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ORIGINAL ARTICLE

Biological activity of *Xanthium strumarium* seed extracts on different cancer cell lines and *Aedes caspius*, *Culex pipiens* (Diptera: Culicidae)

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Abstract Effects of methanol extracts of *Xanthium strumarium* on different cancer cell lines and on the mortality rates of *Aedes caspius*, *Culex pipiens* (Diptera: Culicidae) were investigated. Among the cell lines tested, the Jurkat cell line was the most sensitive to the methanol extract and ethyl acetate fraction, with reported LC₅₀ values of 50.18 and 48.73 µg/ml respectively. Conversely, methanol extracts were not that toxic to the A549 cell line though the toxicity increased on further purification. The percentage of growth inhibition was dose dependent for the methanol extract and ethyl acetate fraction. The ethyl acetate fraction showed higher toxicity to all cell lines tested when compared to the methanol extract. The results showed that methanol extracts of plant seeds caused 100% mortality of mosquito larvae at a concentration of 1000 µg/ml after 24 h of treatment. The LC₅₀ and LC₉₀ values of *X. strumarium* were found to be 531.07 and 905.95 µg/ml against *Ae. caspius* and 502.32 and 867.63 µg/ml against *Cx. pipiens*, respectively. From the investigations, it was concluded that the crude extract of *X. strumarium* showed a weak potential for controlling the larval instars of *Ae. caspius* and *Cx. pipiens*. However, on further purification the extract lost the larvicidal activity. The ethyl acetate fraction showed higher toxicity to all cell lines tested when compared to the methanol extract. The ethyl acetate fraction investigated in this study appears to have a weak larvicidal activity but a promising cytotoxic activity. Future studies will include purification and

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investigation in further detail of the action of *X. strumarium* on Cancer Cell Lines and mosquitoes. © 2016 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Plant extracts have been used by several cultures for their medicinal properties. Active substances in those extracts form the basis of herbal medicine systems that have been practiced for thousands of years and still provide cures for mankind (Gurib-Fakim, 2006; Al-Mekhlafi et al., 2013). According to the World Health Organization (WHO) approximately 65–80% of the developing countries depend on traditional medicine for their health care due to difficulties of accessing modern medicine or poverty (Calixto, 2005). Plants have a long history of use for the treatment of human diseases such as cancer, one of the major causes of mortality worldwide. It is estimated that cancer will cause 83.2 million deaths between 2005 and 2015 (Kabbaj et al., 2012). Medicinal plants play a significant role in cancer treatment (Mark, 2004). It was reported that 40% of anticancer agents between 1940 and 2002 were derived from natural products or their mimics, including Vinca alkaloids, Taxus diterpenes, Camptotheca alkaloids, and Podophyllum lignans (David et al., 1999). Saklani and Kutty (2008) indicated that, flavopiridol, isolated from the Indian tree *Dysoxylum binectariferum* and meisoindigo, isolated from the Chinese plant *Indigo fera tinctoria*, have been shown to exhibit anticancer effects with lesser toxicity than conventional chemotherapeutic drugs (Saklani and Kutty, 2008).

Xanthium strumarium is a medicinal plant used in traditional systems of medicine in China and India for the health improvement and also as an aphrodisiac (Gauthaman and Aداikan, 2005). The spines and seeds are rich in xanthostrumarin which is responsible for the adverse effects. It has also been recommended for treatment of depression, water retention, heart, liver and kidney disease, and is reported to have anti-inflammatory and antiseptic properties.

Mosquitoes (Diptera: Culicidae) are among the most serious insect pests of medical importance. They are well-known vector-borne diseases some of which cause millions of cases of illnesses and deaths in humans and animals each year (Bakr and Al-Ghramh, 2014). In Saudi Arabia, the most common mosquito-borne diseases include filarial (Hawking, 1973), malaria (Abdoon and Alsharani, 2003), Rift valley fever (Jupp et al., 2002; Madani et al., 2003) and dengue (Khan et al., 2008). Local *Culex pipiens* mosquitoes might act as a potential vector of introducing *Bancroftian filariasis* in Saudi Arabia (Omar, 1996). *Aedes caspius* is the most common vector followed by *Cx. pipiens* in the AL-Ahsaa district of eastern Saudi Arabia (Ahmed et al., 2011).

Cancer is an alarming disease and fighting this disease is of great priority to human and public health. There is an inevitable need for search of new anticancer agent as the treatment of cancer with the existing drugs is often cytotoxic to the normal cells. Herbal products have been making fast progress and becoming popular as sources of anticancer compounds (Patel et al., 2010).

