Sains Malaysiana 50(10)(2021): 3085-3094
http://doi.org/10.17576/jsm-2021-5010-21

Zingiber zerumbet (L.) Smith Hexane Crude Extract Caused DNA Damage on *Leptospira* spp.

(Ekstrak Mentah Heksana *Zingiber zerumbet* (L.) Smith Menyebabkan Kerosakan DNA pada *Leptospira* spp.)

FARAH WAHIDA IBRAHIM, NURUL AMIRAH ABDUL AZIZ, LATIFAH IBRAHIM, NURUL FARHANA JUFRI & ASMAH HAMID*

ABSTRACT

Numerous attempts have been made to control leptospirosis by using chemoprophylaxis, but with limited success. The present study was done to investigate the antileptospiral potential of hexane, ethyl acetate and methanol extracts of Zingiber zerumbet *rhizomes. The extracts were assayed for antileptospiral activity using broth microdilution method against* Leptospira interrogans *(serovar Batavie, Canicola, Australis) and* Leptospira biflexa *serovar Patoc. The* Z. zerumbet *hexane extract exhibited antileptospiral activity, with* IC_{50} *values of 248 µg/mL against* L. interrogans *serovar Canicola,* IC_{50} *of 125 µg/mL against* L. interrogans *serovar Australis,* IC_{50} *of 15.63 µg/mL against* L. interrogans *serovar Batavie and* IC_{50} *of 109 µg/mL against* L. biflexa *serovar Patoc. However, both ethyl acetate and methanol extracts did not show any distinct antileptospiral activity. Since the hexane extract of* Z. zerumbet *showed antileptospiral activity, the DNA-damaging properties of this extract were tested according to their* IC_{50} *and* IC_{25} *values that were specific to each serovars. The DNA-damaging properties were determined by treating the selected* Leptospira *spp. with the hexane extract and subjecting its DNA to electrophoresis and analysis on agarose gels. The results demonstrated that the hexane extract had DNA-damaging properties towards* L. biflexa *serovar Patoc and* L. interrogans *serovar Australis, as proven by the appearance of fragmented DNA on the gels. We conclude that the* Z. zerumbet *hexane extract could inhibit the growth of* Leptospira *spp. serovar Patoc and Australis through DNA-damaging activity and thus, could be a potential antileptospiral agent. Further studies are needed to investigate the potential of this hexane extract as an antileptospiral agent using* in vivo *rat models of leptospirosis.*

Keywords: Antileptospiral; DNA damage; growth inhibition; hexane crude extract; Zingiber zerumbet

ABSTRAK

Banyak usaha telah dibuat untuk mengawal leptospirosis dengan menggunakan kemoprofilaksis namun kejayaannya terhad. Penyelidikan ini dijalankan untuk mengkaji potensi antileptospira bagi ekstrak kasar heksana, etil asetat dan metanol rizom Zingiber zerumbet. *Ekstrak telah diuji untuk aktiviti antileptospira menggunakan kaedah mikropencairan kaldu ke atas* Leptospira interogans (*serovar Batavie, Canicola, Australis*) *dan* Leptospira biflexa *serovar Patoc. Ekstrak heksana* Z. zerumbet *menunjukkan aktivti antileptospira dengan nilai* IC_{50} *pada 248 µg/mL terhadap* L. interrogans *serovar Canicola,* IC_{50} *pada 125 µg/mL terhadap* L. interrogans *serovar Australis,* IC_{50} *pada 15.63 µg/mL terhadap* L. interrogans *serovar Batavie dan* IC_{50} *pada 109 µg/mL untuk* L. biflexa *serovar Patoc. Walau bagaimanapun, kedua-dua ekstrak etil asetat dan metanol tidak menunjukkan sebarang aktiviti antileptospira yang ketara. Oleh kerana ekstrak heksana* Z. zerumbet *menunjukkan aktiviti antileptospira, keupayaan merosakkan DNA bagi ekstrak ini diuji mengikut nilai* IC_{50} *dan* IC_{25} *khusus yang diperoleh untuk setiap serovar. Hasil kajian menunjukkan bahawa ekstrak heksana mempunyai keupayaan untuk merosakkan DNA* L. biflexa *serovar Patoc dan* L. interrogans *serovar Australis seperti yang terbukti dengan kehadiran DNA yang berfragmen pada gel. Sebagai kesimpulan, ekstrak heksana* Z. zerumbet *boleh menghalang pertumbuhan* Leptospira *spp. serovar Patoc dan Australis melalui aktiviti merosakkan DNA dan dengan itu, berpotensi sebagai agen antileptospira. Kajian lanjut diperlukan untuk mengkaji potensi ekstrak heksana sebagai agen antileptospira dalam model tikus leptospirosis secara* in vivo.

Kata kunci: Antileptospira; ekstrak kasar heksana; kerosakan DNA; merencat pertumbuhan; Zingiber zerumbet

Introduction

Leptospirosis is a bacterial infection caused by *Leptospira*. The genus *Leptospira* belongs to the phylum Spirochaetes and is divided into three types of species; saprophytic, intermediate and pathogenic (Paster et al. 1991; Schneider et al. 2018). Saprophytic leptospires, such as *Leptospira biflexa*, are free-living organisms found in water or soil and do not infect animal hosts (Faine et al. 1974). On the other hand, pathogenic strains of *Leptospira* infect animal hosts and humans such as *Leptospira interrogans* and *Leptospira borgpetersenii* (Picardeau et al. 2008). Intermediate *Leptospira* (for instance, *Leptospira wolffii* and *Leptospira broomii*) are not as virulent as the pathogenic species, but have been linked to human leptospirosis incidence (Arzouni et al. 2002). *Leptospira* are divided into numerous serovars (sub-species level) that could be distinguished by the characteristics of its antigens. Thirteen serogroups have been identified in Malaysia including Australis, Bataviae and Canicola (Bahaman & Ibrahim 1988).

