# Evaluation of the safe use of the larvicidal fraction of *Capparis cartilaginea* Decne. against *Aedes caspius* (Pallas) (Diptera: Culicidae) larvae

N. Abutaha[1*] & A.F. Al-Mekhlafi[2,3]

[1]*Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia*
[2]*Bioproducts Research Chair, Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia*
[3]*Department of Agricultural Production, College of Agriculture and Veterinary Medicine, Thamar University, Yemen*

Larvicidal activity and cytotoxicity of fruit and leaf extracts of the *Capparis cartilaginea* were evaluated. No previous studies have been reported on the larvicidal activities of crude and column chromatographic fractions of fruit and leaf extracts of *Capparis cartilaginea*. In this study, HPLC analysis of the alkaloidal active fraction F2 of methanolic fruit extract showed the presence of a major peak. Crude aqueous fruit extract of *C. cartilaginea* was less toxic against *Aedes caspius* ($LC_{50}$ = 172.02) compared to its column chromatographic fraction F2 ($LC_{50}$ = 29.17). Cytotoxicity studies on HepG2 and MCF7 at 24 h post-treatment recorded survival rates ranging from 90 to 110 %, respectively. Light and fluorescent microscopy revealed no effect on cell morphology at 100 $\mu$g/ml. The plasma membrane remained well defined and the nuclei were normal and exhibited diffused staining of the chromatin. Most active component(s) responsible for toxic activity against *Aedes caspius* was present in the fraction F2 of *C. cartilaginea*, which showed larvicidal efficacy in a dose-dependent manner. As a result, fraction F2 is a good candidate as an alternative natural larvicidal agent with no hepatotoxicity and at a low cost. Further investigation is required to identify and characterize active compounds.

**Key words**: *Capparis cartilaginea*, chromatography, cell lines, larvicidal activity.

## INTRODUCTION

The WHO considers mosquitoes as a primary public health target, on account of the fact that they transmit more diseases than any other group of arthropods. Mosquito-borne diseases are prevalent in more than 100 countries across the world, infecting over 700 million people every year (Ghosh *et al.* 2012).

Mosquito-borne diseases have been reported in Saudi Arabia with increasing frequency and with an expanding geographic distribution. In Saudi Arabia, the most common mosquito-borne diseases include dengue (Fakeeh & Zaki 2001, 2003; Ayyub *et al*. 2006; Khan *et al*. 2008), filaria (Hawking 1973), malaria (Warrel 1993; Abdoon 2004), and Rift Valley fever (Jupp *et al*. 2002; Miller *et al*. 2002; Balkhy & Memish 2003; Al-Hazmi *et al*. 2003; Madani *et al*. 2003). A survey by Ahmed *et al*. (2011) showed that in Al-Ahsaa, Saudi Arabia, *Ae. caspius* was the most abundant mosquito followed by *Culex pipiens*. *Ae. ca* has also been shown to be widely distributed in different regions of Saudi Arabia, including in Riyadh district (Al-Khreji 2005). Accordingly, recently, Saudi Arabia's Ministry of Health has allocated US$5.5 million as seed funding to develop innovative ways to control major diseases transmitted by mosquito vectors (Almuraisy 2011).

Though the high degree of toxicity and specificity of synthetic insecticides have enabled great advances in insect control, the extensive use of these synthetic organic insecticides have resulted in environmental pollution and resistance in vector species. This has necessitated research and development of low cost, biodegradable, and safer local methods for vector control, especially at the immature stage. During the immature stage, mosquitoes are relatively immobile, and since they remain more concentrated than in the adult stage, they are easier to eradicate (Rutledge *et al*. 2003).

One of the most effective alternative approaches is to use natural sources, and consequently, biological and eco-friendly resources are currently a broad research area for the control of vectors of

*Author for correspondence. E-mail: nabutaha@ksu.edu.sa

medical importance (Singha *et al*. 2012; Chowdhury *et al*. 2008). Plant-based insecticides have been found to be the most efficient, safe and appropriate substitute for chemical insecticides (Ghosh 2012; Banerjee *et al*. 2011; Rawani *et al*. 2009). Several classes of phytochemicals such as alkaloids, phenols, terpenoids and essential oils from different plants have been reported for their potential insecticidal activities.

The Capparaceae family comprises 39 genera and 650 species distributed through warm regions (Boulos 1999). The genus *Capparis comprises* 250 species including shrubs, trees and woody climbers (Tackholm 1974). *Capparis cartilaginea* is a perennial species that produces oval fruits packed with numerous small seeds, which are eaten and dispersed by birds (Elffers *et al*. 1964; Wickens 1979; Rivera *et al*. 2003). The fruits of *C. cartilaginea* (Fig. 1) can be pickled or preserved for consumption. *C. cartilaginea* is found in north and East Africa, the Middle East and southwest Asia, including Pakistan, Afghanistan, Palestine, Iran, Iraq, India, the United Arab Emirates (Rivera *et al*. 2002) and Saudi Arabia. *Capparis cartilaginea* is used by inhabitants of Onaizah province as a disinfectant, wound wash, antitumor medicine, tonic and purgative (Youssef 2013).

The term cytotoxicity means the effect of chemical agents in altering cellular morphology, particularly by causing a failure of the cell to attach to surfaces, and by the changes in the rate of cell growth, cell death and cell disintegration (Slater 2001). Animals are often used to identify potentially hepatotoxic compounds in humans, but interspecies differences limit the ability to produce rapid, accurate and cost-effective safety assessments (Harry *et al*. 2000; Peters 2005). Consequently, the *in vitro* human cell culture system is an essential substitute to enable the assessment of the toxicity of compounds (Gomez-Lechon *et al*. 2007; Horii & Yamada 2007).

