

In vitro antimicrobial activity of some plant extracts against *Arcobacter butzleri* and *Arcobacter cryaerophilus*

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Background: There are increasing reports of resistance to current antibiotics employed in treatment of *Arcobacter* related infections.

Materials and Methods: *Carica papaya*, *Vernonia amygdalina*, *Ocimum gratissimum* and *Momordica charantia* were tested against *A. butzleri* reference strain (ABSH3-1137) and *A. cryaerophilus* isolated from pig and chicken using disk diffusion technique.

Results: The aqueous extract of *C. papaya* inhibited the growth of *A. butzleri* at inhibitory dose (ID) of 0.5 mg and the two *A. cryaerophilus* at ID of 1 mg while the methanol extract did not inhibit the growth of any strains. The aqueous and methanol extracts of *V. amygdalina* inhibited the growth of *A. cryaerophilus* isolated from pig and chicken at ID of 10 mg but did not inhibit *A. butzleri*. The methanol extracts of *O. gratissimum* inhibited *A. butzleri* and *A. cryaerophilus* pig isolate at ID of 2.5 mg and the chicken isolate at ID of 1 mg. The aqueous extracts of *O. gratissimum* inhibited *A. butzleri*, *A. cryaerophilus* of chicken isolate at ID of 5 mg, and *A. cryaerophilus* of pig isolate at 2.5 mg. The methanol extracts of *M. charantia* had ID of 2.5 mg for *A. butzleri* and *A. cryaerophilus* pig isolate and ID of 1 mg for *A. cryaerophilus* chicken isolate while the aqueous extracts had ID of 5 mg for all the three strains tested.

Conclusions: The result obtained from this study showed that both aqueous and methanol extracts of four commonly used herbs in Nigeria inhibited the growth of *Arcobacter* species to a varying degree with the aqueous extract of *C. papaya* leaves reported *in vitro* highest antimicrobial activity and thereby may serve as potential sources for new antimicrobial agents against *Arcobacter*.

Key words: herbs, extracts, antimicrobial, *Arcobacter*, Nigeria

INTRODUCTION

The genus *Arcobacter* was described in 1991 as a second genus within the family *Campylobacteriaceae* to encompass bacteria formerly known as aerotolerant *Campylobacter* (1). *Arcobacter* species are

considered to be emerging pathogens with *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* being linked to disease in humans (2). *Arcobacters* have been isolated from diarrhoeal and bacteraemic cases of which most common symptoms include watery diarrhoea, abdominal pain, nausea and vomiting (3). They have also been detected in drinking water (4), poultry meat (5), beef and pork (6). In two surveillance studies from Belgium and France, *Arcobacter* was identified as the fourth most commonly isolated *Campylobacter*-related organism in diarrhoea illness in humans (7).

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases (5), and reports have shown that *Arcobacter* appears to be resistant to antimicrobial agents typically used in the treatments of diarrhea illnesses caused by *Campylobacter* species. However, some chemical preservatives, spices and herbs were shown to possess anti-infective properties against some strains of *Arcobacter* (8).

In view of increasing reports of resistance to current antibiotics employed in treatment of *Arcobacter* related infections, there is need to develop new or alternative antimicrobial agents effective against *Arcobacter*. In Nigeria, extracts of *Ocimum gratissimum*, *Morinda lucida*, *Carica papaya*, *Phyllanthus amarus* and *Citrus aurantifolia* are among the folkloric herbal remedies that have been scientifically confirmed to possess anti-diarrhoeal, anti-bacterial and anti-protozoan properties (9, 10). Most especially, ethanolic preparation of *C. papaya* and *M. lucida* has been shown to possess tremendous anti-infective potentials against Nigerian isolates of *Helicobacter pylori* (9), a related organism to *Arcobacter*.

Hence this study was undertaken to assess the antimicrobial activity of four prevalent herbal plants in Nigeria, *C. papaya*, *V. amygdalina*, *O. gratissimum* and *M. charantia*, against 2 species of *Arcobacter*: *A. cryaerophilus* and *A. butzleri*.

MATERIALS AND METHODS

Arcobacter test organism

A. butzleri clinical isolate (ABSH3-1137) and 2 field isolates of *A. cryaerophilus* were obtained from healthy pigs (11) and chickens (12). Three *Arcobacter* strains in this study were confirmed by multiplex PCR assay that amplified the 401-bp fragment of *A. butzleri* and the 257-bp fragment of *A. cryaer-*

ophilus (13), and they were maintained in stock cultures at minus/-25 °C in glycerol *Arcobacter* broths at the Department of Medical Microbiology and Parasitology, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria.