The incidence of leptospirosis in tropical countries is higher than the rest of the world and was reported to range from 10 to 100 human cases per 100,000 individuals (Guera 2013). Thus, leptospirosis has become a public health problem due to its potential to become an epidemic (Schneider et al. 2013). In Malaysia, an increasing trend of leptospirosis cases had been observed from 2004 to 2014. From 263 cases in 2004, the number had significantly increased to 7,806 cases in 2014 (Garba et al. 2017). Outbreaks were identified to be seasonal, peaking during the rainy seasons and may be associated with floods (Mohd Radi et al. 2018; Zaki et al. 2018).

Leptospirosis exhibits similar symptoms such as fevers that are common to many other tropical diseases, thus further complicating its early detection. Patients who have been infected are usually treated with antibiotic prophylaxis such as doxycycline (Muhammad Aklil et al. 2018). However, studies on antileptospiral activities of natural products, specifically herbs have gained more interests nowadays. Previously, the *Adhatoda* extract has been proven to damage the inclusion body and motility of *L. interrogans* serovar Louisiana, which led to the loss of the organism's virulence activity (Nelson et al. 2013). In another study, the aqueous extract of *Quercus infectoria* Gall was also reported to have antimicrobial activity against pathogenic *Leptospira* (Husna et al. 2018). The methanol leaves extract of *Dabai* (or *Canarium odontophyllum*), a native plant to Borneo Island, also showed promising antileptospiral activity (Shafariatul Akmar et al. 2019). Of particular interest, one of the identified potential herbal antileptospiral agents is *Zingiber zerumbet*, which is also known as *Lempoyang* among locals and belongs to the family of Zingiberaceae (Yob et al. 2011).

Z. zerumbet is known to have antimicrobial activity against important pathogenic bacteria that are commonly associated with HIV infection such as *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Streptococcus mutants,* and *Salmonella typhi* (da Silva et al. 2018; Kader et al. 2011, 2010; Voravuthikunchai et al. 2005). It also has anti-staphylococcal activity against a series of multi-drug resistant (MDR) *S. aureus* strains (Kader et al. 2010). Although the antimicrobial activity of the *Z. zerumbet* extract on common bacterial infection in humans has been widely studied, its effect against *Leptospira* is still underexplored.

Materials and Methods

Subculture of *Leptospira* spp.

Leptospira samples used in this study were *L. interrogans* serovar Bataviae, Canicola, and Australis as well as *L. biflexa* serovar Patoc. All samples were subcultured into EMJH medium for storage purposes and stored in the dark at 27 °C to 30 °C. All stock cultures of samples were supplied by the Biotechnology Unit, Institute for Medical Research, Kuala Lumpur, Malaysia.

Preparation of *Z. zerumbet* crude extract

The air-dried finely chopped rhizomes of *Z. zerumbet* (Specimen Voucher: UKMB-29952) were sequentially soaked at room temperature in hexane, ethyl acetate and methanol for 72 hours. The extracts were filtered and evaporated to dryness in vacuum to yield crude extracts of hexane, ethyl acetate, and methanol separately. All of the crude extracts were stored at 4 °C for further tests. Prior to use, the *Z. zerumbet* ethyl acetate extract was dissolved in dimethyl sulfoxide (DMSO) and diluted in phosphate buffered saline (pH 7.4).

Percent inhibition test (%) of *Z. zerumbet* Crude extracts

Active *Leptospira* cultures were prepared in EMJH medium and grown at 30 °C for 7 days. For the assay, the density of *Leptospira* was determined based on turbidity by using a spectrophotometer at 280 nm. The culture was then diluted in EMJH medium to reach a bacterial density of 2×10^6 cells/mL. Two-fold serial dilutions of the hexane, ethyl acetate and methanol crude extracts at concentrations ranging from 1.95 to 500 µg/mL were prepared in EMJH medium containing 10% DMSO

in a sterile 96-well round-bottomed plate, with the final volume of 10 µL per well. A total of 190 µL of inoculum containing 2×10^6 cells/mL were transferred into each well. Each plate was prepared with blanks (200 µL of media EMJH without *Leptospira*), negative controls (200 µL of media EMJH containing 2×10^6 *Leptospira*) and positive controls (200 µL of media EMJH containing 2×10^6 *Leptospira* and Doxycycline). The plates were wrapped with aluminium foil and incubated for 3 days at 30 °C in the dark. On the third day, the turbidity of the growth in the plate was determined spectrophotometrically at 280 nm wavelength. The percent inhibition of *Leptospira* growth by *Z. zerumbet* extracts was determined via the following formula (Wong et al. 2010):

$$\text{Percent inhibition (\%)} = \frac{OD_{control} - OD_{test}}{OD_{control}} \times 100$$

where OD_{test} is the OD280 reading average for test sample, and $OD_{control}$ is the OD280 reading average for control sample.

Percentage of inhibition is 0 or no inhibition if the results are negative. The test was conducted in triplicates for each serovar.

DNA PREPARATION AND POLYMERASE CHAIN REACTION FOR *Leptospira* CONFIRMATION

Active *Leptospira* cultures were prepared in EMJH medium and propagated at 30 °C for 7 days. Each type of serovars was then sub-cultured into new EMJH medium containing IC_{25} of hexane extracts for *L. interrogans* serovar Bataviae and IC_{50} of hexane extracts for the other serovars. The inoculums were incubated for 7 days at 30 °C in the dark. Template genomic DNA was prepared using Invitek kit (STRATEC Molecular, Germany). Polymerase chain reaction (PCR) primers LA/LB ([5′-GGC GGC GCG TCT TAA ACA TG-3′] and [5′-TTC CCC CCA TTG AGC AAG ATT-3′]), targeting the 16S rDNA gene, were used to confirm the genus *Leptospira* (Benacer et al. 2013). The reactions were carried out in a thermocycler (Lab Cycler, SensoQuest, Germany). The cycling conditions consisted of an initial denaturation at 94 °C for 3 min followed by 35 cycles of denaturing at 94 °C for 1 min, 35 cycles of annealing at 57 °C for 1 min, 35 cycles of elongation at 72 °C for 2 min, and additional extension at 72 °C for 10 min.