**Fig. 1**. Longitudinal section of *Capparis cartilaginea* fruit.

Assessment of cytotoxicity using *in vitro* cell proliferation assays such as MTT and fluorescent microscopy remain essential tools for the evaluation of the safe use of crude extracts, fractions and purified compounds.

In this study, the larvicidal activity of methanol crude extract of *C. cartilaginea* and its fractions against *Ae. caspius*, and the cytotoxicity of active fractions, were evaluated using the human cell lines.

## MATERIAL AND METHODS

### *Collection and preparation of* C. cartilaginea *methanol extract*

*Capparis cartilaginea* (Capparaceae) was collected from Neaam in Riyadh, Kingdom of Saudi Arabia. The botanical identification was authenticated in the Department of Botany and Microbiology, College of Science, King Saud University, where the voucher specimen was deposited. For the preparation of crude solvent extract, 100 g of fresh leaves and 100 g of unripe fruits of the plant were blended with 500 ml methanol separately before being left to mix overnight at 150 rpm and 30 °C. This procedure was repeated three times, with 500 ml fresh methanol being added to the leftover residue on each occasion. The extract was then centrifuged at 5000 rpm for 10 min and the supernatant was collected and evaporated under reduced pressure using a rotary evaporator at 45 °C.

### *Preparation of aqueous extracts*

Leaves and unripe fruits (100 g each) of *C. cartilaginea* were collected and added separately to 1000 ml of boiling water. The solution was kept in an incubator shaker at 30 °C with gentle swirling at 120 rpm for 2 h. The solution was filtered through a muslin cloth and the filtrate was centrifuged at 5000 rpm for 10 min with the supernatant then being collected and evaporated under reduced pressure using a rotary evaporator at 45 °C for the leaves and fruit extracts separately.

### *Liquid-liquid extraction*

The active extract was subjected to fractionation by partition between ethyl acetate ($F_{EtoAC}$) (3 ×

200 ml). The remaining aqueous layer was evaporated using a rotary evaporator at 45 °C and the aqueous dried residue was further extracted with menthol ($F_{MeOH}$) (3 × 50 ml), with each fraction obtained being evaporated to dryness using a rotary evaporator at 45 °C. The samples were then prepared by dissolving 10 mg of the dried material in 1 ml of high-performance liquid chromatography (HPLC) grade methanol and filtering through a 0.2 $\mu$m (PVDF) syringe filter into sterile vials.

### *Chromatography purification: thin layer chromatography (TLC)*

The ethyl acetate fraction was dissolved in methanol at a concentration of 2 mg/ml. The sample was applied to the plate and TLC separations were performed on 10 × 10 cm silica gel 60 without a fluorescent indicator (Merck, Darmstadt, Germany). The solvent used was chloroform:acetic anhydride (10:0.1, v/v). The developed plates were dried at room temperature and kept in an iodine tank for detection. Bands on the TLC plates were visualized under daylight and the $R_f$ value was recorded. The bands corresponding to the samples were scraped off and the powder was diluted in methanol, with the filtrate being taken to dryness and weighed. The samples were prepared by dissolving 10 mg of the dried material in 1 ml of HPLC grade methanol and filtering through a 0.2 $\mu$m nylon syringe filter into sterile vials to be used for the further tests.

### *Phytochemical analysis of the active fractions*

The phytochemicals included in the study were alkaloid, steroids (Kumar *et al*. 2007) and phenols (Gibbs 1974).

### *High-performance liquid chromatography (HPLC)*

HPLC analysis was performed on a Perkin Elmer system equipped with a binary gradient system and UV detector. A Hypersil Gold phenyl HPLC column (150 mm × 4.6 mm, 5 $\mu$m diameter) was used for the experiments. The active fraction (10 $\mu$l) was injected using an auto sampler. The initial chromatographic conditions were 20:80 (acetonitrile:water) held for 10 min, with these conditions being gradually altered through a linear gradient profile to 80 % acetonitrile and 20 % of the original aqueous mobile phase. The conditions were then kept stable for 20 min before a linear gradient to 100 % acetonitrile for 10 min thereafter before the column was re-equilibrated to the initial conditions. The flow rate was kept constant at 1 ml/min. The samples for HPLC were prepared by dissolving 2 mg of the fraction material in 3 ml of acetonitrile and filtering through a 0.2 $\mu$m (PVDF) filter into HPLC vials.

### *FTIR analysis of bioactive TLC fractions*

The fraction that showed larvicidal activity was subjected to IR spectroscopic analysis for identification of the functional groups.

### *Mosquito culture*

*Aedes caspius* larvae were obtained from a colony maintained in the laboratory for five years without exposure to any insecticide at Department of Zoology, College of Science, King Saud University. They were reared in a plastic tray (24 × 35 × 5 cm) containing fish feed and were kept indoors at 27 ± 2 °C, 50 ± 5 % relative humidity, a 14:10 light:dark photoperiod and fed daily until becoming pupae. The pupae were transferred from the trays to a cup containing tap water and were maintained in our insectary. They were then moved into a mosquito cage where the emergent adults were fed with a 10 % glucose solution in a jar with a cotton wick. A glass Petri dish lined with filter paper with 100 ml tap water was kept inside the cage for oviposition.

### *Larvicidal assay*

Different concentrations of solution ranging between 250 and 12.5 $\mu$g/ml were prepared. Each test solution was placed in multi-well plates (12 well) and left until dried. Later, it was dissolved in 1 ml of tap water and tested against 10 third instar larvae of *Ae. caspius*. Each experiment was conducted in triplicate and tap water was used as a negative control. The number of dead larvae was counted 24 h and 48 h after exposure and the percentage mortality was reported for the average of three replicates.

