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Antibacterial and Anti-Acanthamoebic Properties of *Catha Edulis* (Khat)

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Abstract

Infectious diseases remain a significant threat to human health, contributing to more than 17 million annual deaths thus indicating an urgent need to identify novel molecules for antimicrobial chemotherapy. Here, the antimicrobial activities of aqueous crude extract of *Catha edulis* (khat widely used in Africa and southern parts of Arabia) were tested against a panel of microorganisms including Gram-positive bacteria (*Bacillus magaterium, Micrococcus luteus*), Gram-negative bacteria (*Escherichia coli, Brevundimonas diminuta*), yeast (*Aspergillus varicoluor, Penicillum solitum, Penicillum brevicompactum*) and the protist (*Acanthamoeba castellanii*) *in vitro.* At 100 µg, *C. edulis* extracts exhibited potent antibacterial activity against *B. diminuta* (19 mm ± 2.3), *B. magaterium* (16 mm ± 0.7) and *M. luteus* (22 mm ± 3.1) but not against *E. coli* and yeast (*A. varicoluor, P. solitum, P. brevicompactum*). Notably, *C. edulis* extracts showed amoebicidal effects (>50 % reduction in amoeba numbers of the original inoculum) as evidenced by the uptake of Trypan blue dye. The remaining sub-population of *A. castellanii* remained viable but cultures remained static over longer incubations. These findings show that *C. edulis* extract possess selective antibacterial properties. For the first time, it is shown that *C. edulis* extract exhibit anti-Acanthamoebic properties. Further studies are needed to identify active components and assess their clinical relevance.

Keywords: *Acanthamoeba; Aspergillus*; Antibacterial; Antifungal

Introduction

Infectious diseases result in >17 million deaths worldwide annually, mostly in children and the elderly [1]. The morbidity and mortality associated with infectious diseases has remained significant, particularly food-borne illnesses including diarrhea among children and respiratory infections such as tuberculosis, despite the advances in antimicrobial chemotherapy and supportive care. To make matters worse, the haphazard use of antimicrobials in the treatment of many infectious diseases has inevitably led to the emergence of multiple drug resistant microorganisms [2]. For example, in 1990, almost all cholera isolates in New Delhi (India) were sensitive to furazolidone, ampicillin, co-trimoxazole and nalidixic acid. In 2000, these drugs became largely obsolete in the treatment of cholera. The use of natural products, such as medicinal plants as therapy against infectious diseases is a traditional therapeutic measure especially in developing countries as they contain a combination of potential antimicrobial compound(s) instead of a single purified molecule [3]. Khat, *Catha edulis*, from the family Celastraceae is a natural stimulant that is found as a flowering evergreen tree and is widely cultivated in Africa and southern parts of Arabia [4-6]. The chewing of young shoots and leaves of *C. edulis* is a traditional and social habit in some countries of East Africa and Arab Peninsula [5,6]. It contains the alkaloid called cathinone (an amphetamine-like stimulant) and other polyphenolic compound such as tannins and flavonoids, which generally occur as glycosylated derivatives and are known for their antioxidant effects [7]. Although, the stimulating effects of *C. edulis* are well known, the antimicrobial effects of *C. edulis* remain incompletely understood. The present study was designed to investigate the antimicrobial properties of *C. edulis* extract against Gram-positive bacteria, Gram-negative bacteria, yeast and protists. The results revealed that *C. edulis* extract possess potent antibacterial properties against Gram-positive bacteria tested as well as anti-amoeba activity.

Materials and Methods

Plant materials

Relatively fresh plant leaves and young shoots near the top of the leaves of *Catha edulis* were purchased from a Somali shop on Edgware Road, London, UK. Approximately, 55 g of the plant material were ground and soaked in 100 mL of methanol (100 %) in a water bath at 25°C for 24 h with continuous shaking using Cole Parmer Orbital shaker at 40 g. The extract was filtered, concentrated at 40°C under reduced pressure, and methanol was dried in a freeze-dryer [8]. Finally, the extracts were re-suspended in 20 mL methanol and stored at 4°C until tested for antimicrobial activities.

High performance liquid chromatography (HPLC)

The crude methanolic extract were applied to a reverse phase C18 column with flow rate of 1 mL per min. Using HPLC, methanolic extracts were eluted with 0, 50 and 100 % methanol. Fraction collected from the crude methanolic extract were freeze dried. The lyophilised fractions were reconstituted in 1 mL of methanol and tested for antimicrobial activities [9].

Microbial strains

The microbial strains used in the present study were bacteria [*Escherichia coli* K1 strain RS218 (O18:K1:H7), a cerebrospinal fluid isolate from a neonate with meningitis, and environmental isolates of *Brevundimonas diminuta, Bacillus magaterium, Micrococcus luteus*], yeast (*Aspergillus varicolor, Penicillium solitum, P. brevicompactum*), and the protist (*Acanthamoeba castellanii*, a keratitis isolate belonging to the T4 genotype). All isolates were available in the University microbial collection (stored at -80°C and available upon request)

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except *A. castellanii* that was obtained from the American Type Culture Collection (ATCC 50492).