Preparation of *Arcobacter* inoculum

Arcobacter subcultures were first prepared from the stock cultures on Brain Heart Infusion (BHI) agar supplemented with 5% yeasts and 7% sheep blood. BHI agar plates were incubated at 37 °C in micro-aerophilic atmosphere (1). *Arcobacter* inoculum was prepared by collecting bacteria colonies from BHI agar plates at the exponential growth phase and diluting in 0.85% saline. The resulting bacteria suspension was then standardized by McFarland nephelometry to 10⁵ CFU/ml.

Plant materials and extract preparation

C. papaya (Pawpaw leaf), *V. amygdalina* (Bitter leaf), *O. gratissimum* (Basil leaf: efrin) and *M. charantia* (Coral leaf: ejirin) were obtained from various locations in Osogbo, Nigeria and authenticated by a botanist in Obafemi Awolowo University, Ile-Ife, Nigeria. The leaves of each plant were cut into pieces and ground into powder using a sterile electric blender. Ten (10) grams of the powder were steeped in 100 ml distilled water and another 10 g in 100 ml methanol with thorough shaking at regular intervals at room temperature for a few days. The resulting crude extracts were filtered using Whatman No 1 filter paper (Oxoid, UK) and allowed to dry in an oven to obtain a residue, and each was labeled as the crude extract of an individual plant. For the aqueous extract of each plant, 2.5 mg/ml, 5 mg/ml, 10 mg/ml, 25 mg/ml and 50 mg/ml concentrations were prepared with distilled water in sterile bottles while the similar concentrations of the methanolic extracts were re-constituted with phosphate buffer saline (pH 7.2) to nullify the effect of methanol on the organisms (10).

Preparation of herb disks

Disks for antimicrobial sensitivity tests were prepared by the modification of the Gould and Bowie method (14). Briefly, a 6.25 mm diameter plunger was used to punch Whatman No 1 absorbent filter paper to obtain 6.25 mm diameter paper disks. The disks were labeled, dispensed in screw-capped bottles and sterilized at 40 °C in a hot air oven for 1 hour.

Under aseptic conditions, 1 ml of extracts of each herbal plant at various concentrations (2.5 mg/ml, 5 mg/ml, 10 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml) were added to 10 prepared disks arranged side by side inside a sterile Petri dish for each extract to prepare disks of approximately 0.25 mg, 0.5 mg, 1 mg, 2.5 mg, 5 mg and 10 mg, respectively.

The Petri dishes were placed in the hot air oven at 40 °C for 5 minutes to allow the disks to dry. The impregnated disks were then stored in a sterile screw-capped container with a cap tightly screwed, and the container was kept at 4 °C in a refrigerator until use.

Susceptibility testing procedure

Susceptibility testing of the organism to each plant extract was done in triplicate using the modified Kirby Bauer single disk principle (15). The disks were placed on the surface of the agar plate previously seeded with *Arcobacter* and incubated in micro-aerophilic environment at 37 °C for 24 hours (16). A sterile disk impregnated with sterile phosphate buffer served as a negative control while standard disks of nalidixic acid (30 µg), cephalothin (30 µg) and erythromycin (15 µg) served as a positive control. The zone diameter of inhibition around the disk was measured with a caliper. Interpretation of the zone diameter of inhibition as sensitive or resistant to nalidixic acid, cephalothin and erythromycin was based on the interpretive criteria of the Clinical and Laboratory Standards Institute (16).

RESULTS

The antimicrobial activity of the four herbal extracts (Table 1) against the *Arcobacter* species is summarized in Table 2. The aqueous and methanol extracts of *V. amygdalina* inhibited the growth

of *A. butzleri* and *A. cryaerophilus* (chicken) but methanol extracts did not inhibit the growth of *A. cryaerophilus* (pig) at ID of 10 mg.

The aqueous and methanol extracts of *V. amygdalina* did not inhibit the growth of *A. butzleri* but inhibited the growth of two *A. cryaerophilus* at ID of 10 mg. The methanol extracts of *O. gratissimum* inhibited *A. butzleri* reference strain and *A. cryaerophilus* pig isolate at ID of 2.5 mg and the chicken isolate at ID of 1 mg while the aqueous extracts inhibited *A. butzleri* and *A. cryaerophilus* chicken isolate at ID of 5 mg, and *A. cryaerophilus* pig isolate at 2.5 mg. The methanol extracts of *M. charantia* had ID of 2.5 mg for *A. butzleri* and *A. cryaerophilus* pig isolate and ID of 1 mg for *A. cryaerophilus* chicken isolate while the aqueous extracts had ID of 5 mg for all the three strains. The positive control for this experiment using cephalothin, erythromycin and nalidixic acid is given in Table 3. In general, the methanol extracts showed greater antibacterial activity than the aqueous extracts and the two *A. cryaerophilus* field isolates from pig and chicken were resistant to nalidixic acid and cephalothin but susceptible to erythromycin. The *A. butzleri* reference strain (ABSH3-1137) was resistant to cephalothin and erythromycin but susceptible to nalidixic acid.