### *Cell viability assay*

The human hepatoma HepG2 cell line (DSMZ, Germany) was used to determine the cytotoxicity of the plant extracts. The cells were grown in a 24-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % foetal bovine serum. One millilitre of cell suspension ($10^5$ cells/ml) was seeded in each well and incubated at 37 °C for 24 hours in 5 % $CO_2$. The plates were exposed to various dilutions of the plant extracts. Cell viability was measured using the MTT assay. This tetrazolium salt reduced by viable cells to formazan was measured spectrophoto-

**Table 1**. Larvicidal activity (% mortality) (mean ± S.E.) of different concentrations of fruit aqueous crude extracts of *Capparis cartilaginea* and its fractions against third instar larvae of *Aedes caspius* after 24 and 48 h of exposure. FAQE: fruit aqueous extract, FME: fruit methanol extract, LAQE: leaves aqueous extract, LME: leaves methanol extract, $F_{EtoAC}$: ethyl acetate fraction, $F_{MeOH}$, methanol fraction, $F_{aq}$: aqueous fraction, NT = not tested.

| Extracts | 24 h | | | | 48 h | | | |
|---|---|---|---|---|---|---|---|---|
| | Concentration ( µg/ml) | | | | | | | |
| | 250 | 125 | 65.5 | 32.75 | 250 | 125 | 65.5 | 32.75 |
| FAQE | 80 ± 00 | 23.3 ± 3.33 | 16.7 ± 3.33 | 10.0 ± 5.77 | 100 ± 00 | 30.0 ± 5.77 | 20.0 ± 5.77 | 10.0 ± 5.77 |
| FME | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| LAQE | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| LME | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| $F_{EtoAC}$ | NT | 96.7 ± 3.33 | 53.3 ± 6.67 | 0 ± 0 | NT | 100 ± 0 | 60 ± 577 | 13.3 ± 3.33 |
| $F_{MeOH}$ | NT | 0 ± 0 | 0 ± 0 | 0 ± 0 | NT | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| $F_{aq}$ | NT | 0 ± 0 | 0 ± 0 | 0 ± 0 | NT | 0 ± 0 | 0 ± 0 | 0 ± 0 |

meterically at 540 nm. Controls were maintained throughout the experiment. The assay was performed in triplicate and the mean of the cell viability values was calculated. The percentage of cell viability was plotted against the concentration of the plant extract (Mosmann 1983).

### *Hoechst 33258 staining*

Cells were treated with 100 $\mu$g/ml of the active fraction and incubated for 24 hours. The media was then aspirated and the cells were washed twice with PBS. Then, 3 % paraformaldehyde was added to fix the cells for 20 min. The cells were washed with PBS three times to remove the fixatives before adding the cold (–20 °C) 100 % methanol. They were then left at room temperature for 20 min. The methanol was aspirated and rinsed thoroughly with PBS. The plate was then stained with 0.12 $\mu$g/ml Hoechst dye and incubated for 15 min in the dark. The plates were then rinsed with PBS five times and observed under a fluorescent microscope at 340–380 nm (Papadimitriou & Lelkes 1993).

## RESULTS AND DISCUSSION

No previous studies have been reported on the larvicidal activities of crude and chromatographic fractions of extracts of the fruits and leaves of *C. cartilaginea* (Fig. 1). Only the aqueous crude extract (260 mg) and ethyl acetate fraction (100 mg) of C. cartilagnea fruit resulting from the liquid-liquid extraction showed a larvicidal activity against *Ae. caspius* and the absence of larvicidal activities in the leaf extracts. Hence, ethyl acetate fraction of fruit extract was selected for further purification using thin-layer chromatography (TLC). In order to collect the fractions in large quantities, the spots were scratched from TLC plates, dissolved in methanol and centrifuged, with the supernatant then being vacuum dried to obtain the fractions. TLC of ethyl acetate extract revealed the presence of seven fractions having $R_f$ values of 0.97 (F1), 0.85 (F2), 0.71 (F3), 0.55 (F4), 0.35 (F5), 0.14 (F6) and 0.0 (F7). The yields of these fractions were 14, 4, 11.6, 11.7, 9, 17.3 and 20 mg, respectively.

Only the crude aqueous extract of the fruit showed larvicidal activity at the highest concentration tested. The highest mortality for the crude aqueous extract was recorded at 250 $\mu$g/ml, with 80 % mortality after 24 hours of exposure and 100 % after 48 hours of exposure, while for the ethyl acetate fractions, at the concentration of 125 $\mu$g/ml, mortality was 96.76 % after 24 hours of exposure and 100 % mortality after 48 hours of exposure (Table 1).

For column chromatographic fractions, since it might be expected that the chromatographic fractions contain the larvicidally active compounds, the higher concentrations of 250 and 125 $\mu$g/ml were ignored and larvicidal tests were conducted using lower concentrations ranging from 100 to 12.5 $\mu$g/ml. Fractionation of effective crude extracts by TLC and their bioassay revealed that the fractions F2 and F5 of C. cartilaginea were most effective against *Ae. caspius*. It was found that the mortality of fraction F2 was 100 % after 24 and 48 h of treatment at 50 $\mu$g/ml concentration while the fraction F5 caused 93.33 % and 96.67 % mortality after 24 and 48 h of treatment, respectively (Table 2).