EFFECT OF *Z. zerumbet* HEXANE EXTRACT ON *Leptospira* DNA

The DNAs of treated *L. interrogans* serovar Bataviae, Canicola, Australis and *L. biflexa* serovar Patoc that were isolated and confirmed by PCR assay were mixed with loading buffer, electrophoresed at 100 V and visualized on 1.5% agarose gel containing ethidium bromide. The isolated treated DNAs were analyzed together with the DNAs of the untreated *L. interrogans* serovar Bataviae, Canicola, Australis and *L. biflexa* serovar Patoc, which served as controls. The resulting DNA fragment bands were further visualized and photographed using a gel documentation system (Biorad, Hercules, California, USA).

RESULTS

CONFIRMATION OF *Leptospira* ISOLATES BY PCR WITH SPECIFIC PRIMERS

All spirochete bacteria used in the current study produced PCR products with 331 bp-long fragments of the 16S rDNA gene, a diagnostic target for PCR-based detection of *Leptospira* spp. (Benacer et al. 2013, Mérien et al. 1992) (Figure 1).

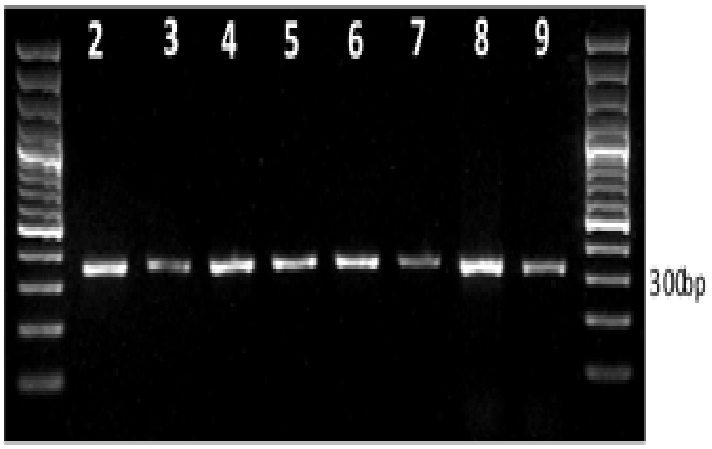

FIGURE 1. PCR product of primer LA/LB on DNA of *Leptospira.* Lane 1 and 10: DNA Ladder (100 bp), Lane 2 and 3: Bataviae (control and grown in 15.63 µg/mL hexane extract), Lane 4 and 5: Canicola (control and grown in 248 µg/mL hexane extract), Lane 6 and 7: Australis (control and grown in in 109 µg/mL hexane extract), Lane 8 and 9: Patoc (control and grown in in 125 µg/mL hexane extract)

INHIBITORY ACTIVITY OF *Z. zerumbet* CRUDE EXTRACTS ON *Leptospira* GROWTH

In general, *L. biflexia* serovar Patoc was the only serovar studied which was affected by hexane, ethyl acetate and methanol extracts of *Z. zerumbet* as evidenced by the 50% growth inhibition (Table 1). At the same percent inhibition, both *L. interrogans* serovar Canicola and *L. interrogans* serovar Australis were susceptible only to the hexane extract of *Z. zerumbet* (more than 50% growth inhibition). Both ethyl acetate and methanol extracts either showed no inhibition or less than 10% growth inhibition activity against *L. interrogans* serovar Australis.

TABLE 1. Percentage of Leptospira growth inhibition (%) in selected range of concentration (µg/mL) using different types of solvents

Concentration of *Z. zerumbet* crude extracts (µg/mL)	Percentage of *Leptospira* growth inhibiton (%) in selected range of concentration (µg/mL) using different solvents											
	Leptospira's Serovar											
	L. interrogans serovar Bataviae			*L. interrogans* serovar Canicola			*L. interrogans* serovar Australis			*L. biflexa* serovar Patoc		
	Hex	EA	Met	Hex	EA	Met	Hex	EA	Met	Hex	EA	Met
1.95	-	-	-	-	-	-	-	-	-	-	-	-
3.91	22	5	-	-	-	-	25	-	-	30	37	39
7.82	23	7	-	-	8	-	26	-	-	31	39	41
15.63	25	10	19	6	28	4	31	-	-	32	56	44
31.25	29	13	21	7	30	6	36	-	-	38	62	45
62.5	32	15	28	40	35	27	45	-	-	42	67	45
125	35	16	34	41	40	29	51	-	4	55	68	49
250	37	18	37	51	45	29	53	-	7	60	69	54
500	39	20	39	75	48	30	66	-	8	74	70	65

Hex: hexane, EA: ethyl acetate, Met: methanol

Dose-response curves (Figure 2(A) - 2(D)) have been constructed to determine the concentration of *Z. zerumbet* extracts that can inhibit the growth of *Leptospira* tested at 10%, 25%, and 50% growth inhibition. The hexane extract was found to have the median inhibitory concentration (IC_{50}) value or able to inhibit 50% of *L. interrogans* serovar Canicola and serovar Australis as well as *L. biflexa* serovar Patoc growth at the concentration of 248 µg/mL, 125 µg/mL, and 109 µg/mL, respectively (Table 2).