The aim of this research was to test bioactivities of methanol extracts and fractions of *X. strumarium*, including the

possible anticancer activity against HepG2 (The human hepatoma), A549 (adenocarcinomic human alveolar basal epithelial cells), L929 (fibrosarcoma) and Jurkat (a human T-lymphocyte) cell lines, and larvicidal activity against *Ae. caspius* and *Cx. pipiens*.

2. Materials and methods

2.1. Collection and processing of seed material

100 grams of dried seeds of different *X. strumarium* plants was collected from wild habitats in Wadi Hanifa, Riyadh, kingdom of Saudi Arabia. The botanical identification was authenticated in the Department of Botany, College of Science, King Saud University, where the voucher specimen was deposited. The dried seeds were powdered in a blender machine (Kenwood, China) and the extract was prepared by mixing 100 g from sample in 500 mL of 95% methanol in a flask. The flask was placed in rotary shaker (Kuhner, Germany) at 30 °C for 24 h. This procedure was repeated three times, adding fresh methanol every time to left over residue. The extracts were pooled together and centrifuged at 4000 rpm for 10 min (Sigma, Germany). The supernatant was collected and evaporated using rotary evaporator (Heidolph, Germany) at 40 °C.

2.2. Preparation and fractionation of ethyl acetate fraction

The crude methanol extract was dissolved in water (500 ml) and exhaustively extracted by liquid/liquid partition with ethyl acetate (3 × 200 mL) using a separating funnel (1000 ml).

The ethyl acetate fraction was centrifuged at 4000 rpm for 10 min. The supernatant was collected and evaporated using a rotary evaporator at 40 °C. The ethyl acetate fraction (200 mg) was chromatographed on Sephadex LH20 with ethyl acetate: Methanol (2:8) to obtain seven fractions. The fractions obtained were weighed, dissolved in methanol and used for the tests.

2.3. Cell viability assay

HepG2 (The human hepatoma cell line), A549 (Human lung adenocarcinoma epithelial cell line), L929 (mouse fibroblast) and Jurkat (a human T-lymphocyte cell line) were used to determine the cytotoxicity of the seed extract. The cells were grown in a 24-well plate in Dulbecco's modified Eagle's medium (DMEM) (invitrogen) supplemented with 10% fetal bovine serum. One mL cell suspension (10^5 cells/mL) was seeded in each well and incubated at 37 °C for 24 h in 5% CO₂ for the formation of a confluent monolayer. The monolayer of cells in the plate was exposed to different concentrations (200, 100, 50, 25, and 12.5 µg/ml) to determine the IC₅₀ by MTT assay. The cell viability was measured using MTT (5 mg/mL) and isopropanol (1 ml). This tetrazolium salt is metabolically reduced by viable cells to yield a blue formazan

product measured at 540 nm spectrophotometer. Methanol control was maintained throughout the experiment. The assay was performed in triplicate for each of the extracts. The mean of the cell viability values was compared to the control to determine the effect of the extract on cells and % cell viability was plotted against concentration of the seed extract (Mosmann, 1983).

2.4. Mosquito culture

Ae. caspius and *Cx. pipiens* larvae were obtained from colonies 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. They were kept indoors at 27 ± 2 °C, 50 ± 5% relative humidity, a 14:10 light: dark photo-period and they were fed daily until they become pupae. Pupae were transferred from the trays to a cup containing tap water and were maintained in our insectary. They were moved into a mosquito cage where emergent adults were fed with a 10% glucose solution in a jar with cotton wick. Glass Petri dish lined with filter paper with 100 ml tap water was kept inside the cage for oviposition.

2.5. Larvicidal assay

Different concentrations were prepared (62.5, 125, 250, 500, and 1000 µg/ml) from stock solution of crude and ethyl acetate fraction of seed extract. Each test solution was placed in Multi-Well Plates (12 Well) and left until dried in an oven at 40 °C. Later, one ml of tap water was added and tested against 10 of 4th instar larvae. Each experiment was conducted in triplicate and tap water was used as a negative control (tap water). The number of dead larvae was counted after 24 h and 48 h of exposure and the percentage of mortality was reported for the average of three replicates and the LD₅₀ and LD₉₀ were calculated.

2.6. Statistical analysis

For cell lines, the data were represented as mean ± Std. LC₅₀ values were calculated using Origin software 6.1 version.