**Table 2**. Larvicidal activity ( % mortality) (mean ± S.E.) of different concentrations of *Capparis cartilaginea* fractions extract against third instar larvae of *Aedes caspius*.

| | 24 h | | | | 48 h | | | |
|---|---|---|---|---|---|---|---|---|
| | Concentration ( $\mu$g/ml) | | | | | | | |
| Fraction | 100 | 50 | 25 | 12.5 | 100 | 50 | 25 | 12.5 |
| F2 | 100 ± 0 | 100 ± 0 | 43.3 ± 3.33 | 6.7 ± 3.33 | | 100 ± 0 | 83.3 ± 3.33 | 6.7 ± 3.33 |
| F5 | 93.3 ± 3.33 | 13.3 ± 3.33 | 0 ± 0 | 0 ± 0 | 96.7 ± 3.33 | 43.3 ± 8.82 | 13.3 ± 6.67 | |

The $LC_{50}$ and $LC_{90}$ values for the crude extract and the fractions are summarized in Table 3. Crude aqueous fruit extract of *C. cartilaginea* had less toxicity against the test larvae ($LC_{50}$ = 172.02 $\mu$g/ml; $LC_{90}$ = 294.85 $\mu$g/ml) when compared with the ethyl acetate fraction ($LC_{50}$ = 74.42 $\mu$g/ml; $LC_{90}$ = 114.06 $\mu$g/ml). The ethyl acetate fraction of *C. cartilaginea* in turn was less toxic against the test larvae when compared to its column chromatographic fraction F2 ($LC_{50}$ = 29.17 $\mu$g/ml; $LC_{90}$ = 45.45 $\mu$g/ml) and F5 ($LC_{50}$ = 69.49 $\mu$g/ml; $LC_{90}$ = 100.37 $\mu$g/ml) (Table 3). When the other fractions were tested on the test larvae and on the cell lines, they exhibited no toxicity in either case (data no shown). In the present study, the TLC fractions of aqueous fruit extract of *C. cartilaginea* and its fractions F2 and F5 showed larvicidal efficacy in a dose-dependent manner, suggesting that the larvicidal activity was not due to synergistic effects. It can be concluded that the most active components responsible for toxic activity against *Ae. caspius* were present in the F2 fraction of *C. cartilaginea*.

The absence of larvicidal activities in the leaf extracts, however, may indicate that the toxic larvicidal fractions are only produced in the unripe fruits of *C. cartilaginea (deleted)*. Nyamoita *et al*. (2013) reported that acetone and methanol extracts of *Vitexpayos* root bark, and acetone column chromatography fractions thereof, exhibited larvicidal activity against the larvae of *Anopheles gambiae* within 72 h. The fractions tested caused larval mortality ranging between 85 and 100 % at a dose of 25 ppm and the $LD_{50}$ values ranged between 7.0 and 19.3 ppm after 24 h. These fractions were all positive for steroids and saponins. Thangaraj *et al*. (2014) meanwhile reported that acetone extract of *Coriandrum sativum* and methanol extract of *Brassica nigra* when used against *Culex quinquefasciatus* exhibited $LC_{50}$ values of 0.015 and 0.32 mg/ml, respectively.

In cytotoxicity studies, HepG2 at 24 h post-treatment with fraction F2 were comparable to that recorded by the untreated culture, with a percentage survival ranging from 90 to 110 % (Fig. 2). This was further confirmed by the similar cell density observed in cell lines tested in the presence or absence of fractions F2 and F5 (Fig. 3).

To verify whether the fraction F2 of *C.cartilaginea* has an effect on the morphology of HepG2 cells, light and fluorescent microscopy were used to evaluate the cells after treatment with 100 $\mu$g/ml of fraction F2 compared with a control. There appeared to be no effect on the cell morphology of HepG2 cells treated at this dosage – the plasma membrane remained well defined with no discernible effect on the adhesion of cultured epithelial cells (Fig. 3).

**Table 3**. Toxicity of *Capparis cartilaginea* plant extracts on *Aedes caspius* third instar larvae after 24 h and 48 h of exposure.

| Extracts | 24 h | | 48 h | |
|---|---|---|---|---|
| | $LC_{50}$ ( µg/ml) | $LC_{90}$ (µg/ml) | $LC_{50}$ ( µg/ml) | $LC_{90}$ (µg/ml) |
| FAQE | 172.02 | 294.85 | 142.31 | 238.30 |
| $F_{EtoAC}$ | 74.42 | 114.06 | 65.82 | 109.94 |
| Fraction 2 | 29.17 | 45.45 | 23.18 | 41.13 |
| Fraction4 | 69.03 | 98.59 | 69.03 | 98.59 |
| Fraction5 | 69.49 | 100.37 | 57.33 | 93.53 |

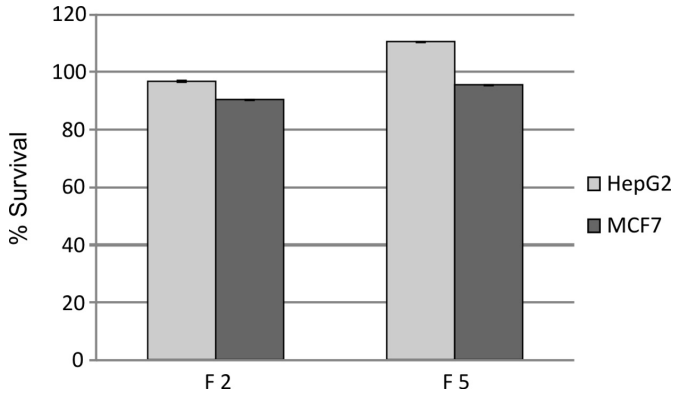


**Fig. 2**. Antiproliferative effects of fractions F2 and F5 of methanol extract towards HepG2 and MCF7 after 24 hours of exposure. The columns represent the mean percentage of live cells with bar showing ± 1 standard deviation.