Both ethyl acetate and methanol extracts showed IC_{50} values of 13 µg/mL and 156.25 µg/mL for *L. biflexa* serovar Patoc, respectively. Meanwhile, for *L. interrogans* serovar Canicola, IC_{25} values of 13.69 µg/mL and 61 µg/mL were observed for both ethyl acetate and methanol extracts, respectively. The methanol extract inhibited 25% growth of *L. interrogans* serovar Bataviae (IC_{25}) at the concentration of 46.88 µg/mL, while the ethyl acetate extract was not active against this serovar. However, 25% of the bacterial growth (IC_{25}) of *L. interrogans* serovar Bataviae was inhibited by 15.63 µg/mL hexane extract, though the inhibition was not dose-dependent as exposure to higher concentrations of the hexane extract failed to achieve 50% inhibition against this serovar (Table 2). Unlike the other three *Leptospira* spp., the growth of *L. interrogans* serovar Australis was not affected by the ethyl acetate and methanol extract of *Z. zerumbet*. This was clearly evidenced by absence of inhibitory concentration values as shown in Figure 2(C) and tabulated in Table 2.

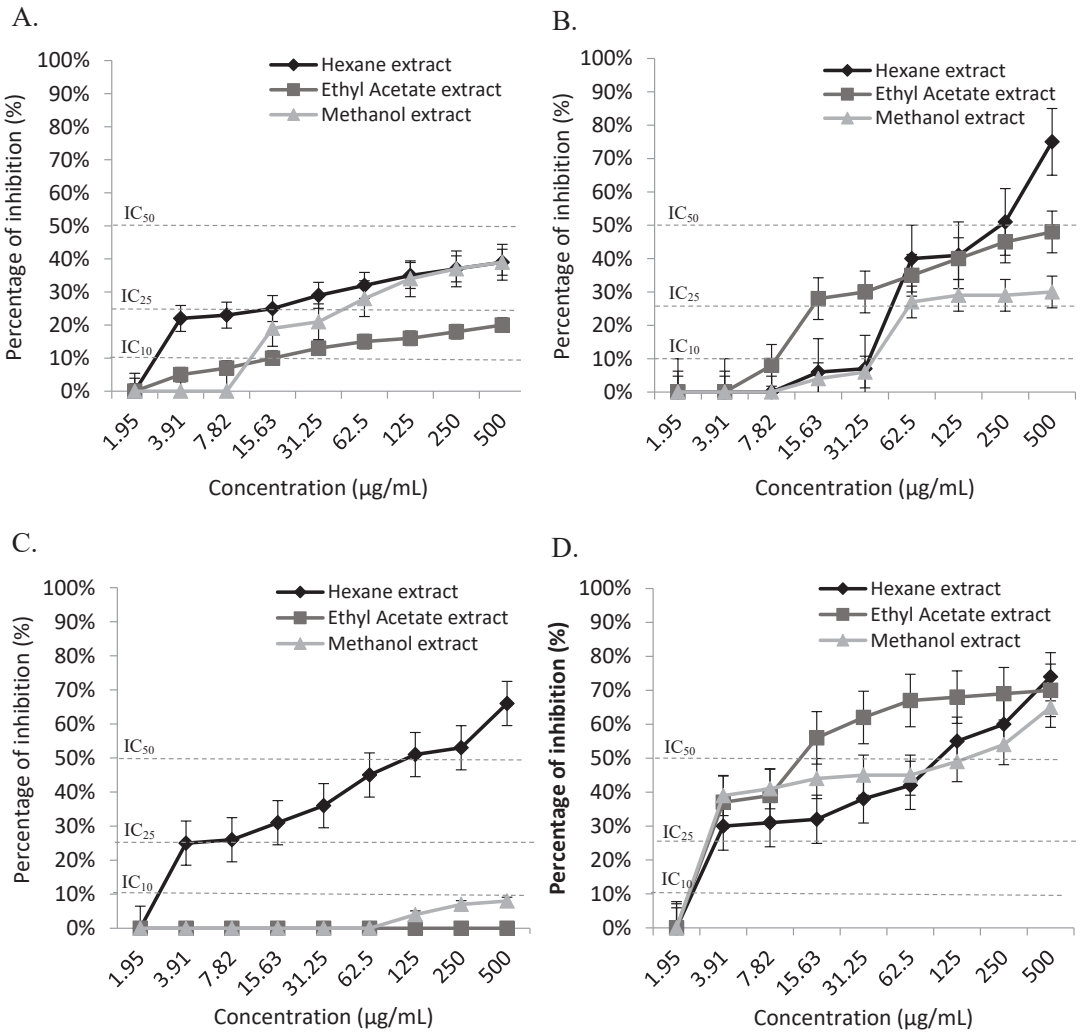

FIGURE 2. The growth inhibition concentration of hexane, ethyl acetate, and methanol extracts of *Zingiber zerumbet* on different *Leptospira* spp.; *L. interrogans* serovar Bataviae (A), B-*L. interrogans* serovar Canicola (B), *L. interrogans* serovar Australis (C) and *L. biflexa* serovar Patoc (D)

TABLE 2. The *Leptospira* spp. growth inhibitory concentrations of Z. *zerumbet* crude extracts (µg/mL) at 10%, 25% and 50%

Inhibitory concentration (IC) / Inhibitory Concentration (IC)	Inhibitory concentration values of *Z. zerumbet* crude extracts (µg/mL)											
	Leptospira's Serovar											
	L. interrogans serovar Bataviae			*L. interrogans* serovar Canicola			*L. interrogans* serovar Australis			*L. biflexa* serovar Patoc		
	Hex	EA	Met	Hex	EA	Met	Hex	EA	Met	Hex	EA	Met
IC_{10}	2.93	15.63	11.73	34	8	2.44	2.93	-	-	2.44	2.44	2.44
IC_{25}	15.63	-	46.88	47	13.69	61	3.91	-	-	3.66	3.42	3.42
IC_{50}	-	-	-	248	-	-	125	-	-	109	13	156.25

Hex: hexane, EA: ethyl acetate, Met: methanol

GENOMIC DNA-DAMAGING EFFECT OF *Zingiber zerumbet* EXTRACTS

The genomic DNA extracted from *L. interrogans* serovar Bataviae and serovar Canicola was exposed to 15.63 µg/mL (Figure 3(A)) and 248 µg/mL (Figure 3(B)) of hexane extracts, respectively, and was shown to be all intact, comparable to the untreated genomic DNA. However, when compared to the untreated DNA of *L. interrogans* serovar Australis (control), the treatment of *Z. zerumbet* hexane extract at 125 µg/mL caused fragmentation to the bacterial genomic DNA (Figure 4(A)). Subculturing of *L. biflexa* serovar Patoc with the IC_{50} concentration of *Z. zerumbet* hexane extract (109 µg/mL) showed fragments of DNA being produced, indicating DNA-damaging effects of the hexane extract (Figure 4(B)).

