *Dombeya torrida* (J. F. Gmel.) Bamps. Record from Protabase. Louppe, D., Oteng-Amoako, A. A. & Brink, M. (Editors). PROTA (Plant Resources of Tropical Africa), Wageningen, Netherlands.
Available at http://database.prota.org/search.htm Accessed 29-04-2013.

Cragg G.M. and Newman, D.J. (2001). Medicinals for millenia; the historical record. *In:* New vistas in therapeutics; from drug design to gene therapy. Eds. Skarlatos, I. S., Velletri, P. and Morris, M. New York Academy of Sciences. pp 3-13.

Gachathi F. N. (1989). *The Kikuyu botanical dictionary*. Africa Medical Research Foundation Printing Department, Nairobi Kenya.p 117.

Kokwaro J. O. (2009). Medicinal plants of East Africa. University of Nairobi Press, Nairobi, Kenya. pp. 140, 273, 325-380.

Musila W., Kisangau D. and Muema, J. (2004). Conservation status and use of medicinal plants by traditional medical practitioners in Machakos District, Kenya. National Museums of Kenya.
Available at Http://www.ed.psu.edu/ick2004proceedings/ Accessed 29-04-2005.

Mwangi J.W., Kibwage, I.O. and Gathumbi P.K. (2001). Personal communication with herbalists in Embu District, Kenya.

Saadabi A.M.A. and Ayoub S.M.H. (2009). Comparative bioactivity of *Hydnora abyssinica* A. Braun against different groups of fungi and bacteria. *J. Med. Plants Res.* **3** (4): 262-265.

 ISSN 2303-9841