The Hoechst 33342 test was used to assess changes in nuclear morphology following fraction F2 treatment. The nuclei in control and treated cells were normal and exhibited diffused staining of the chromatin and no morphological changes suggestive of apoptosis, such as chromatin condensation and a shrunken nucleus (Fig. 4). Similar result was obtained by Machana, *et al*. (2011) who reported that ethanol-water crude extract of *Pinuskesiya* and *Catimbiums peciosum* showed potent cytotoxicity in the HepG2 cell line, with an $IC_{50}$ value of 52.0 ± 5.8 $\mu$g/ml and $IC_{50}$ value of 55.7 ± 8.1 $\mu$g/ml, respectively. Ethanol-water crude extract of *Glochidiondaltonii*, *Cladogynosorientalis*, *Acorustatarinowii* and *Amomumvillosum* exhibited cytotoxicity with $IC_{50}$ values ranging from 100–500 $\mu$g/ml. Extracts of *P. kesiya*, *C. speciosum* and *C. orientalis* caused morphological changes in the nuclei. *C. speciosum* and *P. kesiya* had apoptotic bodies from nuclear fragmentation (Machana *et al*. 2011).

Human hepatocellular carcinoma cell line (HepG2) is an effective and widely used cellular model for *in vitro* cytotoxicity studies for determination of hepatotoxicity (Mersch-Sundermann *et al*. 2004; Knasmuller *et al*. 2004). In addition, screening for hepatotoxicity using human HepG2 is expected to be a more reliable indicator of human hepatotoxicity than animal models (O'Brien *et al*. 2006). The fact that these morphological studies suggested no toxicity to HepG2 cells after being treated with fraction F2 makes it a good candidate as an alternative natural larvicidal agent with no hepatotoxicity and at a low cost.

Spectroscopic analysis was performed for the TLC fractions which showed a larvicidal activity. A comparison between the FTIR spectrum obtained and that of the reference chart revealed that fraction F2 was aliphatic and showed the presence of different functional groups such the hydroxyl group, carbonyl group, amine group etc. (Fig. 5).

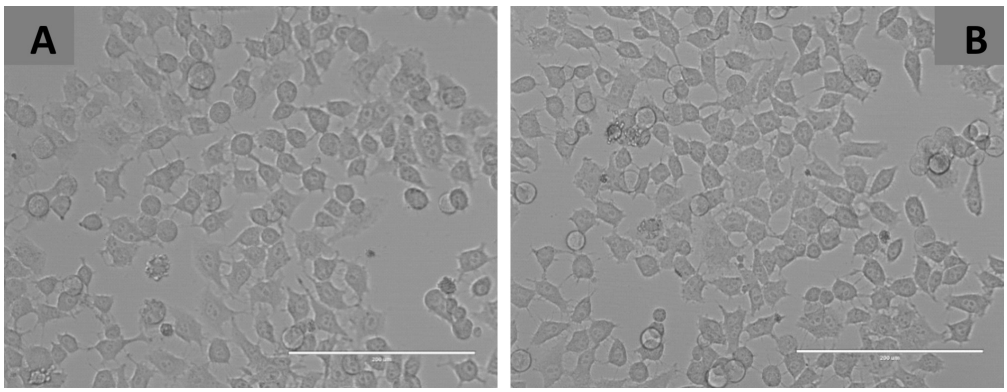


**Fig. 3**. Photomicrograph of the HepG2 cultures following treatment with fraction F2. No effect on HepG2 morphology was observed after 24 h culture periods visualized under light microscope. **A** = Control, **B** = treated.

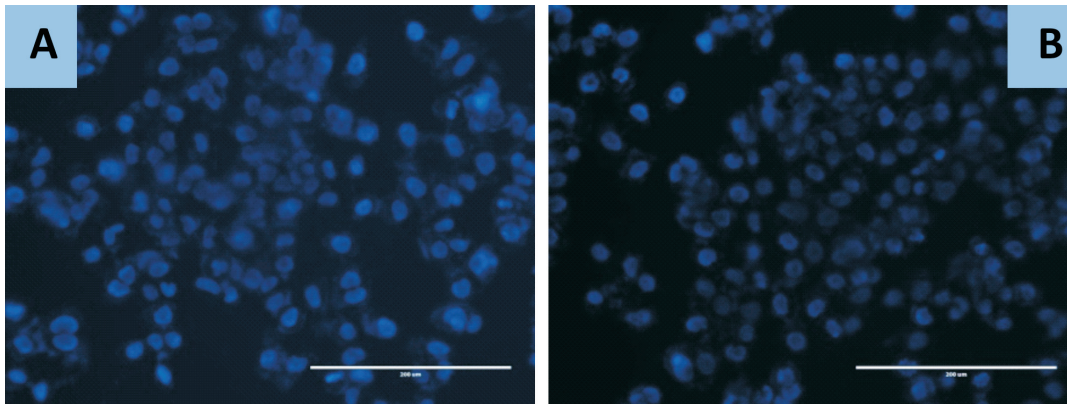


**Fig. 4**. Fluorescence photomicrographs of HepG2 cells stained by Hoechst 33342 after being treated with fraction F2. Cells were incubated with fraction F2 for 24 h. **A** = control; **B** = treated.

The phytochemical screening for fraction F2 only showed a positive result for alkaloids ($R_f$ = 0.85) which is in accordance with the IR chart. HPLC analysis (Fig. 6) of fraction F2 at 254 nm, showed the presence of a major peak and various minor constituents, as evidenced by the chromatogram obtained at various retention times (6.25, 6.80, 7.64, 9, 9.71).