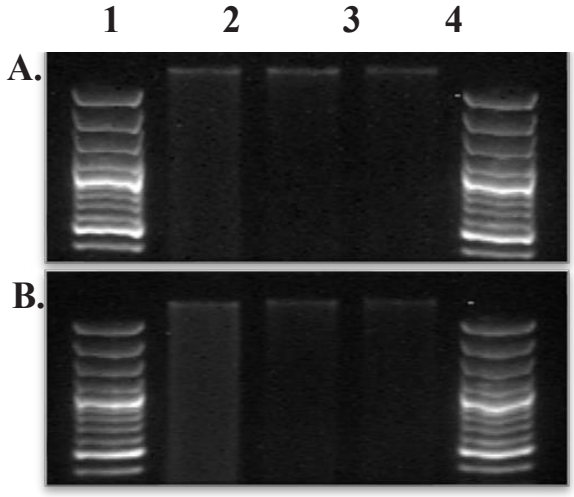

FIGURE 3. The effect of *Z. zerumbet* hexane extract on the DNA of *Leptospira* spp. The *Leptospira* was grown in EMJH media with 15.63 µg/mL of hexane extract against *L. interrogans* serovar Bataviae (A) and 248 µg/mL hexane extract against *L. interrogans* serovar Canicola (B) for 7 days before the DNA was extracted and electrophoresed. Lane 1 and 5: DNA ladder (100 bp), Lane 2: Normal DNA, Lane 3 and 4: *Leptospira* with hexane extract

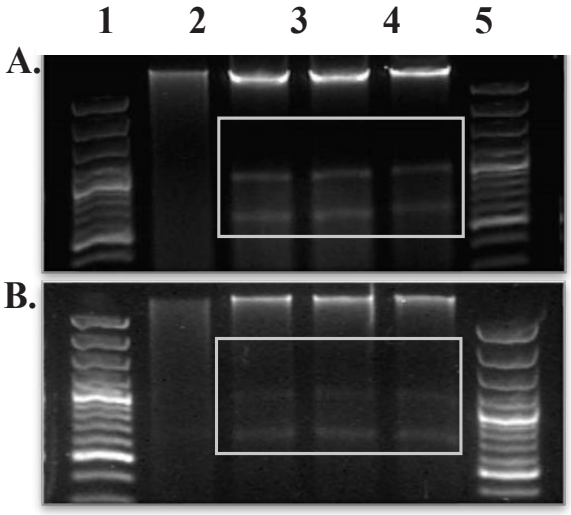

FIGURE 4. The effect of *Z. zerumbet* hexane extract on the DNA of *Leptospira* spp. The Leptospira was grown in EMJH media with 125 µg/mL against *L. interrogans* serovar Australis (A) and with 109 µg/mL hexane extract against *L. biflexa* serovar Patoc for 7 days before the DNA was extracted and electrophoresed. Note the presence of fragmented DNA as indicated in the white box in Lane 3-5 of both Figure 4(A) and 4(B). Lane 1 and 6: DNA ladder, Lane 2: Normal DNA, Lane 3, 4 and 5: *Leptospira* with hexane extract

DISCUSSION

In the current study, the LA/LB 16S rDNA primers were used to identify the *Leptospira* isolates via PCR. The presence of bands at 331 bp demonstrated the extracted DNA was of *Leptospira* origin (Benacer et al. 2013). The pathogenicity status of *Leptospira* spp. could be further confirmed by detecting the expression of lipoprotein LipL32 protein (a virulence factor) via PCR (Dive et al. 1987; Latifah et al. 2017). This particular protein is highly expressed in *Leptospira* during fatal acute infections compared to *in vitro* culture (Nally et al. 2007). Although the pathogenicity of the *Leptospira* spp. used in the current study was not determined, though the pathogenicity of the original stocks has been confirmed. Furthermore, previous reports have stated that *L. interrogans* is a pathogenic species whilst *L. biflexia* is saprophytic (Benacer et al. 2016; Evangelista & Coburn 2010; Saengjaruk et al. 2007).

The aim of the study was to investigate the antileptospiral activity of three different *Zingiber zerumbet* extracts, which were hexane, ethyl acetate, and methanol. Based on the percent inhibition results, each tested extract showed different inhibitory activities to different *Leptospira* serovars. Similarly, a study conducted by Murray and Hospenthal (2004) also showed the diverse susceptibilities of 12 different *Leptospira* serovars towards three common antibiotics used in the treatment of leptospirosis: penicillin, doxycycline and chloramphenicol. Their study showed that the currently used antibiotics have different inhibitory concentrations against selected *Leptospira* spp. The diversity of response of *Leptospira* spp. towards different treatments and doses used could be explained by the lipopolysaccharide expressions on the surface of the bacteria (Adler et al. 2010), or could be due to the formation of biofilms (Vinod et al. 2018).