DISCUSSION

The use of herbal extracts as alternative medicine and natural therapies has been documented for ages. In Nigeria, more than 70% of the populace depend on various forms of plant extracts used as herbal concoctions for treatment of many ailments including infectious diseases (17). The result obtained from this study showed that both aqueous and methanol extracts of four commonly used

Table 1. Herbal extracts used in the study

Botanical name	English name	Local name
<i>Carica papaya</i>	Pawpaw	Ibepe
<i>Vernonia amygdalina</i>	Bitter leaf	Ewuro
<i>Ocimum gratissimum</i>	Basil leaf	Efirin
<i>Momordica charantia</i>	Coral leaf	Ejirin

Table 2. Zone inhibition of 4 plant extracts against *Arcobacter* strains

Concentration of extracts / <i>Arcobacter</i> strains	* Zone diameter of inhibition (mm)											
	0.25 mg		0.5 mg		1.0 mg		2.5 mg		5 mg		10 mg	
	W	M	W	M	W	M	W	M	W	M	W	M
<i>Carica papaya</i>												
<i>A. butzleri</i> (ABSH3-1137)	-	-	++	-	++	-	++	-	++	-	+++	-
<i>A. cryaerophilus</i> (pig)	-	-	++	-	++	-	++	-	++	-	++	-
<i>A. cryaerophilus</i> (chicken)	-	-	-	-	++	-	++	-	++	-	++	-
<i>Vernonia amygdalina</i>												
<i>A. butzleri</i> (ABSH3-1137)	-	-	-	-	-	-	-	-	-	-	+	+
<i>A. cryaerophilus</i> (pig)	-	-	-	-	-	-	-	-	-	-	+	-
<i>A. cryaerophilus</i> (chicken)	-	-	-	-	-	-	-	-	-	-	+	+
<i>Ocimum gratissimum</i>												
<i>A. butzleri</i> (ABSH3-1137)	-	-	-	-	-	-	-	+	++	+	++	++
<i>A. cryaerophilus</i> (pig)	-	-	-	-	-	-	+	+	+	+	+	++
<i>A. cryaerophilus</i> (chicken)	-	-	-	-	-	++	-	++	+	++	++	++
<i>Momordica charantia</i>												
<i>A. butzleri</i> (ABSH3-1137)	-	-	-	-	-	-	-	+	+	+	+	+
<i>A. cryaerophilus</i> (pig)	-	-	-	-	-	-	-	+	+	+	+	+
<i>A. cryaerophilus</i> (chicken)	-	-	-	-	-	+	-	+	+	+	+	+
Mueller Hinton Broth (control)												
<i>A. butzleri</i> (ABSH3-1137)	-	-	-	-	-	-	-	-	-	-	-	-
<i>A. cryaerophilus</i> (pig)	-	-	-	-	-	-	-	-	-	-	-	-
<i>A. cryaerophilus</i> (chicken)	-	-	-	-	-	-	-	-	-	-	-	-

M = methanol extract; W = water extract

* 1–5 mm = +; 6–10 mm = ++; 11–15 mm = +++; - = No inhibition zone

Table 3. Zone of inhibition of *Arcobacter* using selected antimicrobials

Positive controls	Zone diameter of inhibition (mm)		
	<i>A. butzleri</i> (ABH3-1137)	<i>A. cryerophilus</i> (Pig isolate)	<i>A. cryaerophilus</i> (Chicken isolate)
Nalixidic acid (30 µg/disk)	20.7–21.3	10–11	9.3–10.4
Cephalothin (30 µg/disk)	6–7	5–6	5–6.5
Erythromycin (15 µg/disk)	15.5–16.2	22.5–23	21.2–22

herbs in Nigeria inhibited the growth of *Arcobacter* to a varying degree. The aqueous and methanol extracts of *V. amygdalina* appeared to have the least antimicrobial potential at an inhibitory dose of 10 mg of the leaf extract although its root has been demonstrated to contain a proven antimicrobial activity on micro-organisms causing gingivitis, despite the fact that methanol extracts are more efficient substances that are related to antimicrobial activity of various herbs (18). The aqueous extract of leaves of *C. papaya* in this study showed the highest antimicrobial activity while the methanol extract did not have inhibitory activity on any of the three *Arcobacter* strains. This finding may be attributed to the high phenolic compound in *C. papaya* leaf which is responsible for its antimicrobial activity (19), and which appears better extracted with water than methanol. *C. papaya* has been shown to possess several biological functions with its seeds having demonstrable anti-fertility effect in rats (20) and its leaf with anti-*H. pylori* activity *in-vitro* (9). Our result is an extension of many biological activities of this herb.