Further work needs to be done to identify the active compounds and their mechanism of action and field trials are also required on the active fraction identified here so that environmentally safe, biodegradable and target-specific agents for the control of mosquitos can be developed.

## ACKNOWLEDGEMENT

This Project was supported by King Saud University, Deanship of Scientific Research, College of Sciences Research Center.

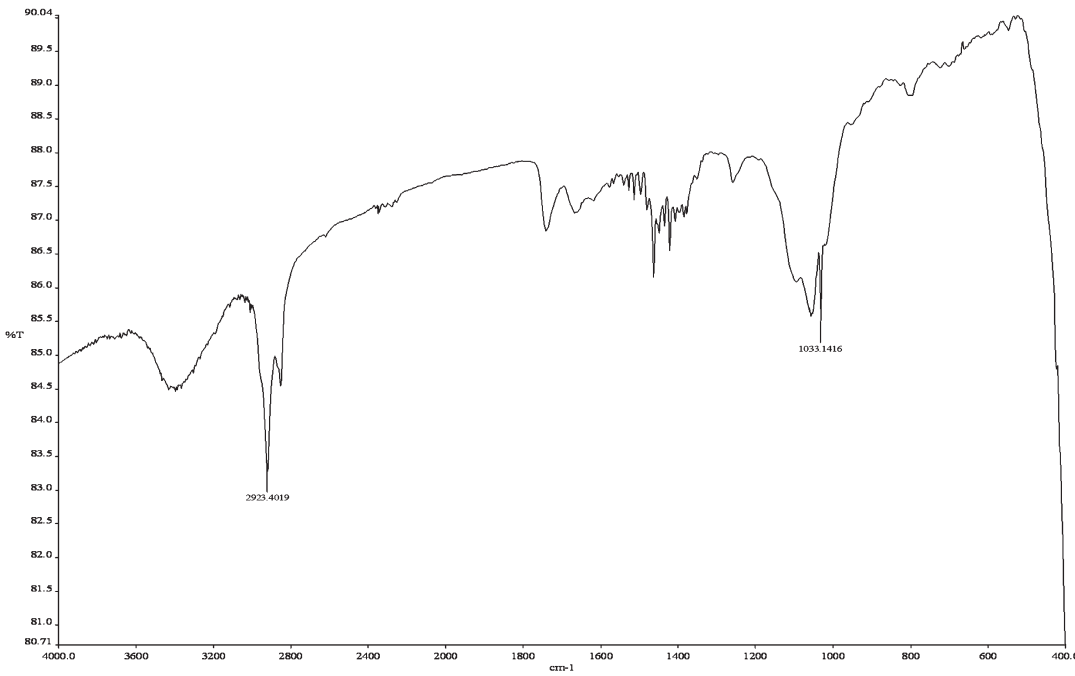


**Fig. 5**. FTIR (Fourier-transformed infrared) spectrum of TLC fraction 2 of fruit extract of *Capparis cartilaginea.*

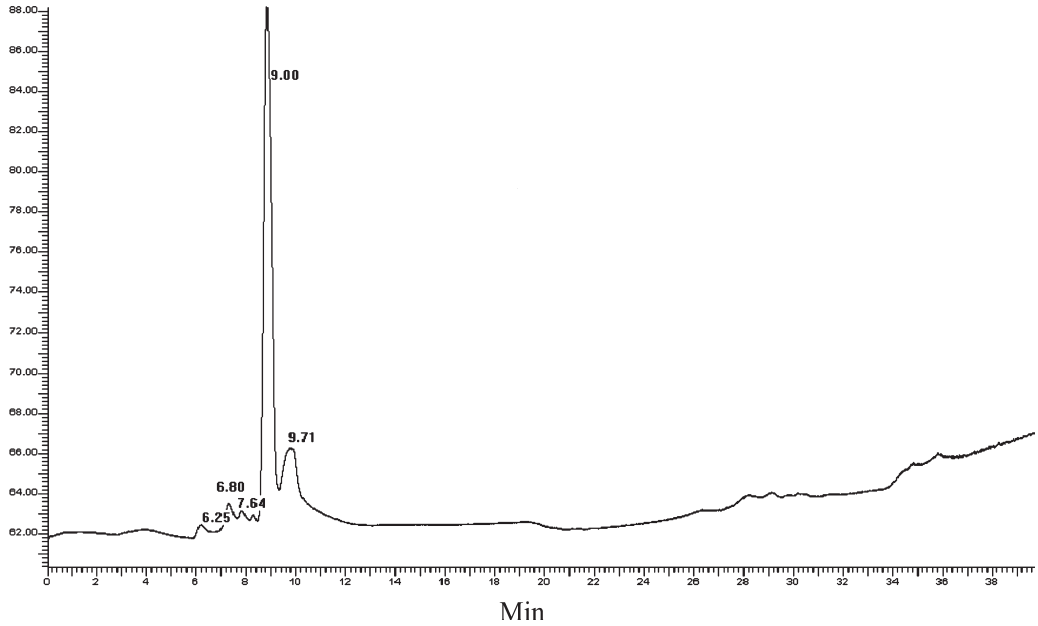


**Fig. 6**. HPLC chromatogram of TLC fraction F2 of fruit extract of *Capparis cartilaginea* at 254 nm.

## REFERENCES


ABDOON, A.M.M.O. 2004. First record of three Afrotopical *Culex* species (Diptera: Culicidae) in Saudi Arabia. *The Annals of Medical Entomology* **13**(1–2): 1–9.