Antibacterial test

The antibacterial activities of *C. edulis* extract were determined using the antibiotic disc diffusion assay ("Kirby-Bauer" assay) [10]. Briefly, bacterial strains (*E. coli, B. diminuta, B. magaterium* and *M. luteus*) were grown at 37°C in Luria-Bertani broth and their optical density adjusted to 1.0 at 595 nm. The bacterial cultures were evenly spread on the surface of nutrient agar plates. Sterile Whatman filters of 60 mm size were impregnated with 100 µL (0.1 mg protein) of methanolic extract and placed on agar containing bacteria. For negative controls, filters were impregnated with 100 µL of the solvent alone and for positive controls filters were impregnated with 100 µg per mL gentamicin. The plates were incubated at 37°C overnight, following this the zones of inhibition were measured. An increase in the diameter of the inhibition zone was used to determine whether *C. edulis* extract have the potential to target the bacteria investigated.

Antifungal activity test

Sporing fungus (*A. varicoluor, P. solitum* and *P. brevicompactum*) were suspended in 1 mL of sterile water and evenly spread on the surface of potato dextrose agar (PDA). The antifungal activity of the *C. edulis* extract was determined by disk diffusion method. Sterile Whatman filters of 60 mm size were impregnated with 100 µL (0.1 mg protein) of methanol extracts and placed on PDA containing yeast. For negative controls, filters were impregnated with 100 µL of the solvent alone and for positive controls filters were impregnated with 100 µg per mL amphotericin B. Plates were incubated at 25°C for 24 h, following this the zones of inhibition were measured.

Amoebistatic and Amoebicidal assays

Acanthamoeba castellanii were grown to confluency in 24-well plates. The next day, plates were washed with PBS to remove unbound amoebae. Amoebae were incubated with various concentrations of methanol extracts of *C. edulis* (50 µg and 100 µg protein) or solvent alone and the plates incubated at 30°C for 24 h. The effects on amoeba growth and viability were determined using haemocytometer counting and Trypan blue exclusion testing. The normal growth rates of *A. castellanii* were determined using growth medium alone, i.e., PYG. For positive control, amoebae were treated with 100 µg per mL of chlorhexidine (anti-amoeba drug).

Results

C. edulis extracts exhibited antibacterial but no antifungal properties

The crude metanolic extract of *C. edulis* were tested against bacteria and yeast using the disc diffusion assay [10]. The results revealed that *C. edulis* extract inhibited growth of *B. diminuta, M. luteus and B. magaterium*. In the presence of extract, the results showed that the zone of inhibition for *B. diminuta* was 19 mm ± 2.3; 22 mm ± 3.1 for *M. luteus* and 16 mm ± 0.7 for *B. magaterium*. Notably, *C. edulis* extract had no effect against the clinical isolate of *E. coli*. The zone of inhibition for *E. coli* around the *C. edulis* extract disc was <11 mm, even though the zone of inhibition for *E. coli* around the gentamicin antibiotic disc (positive control) was >18 mm. The breakpoint inhibition zone was considered 14 mm as per EUCAST guidelines. In contrast, *C. edulis* extract had no effect on *A. varicolor, P. solitum* and *P. brevicompactum* in the disc diffusion assay even though the zone of inhibition around the amphotericin B disc (positive control) was >15 mm.. The results are representative of at least three independent experiments performed in duplicate.

C. edulis extract showed potent amoebicidal and amoebistatic effects

To determine the effects of crude methanolic extract of *C. edulis* on *A. castellanii* growth and viability, amoebicidal and amoebistatic assays were performed. 100 µg per mL of chlorhexidine exhibited 100% kill. For *C. edulis* extract, the results revealed that *C. edulis* extract exhibited amoebicidal effects with an initial reduction in the viability of amoeba numbers as evidenced by the uptake of Trypan blue dye. However, a sub-population of *A. castellanii* remained viable, but cultures remained static over longer incubations (i.e., 48 h) even in growth medium. At 100 µg, the addition of *C. edulis* extract produced 55 % ± 6.7 reduction in viable cell numbers within 24 h. At 50 µg, the addition of *C. edulis* extract produced 23.5 % ± 3.3 reduction in viable cell numbers of the original inoculum. The results are representative of at least three independent experiments performed in duplicate.