Furthermore, the differences in the inhibitory responses shown by the *Leptospira* spp. used in the current study could also be attributed to the presence of different active compounds in each of the extraction solvent used. The types and amounts of phytochemical compounds in the crude extracts depend on the polarity of the solvent used for extraction. Among the three solvents used, methanol was the most polar whilst hexane was the least. In the current study, the hexane extract showed more effective inhibitory activities as proven by the IC_{50} values against all selected *Leptospira* spp. compared to the limited effectiveness of methanol and ethyl acetate extracts. Although the inhibition was not total (100% inhibition), the fact that the hexane extract could inhibit 50% growth of the pathogenic *L. interrogans* strains used in the current study showed that it has great potential as antileptospiral agent. Further investigation showed that only *L. interrogans* serovar Australis exhibited DNA damage upon exposure to *Z. zerumbet* hexane extract at 125 µg/mL compared to serovar Canicola that was exposed to double the concentration of similar solvent extract. The results suggest that there could be other mechanism besides bacterial DNA damage that induced bacterial growth inhibition in *L. interrogans* serovar Canicola. The primary modes of action of therapeutic agents generally used in the treatment of leptospirosis include inhibiting the synthesis of nucleic acid, protein and bacterial cell wall; altering the bacterial cell membrane or having antimetabolite activity.

Despite the fact that *L. biflexa* serovar Patoc is a saprophytic strain and non-pathogenic, this bacterium was vulnerable towards all three *Z. zerumbet* extracts used in this study. Among the three solvents used, ethyl acetate extract was the only extract that could inhibit 50% of the growth of this bacterium and at the lowest concentration compared to the other two solvents. Furthermore, at an IC_{50} of 109 µg/mL, the hexane extract of *Z. zerumbet* was also shown to cause DNA damage to *L. biflexa* serovar Patoc, similar to the effect of the hexane extract towards *L. interrogans* serovar Australis.

The results of the current study focused more on the effect of the hexane extract rather than the other two solvent extracts as this particular extract was shown to be active against all of the *Leptospira* spp. investigated in this study. This shows that the hexane extract is more practical to be developed as an antileptospiral agent due to its broader spectrum. Hexane is a non-polar solvent known to extract lipophilic compounds (Musa et al. 2015). Lipophilicity of an antibacterial compound is an important characteristic as it can determine the success or failure of the compound to access its target (Echeverría et al. 2017).

According to Patial et al. (2019), among the phytochemical constituents obtained using the soxhlet extraction method with hexane as a solvent are triterpenoids, glycosides and steroids. Plant-based terpenoid, flavanoid and steroid compounds are commonly associated with antimicrobial and anti-inflammatory activities (Cushnie & Lamb 2005; Patel & Saviani 2015; Prakash 2017). Zerumbone, a sesquiterpenoid that is lipophilic in nature, is the main bioactive compound present in the rhizomes of *Z. zerumbet* (L.) Smith. The reported activities of zerumbone that have merits and relevant to the findings of the current study are antimicrobial and anti-inflammatory activities (Abdul et al. 2008; Haque et al.

2018). Doxycycline, one of the antibiotics used for the treatment of leptospirosis is both antibacterial and anti-inflammatory. This dual characteristic is beneficial and more effective as a therapeutic agent as *Leptospira* may induce overexpression of inflammatory responses that are often associated with poor prognosis of leptospirosis (Matsui et al. 2011; Zhang et al. 2017). Furthermore, *Z. zerumbet* crude extracts have also been shown to be neuroprotective, hepatoprotective, and nephroprotective against pathological-induced conditions (Abdul Hamid et al. 2012; Hamid et al. 2018, 2011), thus the usage might be able to delay functional deterioration of affected organs in leptospirosis.

Conclusion

The hexane extract of *Z. zerumbet* was found to have a broader antileptospiral activity compared to ethyl acetate and methanol extracts. The hexane extract was also shown to cause damage to the DNA of *L. interrogans* serovar Australis, which is one of the pathogenic serovars of *Leptospira* spp. Based on the results of the two tests, it was found that the hexane extract of *Z. zerumbet* has the potential to be developed as an antileptospiral agent in the future. To achieve this, future work should focus on investigating both antileptospiral and anti-inflammatory activities of the *Zingiber zerumbet* hexane crude extract of the rhizomes using the *in vivo* model of leptospirosis-induced rats.

Acknowledgements

We are thankful to the Biotechnology Unit, Institute for Medical Research, Kuala Lumpur for providing the *Leptospira* spp. samples as well as providing us with the laboratory facilities to partially conduct the research.

References

Abdul, A., Siddiq, I.A., Adel, S.A., Manal, M.E. & Syam, M.M. 2008. Anti-cancer and anti-microbial activities of zerumbone from the rhizomes of *Zingiber zerumbet*. *Int. J. Pharm.* 4(4): 301-304.

Abdul Hamid, Z., Budin, S.B., Ng, W.J., Hamid, A., Husain, K. & Mohamed, J. 2012. Nephroprotective effects of *Zingiber zerumbet* Smith ethyl acetate extract against paracetamol-induced nephrotoxicity and oxidative stress in rats. *J. Zhejiang Univ. Sci. B* 13(3): 176-185.

Adler, B. & de la Pena Moctezuma, A. 2010. Leptospira and leptospirosis. *Vet. Microbiol.* 140(3-4): 287-296.

Arzouni, J.P., Parola, P., La Scola, B., Postic, D., Brouqui, P. & Raoult, D. 2002. Human infection caused by *Leptospira fainei*. *Emerg. Infect.* 2002: 865-868.

Bahaman, A.R. & Ibrahim, A.L. 1988. A review of leptospirosis in Malaysia. *Vet. Res. Commun*. 12(2): 179-189.

Benacer, D., Siti, N.M.Z., Zhu Sim, S., Nizam, M.K.M.K., Galloway, R.L., Souris, M. & Thong, K.L. 2016. Determination of *Leptospira borgpetersenii* serovar Javanica and *Leptospira interrogans* serovar Bataviae as the persistent *Leptospira* serovars circulating in the urban rat populations in Peninsular Malaysia. *Parasit Vectors* 9: 117.

Benacer, D., Who, P.Y., Mohd Zain, S.N., Amran, F. & Thong, K.L. 2013. Pathogenic and saprophytic *Leptospira* species in water and soils from selected urban sites in Peninsular Malaysia. *Microbes. Environ.* 28(1): 135-140.