AHMED, A.M., SHAALAN, E.A., ABOUL-SOUD, M.A.M., TRIPET, F. & AL-KHEDHAIRY, A.A. 2011. Mosquito vectors survey in the Al-Ahsaa district of eastern Saudi Arabia. *Journal of Insect Science* **11**: Article 176.

AL-HAZMI, M., AYOOLA, E.A., ABDURAHMAN, M., BANZAL, S., ASHRAF, J., EL-BUSHRA, A., HAZMI, A., ABDULLAH, M., ABBO, H., ELAMIN, A., ALSAMMANI, E., GADOUR, M., MENON, M. HAMZA, I., RAHIM, M. HAFEZ, M., JAMBAVALIKAR, H., ARISHI, A. & AQEEL, C. 2003. Epidemic Rift Valley fever in Saudi Arabia: a clinical study of severe illness in humans. *Clinical Infectious Diseases* **36**: 245–252.

AL-KHREJI, M.A. 2005. Survey and distribution of mosquito species (Diptera: Culicidae) and description of its habitat in Riyadh district, Kingdom of Saudi Arabia. M.Sc. thesis, King Saud University, Riyadh, Kingdom of Saudi Arabia.

ALMURAISY, M. 2011. Malaria: Saudi research centre to target malaria and dengue. Online at: http://www.scidev.net/global/health/news/saudi-research-centre-to-target-malaria-and-dengue-1.html

AYYUB, M., KHAZINDAR, A.M., LUBBAD, E.H., BARLAS, S., ALFI, A.Y. AND AL-UKAYLI, S. 2006. Characteristics of dengue fever in a large public hospital, Jeddah, Saudi Arabia. *Journal of Ayub Medical College Abbottabad* **18**: 9–13.

BALKHY, H.H. & MEMISH, Z.A. 2003. Rift Valley Fever: an uninvited zoonosis in the Arabian peninsula. *International Journal of Antimicrobial Agents* **21**: 153–157.

BANERJEE, S., SINGHA, S., LASKAR, S. & CHANDRA, G. 2011. Efficacy of *Limonia acidissima* L. (Rutaceae) leaf extract on larval immatures of *Culex quinquefasciatus* Say 1823, *Asian Pacific Journal of Tropical Medicine* **4**(9): 711–717.

BOULOS, L. 1999. *Flora of Egypt*. Vol. 1, 1st edn. Al Hadara Publishing, Cairo, Egypt.

CHOWDHURY, N., LASKAR, S. & CHANDRA, G. 2008. Mosquito larvicidal and antimicrobial activity of protein of *Solanum villosum* leaves. *BMC Evidence-based and Complementary Alternative Medicine* **8**: 62. doi:10.1186/1472-6882-8-62.

ELFFERS, J., GRAHAM, R.A. & DEWOLF, G.P. 1964. Capparidaceae. In: *Flora of Tropical East Africa*. 1–85. Crown Agents for Overseas Governments and Administrations, London, U.K.

FAKEEH, M. & ZAKI, A.M. 2001. Virologic and serologic surveillance for dengue fever in Jeddah, Saudi Arabia, 1994–1999. *American Society of Tropical Medicine and Hygiene* **65**: 764–767.

FAKEEH, M. & ZAKI, A.M. 2003. Dengue in Jeddah, Saudi Arabia, 1994–2002. *Dengue Bulletin* **27**: 13–18.

GHOSH, A., CHOWDHURY, N. & CHANDRA, G. 2012. Plant extracts as potential mosquito larvicide, *Indian Journal of Medical Research* **135**: 581–598.

GIBBS, R.D. 1974. *Chemotaxonomy of Flowering Plants*. Vol. 1. McGill Queen's University Press, Montreal, Canada, and London, U.K.

GOMEZ-LECHON, M.J., CASTELL, J.V. & DONATO, M.T. 2007. Hepatocytes – the choice to investigate drug metabolism and toxicity in man: *in vitro* variability as a reflection of *in vivo*. *Chemico-Biological Interactions* **168**(1): 30–50.

HARRY, O., GRAHAM, B., DENISE, R., KARLUSS,T., ALASTAIR, M., GERALD, K., PATRICK, L., JAMES, S., GLENN, S., WILLIAM, B., MICHAEL, D., KOEN, V.D., PETER, S., BRUCE, B. & ALLEN, H. 2000. Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regulatory Toxicology and Pharmacology* **32**(1): 56–67.

HAWKING, F. 1973. The distribution of human filariasis throughout the world. II. Asia. *WHO mimeograph* WHO/FIL/73.114.

HORII, I. & YAMADA, H. 2007. *In vitro* hepatotoxicity testing in the early phase of drug discovery. *Alternatives to Animal Testing and Experimentation* **14**: 437–441.

JUPP, P.G., KEMP, A., GROBBELAAR, A., LEMAN, P., BURT, F.J., ALAHMED, A.M., ALMUJALLI, D., ALKHAMEES, M. & SWANEPOEL, R. 2002. The 2000 epidemic of Rift Valley fever in Saudi Arabia: mosquito vector studies. *Medical and Veterinary Entomology* **16**: 245–252.

KHAN, N.A., AZHAR, E.I., EL-FIKY, S., MADANI, H.H., ABULJADIAL, M.A., ASHSHI, A.M, TURKISTANI, A.M. & HAMOUH, E.A. 2008. Clinical profile and outcome of hospitalized patients during first outbreak of dengue in Makkah, Saudi Arabia. *Acta Tropica* **105**: 39–44.