Both aqueous and methanol extracts of *O. gratissimum* exerted relatively mild antimicrobial activity on the *Arcobacter* strains in the study. *O. gratissimum* has been shown to possess antioxidant property (21) and its leaf has been demonstrated both *in-vitro* and *in-vivo* to inhibit aetiologic agents of diarrhoea (22). The anti-*H. pylori* activity of *O. gratissimum* was also demonstrated in the study by Smith et al. (9). The present findings in this study are in agreement with those of others and indicate that *O. gratissimum* may be a candidate herb that needs to be further studied to characterize the anti-infective ingredients present in the plant.

M. charantia leaf extracts demonstrated moderate antibacterial activity on the *Arcobacter* strains in this study although there are no previous reports of the antimicrobial property of this herb. However, the plant has been reported to possess immunostimulatory and anti-diabetic activity (24), and our findings revealed that this plant may possess additional anti-infective properties on *Arcobacter* and probably other related bacteria.

Although the ethanolic acid extraction method has been shown to yield more active ingredients from medicinal plants (25), both water and methanol extracted the anti-*Arcobacter* property of *V. amygdalina*, *O. gratissimum* and *M. charantia*

in this study with methanol extraction appearing more effective. However, aqueous extraction was the only effective method for the anti-*Arcobacter* property of *C. papaya* leaf. This sharply contrasts the study of Smith et al. (9), where ethanolic and not aqueous extraction method was effective for anti-*H. pylori* activity of *C. papaya* and *M. lucida*. The *A. butzleri* reference strain was resistant to erythromycin and cephalothin while the two *A. cryaerophilus* field isolates demonstrated resistance to nalidixic acid and cephalothin. These three antibiotics are known to be effective against *Campylobacter*-like organisms, although there have been reports of field isolates of *Arcobacter* resistant to erythromycin (26). With the demonstration of resistance to erythromycin, nalidixic acid and cephalothin in this study, the need to develop new effective antibacterial agents becomes highly imperative. *C. papaya*, *O. gratissimum* and *M. charantia* possess anti-*Arcobacter* properties and may serve as potential sources for new antimicrobial agents effective against *Arcobacter*.

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**ANTIMIKROBINIS AUGALŲ EKSTRAKTŲ
IN VITRO AKTYVUMAS PRIEŠ ARCOBACTER
BUTZLERI IR ARCOBACTER CRYAEROPHILUS**

Santrauka

Įvadas. Daugėja pranešimų apie su *Arcobacter* susijusių infekcijų atsparumą antibiotikams.

Medžiaga ir metodai. Tirta, kaip *Carica papaya*, *Vernonia amygdalina*, *Ocimum gratissimum* ir *Momordica charantia* augalų ekstraktai slopino *A. butzleri* (ABSH3-1137) ir *A. cryaerophilus* atmainas, išskirtas iš kiaulių ir vištų, naudojant diskų difuzijos metodą.

Rezultatai. *C. papaya* vandeninis ekstraktas sustabdė *A. butzleri* augimą naudojant slopinančią 0,5 mg ir *A. cryaerophilus* augimą naudojant 1 mg dozę, o metanolio ekstraktas taip neveikė. Vandeninis ir metanolio *V. amygdalina* ekstraktas nuslopino *A. cryaerophilus* atmainų, išskirtų iš kiaulių ir vištų, augimą naudo-

jant 10 mg dozę, tačiau neveikė *A. butzleri*. *O. gratissimum* metanolio ekstraktas nuslopino *A. butzleri* ir *A. cryaerophilus* atmainą, išskirtą iš kiaulių, naudojant 2,5 mg dozę ir *A. cryaerophilus* atmainą, išskirtą iš vištų, naudojant 1 mg; vandeninis ekstraktas slopino *A. butzleri* ir *A. cryaerophilus* atmainą, išskirtą iš vištų, naudojant 5 mg, o iš kiaulių išskirtą *A. cryaerophilus* atmainą – 2,5 mg dozę. *M. charantia* metanolio ekstrakto 2,5 mg dozė slopino *A. butzleri* ir *A. cryaerophilus* atmainą iš kiaulių, o 1 mg – *A. cryaerophilus* iš vištų išskirtą izoliatą; visas atmainas slopino 5 mg vandeninio ekstrakto dozė.

Išvados. Šio tyrimo rezultatai rodo, kad Nigerijoje naudojami keturių augalų vandeniniai ir metanolio ekstraktai slopino *Arcobacter* atmainų augimą. *C. papaya* vandeninis ekstraktas buvo efektyviausias. Visi tirti augalai gali būti naujas antimikrobinių agentų prieš *Arcobacter* šaltinis.

Raktažodžiai: augalai, ekstraktai, antimikrobinis, *Arcobacter*, Nigerija