Cushnie, T.P. & Lamb, A.J. 2005. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* 26(5): 343-356.

da Silva, T.M., Pinheiro, C.D., Orlandi, P.P., Pinheiro, C.C. & Pontes, G.S. 2018. Zerumbone from *Zingiber zerumbet* (L.) Smith: A potential prophylactic and therapeutic agent against the cariogenic bacterium *Streptococcus mutans*. *BMC Complement Altern. Med.* 18: 301.

Dive, A.M., Bigaignon, G. & Reynaert, M. 1987. Adult respiratory distress syndrome in leptospira icterohaemorrhagiae infection. *Intensive Care Med.* 13(3): 214.

Echeverría, J., Opazo, J., Mendoza, L., Urzúa, A. & Wilkens, M. 2017. Structure-activity and lipophilicity relationships of selected antibacterial natural flavones and flavanones of Chilean flora. *Molecules* 22(4): E608. doi: 10.3390/molecules22040608.

Evangelista, K.V. & Coburn, J. 2010. Leptospira as an emerging pathogen: A review of its biology, pathogenesis and host immune responses. *Future Microbiol.* 5(9): 1413-1425.

Faine, S., Adler, B. & Ruta, G. 1974. A mechanism of immunity to leptospirosis. *Aust. J. Exp. Biol. Med. Sci.* 52(2): 301-310.

Garba, B., Bahaman, A.R., Khairani-Bejo, S., Zakaria, Z. & Mutalib, A.R. 2017. Retrospective study of leptospirosis in Malaysia. *EcoHealth* 14(2): 389-398.

Guerra, M.A. 2013. Leptospirosis: Public health perspectives. *Biologicals* 41(5): 295-297.

Hamid, A., Ibrahim, F.W., Teoh, H.M., Nasrom, M.N., Eusoff, N., Husain, K. & Abdul Latif, M. 2018. *Zingiber zerumbet* L. (Smith) extract alleviates the ethanol-induced brain damage via its antioxidant activity. *BMC Complement Altern. Med.* 18(1): 101.

Hamid, A., Budin, S.B., Mohamed, R.A.P., Manaf, N.A., Yuhana, N.Y., Husain, K., Abd Hamid, Z. & Mohamed, J. 2011. Role of oxidative stress in the protective effects of *Zingiber zerumbet* Smith ethyl-acetate extract against paracetamol-induced hepatotoxicity in Sprague-Dawley rat. *Aust. J. Basic Appl. Sci.* 5(8): 1519-1525.

Haque, M.A., Jantan, I. & Harikrishnan, H. 2018. Zerumbone suppresses the activation of inflammatory mediators in LPS-stimulated U937 macrophages through MyD88-dependent NF-κB/MAPK/PI3K-Akt signaling pathways. *Int. Immunopharmacol.* 55: 312-322.

Husna, M., Nabilah, I. & Wan Nor Amilah, W.A.W. 2018. Anti-microbial activity of aqueous *Quercus infectoria* gall extract against pathogenic Leptospira. *Malays. J. Med. Sci.* 25(4): 42-50.

Kader, G., Nikkon, F., Rashid, M.A. & Yeasmin, T. 2011. Antimicrobial activities of the rhizome extract of *Zingiber zerumbet* Linn. *Asian Pac. J. Trop. Biomed.* 1(5): 409-412.

Kader, M.G., Habib, M.R., Nikkon, F., Yeasmin, T., Rashid, M.A., Rahman, M.M. & Gibbons, S. 2010. Zederone from the rhizomes of *Zingiber zerumbet* and its anti-staphylococcal activity. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* 9(1): 63-68.

Latifah, I., Abdul Halim, A., Rahmat, M.S., Faten, N.M., Ubil, Z.E., Asmah, H., Shafariatul Akmar, I., Picardeau, M., Siti Haslina, O. & Nasir, M.A. 2017. Isolation by culture and PCR identification of LipL32 gene of pathogenic *Leptospira* spp. in wild rats of Kuala Lumpur. *Malays. J. Pathol.* 39(2): 161-166.

Matsui, M., Rouleau, V., Bruyère-Ostells, L. & Goarant, C. 2011. Gene expression profiles of immune mediators and histopathological findings in animal models of leptospirosis: Comparison between susceptible hamsters and resistant mice. *Infect. Immun.* 79: 4480-4492.

Mérien, F., Amouriaux, P., Perolat, P., Baranton, G. & Saint Girons, I. 1992. Polymerase chain reaction for detection of *Leptospira* spp. in clinical samples. *J. Clin. Microbiol.* 30(9): 2219-2224.

Mohd Radi, M.F., Hashim, J.H., Jaafar, M.H., Hod, R., Ahmad, N., Nawi, M., Baloch, G.M., Ismail, R. & Farakhin Ayub, N.I. 2018. Leptospirosis outbreak after the 2014 major flooding event in Kelantan, Malaysia: A spatial-temporal analysis. *Am. J. Trop. Med. Hyg.* 98(5): 1281-1295.

Muhammad Aklil, A.R., Aznida, M.Z., Azman, A., Muhammad Haneef, A., Shaifuddin, N.H., Syed Syarizman, S.A.R., Mohammad Safree, J., Norfazilah, A. & Mohd Rohaizat, H. 2018. Effectiveness of antibiotic prophylaxis for leptospirosis among adults: A systematic review. *Malays. J. Appl. Sci.* 3(2): 46-56.

Murray, C.K. & Hospenthal, D.R. 2004. Determination of susceptibilities of 26 *Leptospira* sp. serovars to 24 antimicrobial agents by a broth microdilution technique. *Antimicrob. Agents Chemother.* 48(10): 4002-4005.

Musa, A.M., Ibrahim, M.A., Aliyu, A.B., Abdullahi, M.S., Tajuddeen, N., Ibrahim, H. & Oyewale, A.O. 2015. Chemical composition and antimicrobial activity of hexane leaf extract of *Anisopus mannii (Asclepiadaceae*). *J. Intercult. Ethnopharmacol*. 4(2): 129-133.