HPLC fractions of *C. edulis* extract showed anti-Acanthamoebic activities

A total of 35 fractions were collected from the crude methanolic extract and were tested for antimicrobial activity against *A. castellanii*. The results revealed that fractions eluted with 50 % methanol of *C. edulis* extract exhibited amoebicidal effects corresponding to crude methanolic extract. The addition of 100 µg of *C. edulis* extract produced 63.4 % ± 5.4 amoebicidal effects within 24 h. The residual amoebae, although viable, remained static in growth medium for up to 48 h. In contrast, fractions eluted with 0 and 100 % methanol (i.e., PBS alone) of *C. edulis* extract did not exhibit amoebicidal and/or amoebistatic effects, even though both fractions contained approximately similar amount of protein (100 µg). The results are representative of at least three independent experiments performed in duplicate.

Discussion

Although *C. edulis* is widely studied for its psychoactive properties, little work has been done to test its antimicrobial properties. Our findings revealed that the methanolic crude extract of *C. edulis* exhibit antibacterial properties against *B. diminuta, M. luteus* and *B. magaterium* but not *E. coli* and they have no antifungal properties against *A. varicolor, P. solitum* and *P. brevicompactum* using the disc diffusion assay. These results are consistent with previous findings, which demonstrated the antimicrobial properties of *C. edulis* extract against bacteria (*Porphyromonas gingivalis, Tannerella forsythensis, Streptococcus pyogenes* with the zone of inhibition in the range of 10 to 14 mm at a concentration of 10 mg per cm^3 but showed no effect against either *Staphylococcus aureus*), or yeast (*Candida albicans*) [11]. The selective targeting of certain bacterial species by *C. edulis* extract is not clear. Given the potent antibacterial activities of *C. edulis* extract against some Gram-positive but none of the Gram-negative bacteria tested, suggest that they possibly target(s) the bacterial outer membrane and this needs to be investigated in future studies.

More importantly, for the first time the results revealed potent anti-Acanthamoebic properties of *C. edulis* extracts. This is interesting as *C. edulis* extracts had no effect against the eukaryotic yeast (*Candida albicans*) suggesting its selective targeting. Although, the mechanisms of *C. edulis*-mediated *A. castellanii* death is unknown, previous studies have shown that *C. edulis*-induced apoptosis in various human

leukaemia cell lines including HL-60, NB4, Jurkat cells in a caspase-1 and -8 dependent manner [4]. As the single-celled *A. castellanii* is not yet known to undergo programmed cell death, future studies are needed to understand the molecular mechanisms of *C. edulis*-mediated *A. castellanii* death as well as its antibacterial properties. Further investigations are in progress to identify the active component(s) and to assess their clinical relevance.

Conclusion

In conclusion, this study showed the selective antibacterial properties against Gram-positive but not Gram-negative bacteria tested. For the first time, it is shown that *C. edulis* extracts possess anti-Acanthamoebic but not antifungal properties, albeit weak compared to that of the positive control (chlorhexidine).

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References

1. Butler MS, Buss AD (2006) Natural products--the future scaffolds for novel antibiotics? Biochem Pharmacol 71: 919-929.
2. Powers JH (2004) Antimicrobial drug development--the past, the present, and the future. Clin Microbiol Infect 10: 23-31.
3. Helvaci S, Kökdil G, Kawai M, Duran N, Duran G, et al. (2010) Antimicrobial activity of the extracts and physalin D from *Physalis alkekengi* and evaluation of antioxidant potential of physalin D. Pharm Biol 48: 142-150.
4. Dimba EA, Gjertsen BT, Bredholt T, Fossan KO, Costea DE, et al. (2004) Khat (*Catha edulis*)-induced apoptosis is inhibited by antagonists of caspase-1 and -8 in human leukaemia cells. Br J Cancer 91: 1726-1734.
5. Elmi AS (1983) The chewing of Khat in Somalia. J Ethnopharmacol 8: 163-176.
6. Kalix P (1988) Khat: a plant with amphetamine effects. J Subst Abuse Treat 5: 163-169.
7. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the zutphen elderly study. Lancet 342: 1007-1011.
8. Riaz S, Faisal M, Hasnain S, Khan NA (2011) Antibacterial and cytotoxic activities of *Acacia nilotica* (Lam.) family Mimosaceae methanolic extracts against extended spectrum beta-lactamase producing *Escherichia coli*, and *Klebsiella* spp. Trop J Pharmaceut Res 10: 785-791.
9. Sissons J, Alsam S, Goldsworthy G, Lightfoot M, Jarroll EL, et al. (2006) Identification and properties of proteases from an Acanthamoeba isolate capable of producing granulomatous encephalitis. BMC Microbiol 6: 42.
10. Ho PL, Chow KH, Yuen KY, Ng WS, Chau PY (1998) Comparison of a novel, inhibitor-potentiated disc-diffusion test with other methods for the detection of extended-spectrum beta-lactamases in *Escherichia coli* and *Klebsiella pneumoniae*. J Antimicrob Chemother 42: 49-54.
11. Al-hebshi N, Al-haroni M, Skaug N (2005) *In vitro* antimicrobial and resistance-modifying activities of aqueous crude khat extracts against oral microorganisms. Arch Oral Biol 51: 183-188.