KNASMULLER, S., MERSCH-SUNDERMANN, V., KEVEKORDES, S., DARROUDI, F., HUBER, W.W., HOELZL, C., BICHLER, J. & MAJER, B. 2004. Use of human-derived liver cell lines for the detection of environmental and dietary genotoxicants; current state of knowledge. *Toxicology* **198**: 315–328.

MACHANA, S., WEERAPREEYAKUL, N., BARUSRUX, S., NONPUNYA, A., SRIPANIDKULCHAI, B. & THITIMETHAROCH, T. 2011. Cytotoxic and apoptotic effects of six herbal plants against the human hepatocarcinom (HepG2) cell line. *Chinese Medicine* **6**: 39.

MADANI, T.A., AL-MAZROU, Y.Y., AL-JEFFRI, M.H., MISHKHAS, A.A., AL-RABEAH, A.M., TURKISTANI, A.M., AL-SAYED, M.O., ABODAHISH, A.A., KHAN, A.S., KSIAZEK, T.G. & SHOBOKSHI, O. 2003. Rift Valley fever epidemic in Saudi Arabia: epidemiological, clinical, and laboratory characteristics. *Clinical and Infectious Diseases* **37**: 1084–1092.

MERSCH-SUNDERMANN, V., KNASMULLER, S., DARROUDI, F. & KASSIE, F. 2004. Use of a human-derived liver cell line for the detection of cytoprotective, antigenotoxic and cogenotoxic agents. *Toxicology* **198**: 329–340.

MILLER, B.R., GODSEY, M.S., CRABTREE, M.B., SAVAGE, H.M., AL-MAZRAO, Y., AL-JEFFRI, M.H., ABDOON, A.M., AL-SEGHAYER, S.M., ALSHAHRANI, A.M. & KSIAZEK, T.G. 2002. Isolation and genetic characterization of Rift Valley fever virus from *Aedes vexansarabiensis*, Kingdom of Saudi Arabia. Emerging *Infectious Disiseases* **8**: 1492–1494.

MOSMANN, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* **65**: 55–63.

NYAMOITA, M.G., MBWAMBO, Z.H., OCHOLA, B.J., INNOCENT, E., LWANDE, W. & HASSANALI, A. 2013. Chemical composition and evaluation of mosquito larvicidal activity of *Vitex payos* extracts against *Anopheles gambiae* Giles S.S larvae. *Spatula DD* **3**(3): 113–120.

O'BRIEN, P.J., IRWIN, W., DIAZ, D., HOWARD-COFIELD, E., KREJSA, C.M., SLAUGHTER, M.R., GAO, B., KALUDERCIC, N., ANGELINE, A., BERNARDI, P., BRAIN, P. & HOUGHAM, C. 2006. High concordance of drug-induced human hepatotoxicity with *in vitro* cytotoxicity measured in a novel cell-based model using high content screening. *Archives of Toxicology* **80**: 580–604.

PAPADIMITRIOU, E. & LELKES P.I. 1993. Measurement of cell numbers in microtiter culture plates using the fluorescent dye Hoechst 33258. *Journal of Immunological Methods* **162**: 41–45.

PETERS, T.S. 2005. Do preclinical testing strategies help predict human hepatotoxic potentials? *Toxicologic Pathology* **33**(1): 146–154.

RAWANI, A., HALDAR, K.M., GHOSH, A. & CHANDRA, G. 2009. Larvicidal activities of three plants against filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitology Research* **105**(5): 1411–1417.

RIVERA, D., FRIIS, I., INOCENCIO, C., OBÓN, C., ALCARAZ, F. & REALES, A. 2003. The typification of *Capparis inermis* Forssk., *C. sinaica* Veill. and *C. cartilaginea* Decne. (Capparaceae). *Taxon* **52**: 307–311.

RIVERA, D., FRIIS, I., INOCENCIO, C., OBÓN, C., REALES, A. & ALCARAZ, F. 2002. Archaeobotany of capers (*Capparis*) (Capparaceae). *Vegetation History and Archaeobotany* **11**: 295–313.

RUTLEDGE, C.R., CLARKE, F., CURTIS, A. & SACKETT, S. 2003. Larval mosquito control. *Technical Bulletin of the Florida Mosquito Control Association* **4**: 16–19.

SINGHA, S., ADHIKARI, U., GHOSH, A. & CHANDRA, G. 2012. Mosquito larvicidal potentiality of *Holoptelea integrifolia* leaf extract against Japanese encephalitis vector, *Culex vishuni* group. *Journal of Mosquito Research* **2**(4): 25–31.

SLATER, K. 2001. Cytotoxicity tests for high-throughput drug discovery. *Current Opinion in Biotechnology* **12**: 70–74

TACKHOLM, V. 1974. *Student's Flora of Egypt*. 2nd edn. Cairo University, Cairo, Egypt.

THANGARAJ, D., RAVICHANDRAN, R. & ALWARSAMY, M. 2014. Mosquito larvicidal activity of *Coriandrum sativum* and *Brassica nigra* seed extract against the filarial vector, *Culex quinquefasciatus* Say. *International Journal of Innovative Research in Science, Engineering and Technology* **3**(1): 8632–8638.

WARREL, D.A. 1993. Leishmaniasis, malaria and schistosomiasis in Saudi Arabia. *Saudi Medical Journal* **14**: 203–208.

WICKENS, G.E. 1979. Thepropagules of the terrestrial flora of the Aldabra archipelago, western Indian Ocean. *Atoll Research Bulletin* **229**: 1–40.

YOUSSEF, R.S.A. 2013. Medicinal and non-medicinal uses of some plants found in the middle region of Saudi Arabia. *Journal of Medicinal Plants Research* **7**(34): 2501–2513.

Accepted 3 September 2014