Nally, J.E., Whitelegge, J.P., Bassilian, S., Blanco, D.R. & Lovett, M.A. 2007. Characterization of the outer membrane proteome of *Leptospira interrogans* expressed during acute lethal infection. *Infect. Immun.* 75(2): 766-773.

Nelson, J., Chairman, K., Ranjit Singh, A.J.A., Padmalatha, C. & Hepsibah, B. 2013. Cytomorphological changes and inhibition of inclusion body formation in *Leptospira Interrogans* on treatment with the extracts of Adhatodavasica. *Adv. Tech. Biol. Med.* 1(1): 1-4.

Paster, B.J., Dewhirst, F.E., Weisburg, W.G., Tordoff, L.A., Fraser, G.J., Hespell, R.B., Stanton, T.B., Zablen, L., Mandelco, L. & Woese, C.R. 1991. Phylogenetic analysis of the spirochetes. *J. Bacteriol*. 173(19): 6101-6109.

Patel, S.S. & Savjani, J.K. 2015. Systematic review of plant steroids as potential antiinflammatory agents: Current status and future perspectives. *J. Phytopharmacol.* 4(2): 121-125.

Patial, P.K., Sharma, A., Kaur, I. & Cannoo, D.S. 2019. Correlation study among the extraction techniques, phytochemicals, and antioxidant activity of *Nepeta spicata* aerial part. *Biocatalysis and Agricultural Biotechnology* 20: 101275.

Picardeau, M., Bulach, D.M., Bouchier, C., Zuerner, R.L., Zidane, N., Wilson, P.J., Creno, S., Kuczek, E.S., Bommezzadri, S., Davis, J.C., McGrath, A., Johnson, M.J., Boursaux-Eude, C., Seemann, T., Rouy, Z., Coppel, R.L., Rood, J.I., Lajus, A., Davies, J.K., Médigue, C. & Adler, B. 2008. Genome sequence of the saprophyte *Leptospira biflexa* provides insights into the evolution of *Leptospira* and the pathogenesis of leptospirosis. *PLoS ONE* 3(2): e1607.

Prakash, V. 2017. Terpenoids as source of anti-inflammatory compounds. *Asian J. Pharm. Clin. Res.* 10(3): 68-76.

Saengjaruk, P., Sakolvaree, Y., Maneewatch, S., Tomanakan, K., Tongtawe, P., Tapchaisri, P. & Chaicumpa, W. 2007. Components of pathogenic *Leptospira* spp. with potentials for diagnosis of human leptospirosis. *Asian Pac. J. Allergy Immunol.* 25(4): 225-232.

Schneider, A.G., Casanovas-Massana, A., Hacker, K.P., Wunder, E.A.J., Begon, M., Reis, M.G., Childs, J.E., Costa, F., Lindow, J.C. & Ko, A.I. 2018. Quantification of pathogenic *Leptospira* in the soils of a Brazilian urban slum. *PLoS Negl. Trop. Dis.* 12(4): e0006415.

Schneider, M.C., Jancloes, M., Buss, D.F., Aldighieri, S., Bertherat, E., Najera, P., Galan, D.I., Durski, K. & Espinal, M.A. 2013. Leptospirosis: A silent epidemic disease. *Int. J. Environ. Res. Public Health* 10(12): 7229-7234.

Shafariatul Akmar, I., Syahirah, S., Fifi Fariza, A., Asmah, H., Latifah, I. & Dayang Fredalina, B. 2019. *In vitro* antileptospiral activity of *Canarium odontophyllum* Miq. (Dabai) leaves extract. *Malays. J. Microb.* 15(3): 220-225.

Vinod, K.K., Lall, C., Vimal, R.R., Vedhagiri, K., Sunish, I.P. & Vijayachari, P. 2018. Can subminimal inhibitory concentrations of antibiotics induce the formation of biofilm in Leptospira? *Microb. Drug Resist.* 24(7): 1040-1042.

Voravuthikunchai, S.P., Phongpaichit, S. & Subhadhirasakul, S. 2005. Evaluation of antibacterial activities of medicinal plants widely used among aids patients in Thailand. *Pharm. Biol.* 43(8): 701-706.

Wong, S.K., Lim, Y.Y. & Chan, E.W.C. 2010. Evaluation of antioxidant, anti-tyrosinase and antibacterial activities of selected Hibiscus species. *Etnobotanical Leaflets* 14: 781-796.

Yob, N.J., Jofrry, S.M., Affandi, M.M.L.K., Salleh, M.Z. & Zakaria, Z.A. 2011. *Zingiber zerumbet* (L.) Smith: A review of its ethnomedicinal, chemical, and pharmacological uses. *Evid. Based Complement Alternat. Med*. 2011: 543216.

Zaki, A.M., Rahim, M.A.A., Azme, M.H., Mahmood, N.A., Jeffree, M.S., Ghazi, H.F. & Hassan, M.R. 2018. Animal reservoirs for *Leptospira* spp. in South-East Asia: A meta-analysis. *J. Adv. Res. Med.* 5(3): 23-31.

Zhang, W., Xie, X., Wu, D., Jin, X., Liu, R., Hu, X., Fu, Y., Ding, Z., Zhang, N. & Cao, Y. 2017. Doxycycline attenuates leptospira-induced il-1β by suppressing nlrp3 inflammasome priming. *Front Immunol.* 8: 857.

Farah Wahida Ibrahim, Nurul Amirah Abdul Aziz, Nurul Farhana Jufri & Asmah Hamid*
Center for Toxicology and Health Risk Studies (CORE)
Faculty of Health Sciences
Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz
50300 Kuala Lumpur, Federal Territory
Malaysia

Latifah Ibrahim
Biotechnology Unit
Institute for Medical Research, Jalan Pahang
50588 Kuala Lumpur, Federal Territory
Malaysia

*Corresponding author; email: asmah0901@ukm.edu.my

Received: 25 March 2020
Accepted: 18 February 2021