3. Results

Results of MTT assay of different concentrations are graphically represented in Figs. 1 and 2 for methanol extract and ethyl acetate fraction, respectively. Among the cell lines tested, Jurkat cell line was the most sensitive to the methanol extract and ethyl acetate fraction with LC₅₀ value of 50.18 µg/ml and 48.73 µg/ml, respectively. The next cell line followed was HepG2 with LC₅₀ values of 112.9 and 68.739 µg/ml for the methanol extract and ethyl acetate fraction, respectively. LC₅₀ value of L929 cell line was 163 and 111 µg/ml to the methanol extract and ethyl acetate fraction, respectively. Conversely, methanol extract was not that toxic to A549 cell line though the toxicity increased on further purification. However, the highest concentration tested resulted in 84.34% and 57.90% inhibition for methanol and ethyl acetate extract, respectively and therefore, the LC₅₀ was not calculated. In

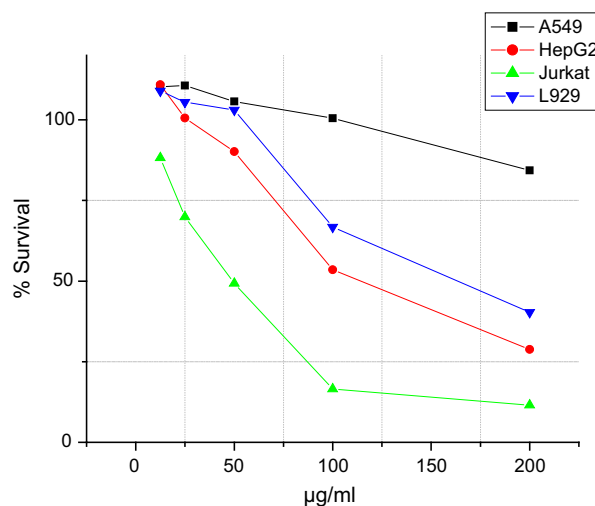


Figure 1 Cytotoxic activity of methanol crude extract of *Xanthium strumarium* seeds against different cancer cell lines. The values presented are mean ± SE. ●HepG2 (The human hepatoma cell line), ■A549 (Human lung adenocarcinoma epithelial cell line), ▼L929 (mouse fibroblast) ▲ Jurkat (a human T-lymphocyte cell line).

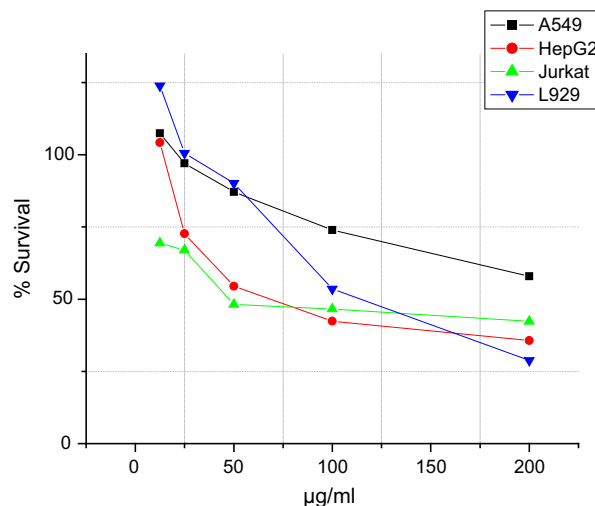


Figure 2 Cytotoxic activity of ethyl acetate fraction of methanol extract of *Xanthium strumarium* seed extract against different cancer cell lines. The values presented are mean ± SE. ●HepG2 (The human hepatoma cell line), ■A549 (Human lung adenocarcinoma epithelial cell line), ▼L929 (mouse fibroblast) ▲ Jurkat (a human T-lymphocyte cell line).

further purification of the ethyl acetate fraction using Sephadex as stationary phase, all the fractions tested exhibited no activity up to the concentration of 400 µg/ml.

The results of the larvicidal activity of methanol seed extract from *X. strumarium* against the larvae of important vector mosquito *Ae. caspius* and *Cx. Papiens* were represented in the Table 1. The crude extract tested showed the larvicidal efficacy within 24 h of exposure. Mortality rate was found to be directly proportional to the concentration of dose indicating that mortality rate increases with the increasing dose.

Table 1 Larvicidal activity of different concentrations of methanol crude seed extracts of *Xanthium strumarium* seeds against 4th instar larvae of *Cx. pipiens* and *Ae. Caspius* after 24 h. The values presented are mean \pm SE.

Mosquito Species	Mortality (%)					LD50 \pm SE (μ g/ml)	LD90 \pm SE (μ g/ml)
	Concentration (μ g/ml)						
	1000	500	250	125	62.5		
<i>Ae. caspius</i>	100 \pm 00	46.67 \pm 1.23	20.00 \pm 1.23	13.33 \pm 1.23	0	531.07	905.95
<i>Cx. pipiens</i>	100 \pm 00	63.33 \pm 1.23	13.33 \pm 1.23	0	0	502.32	867.63

Efficacy of the seed extract from methanol was evaluated against both the targets which showed LD₅₀ values of 531.07 μ g/ml against IVth instar larvae of *Ae. caspius* and 502.32 μ g/ml for IVth instar larvae of *Cx. pipiens*. Also, results showed LD₉₀ values 905.95 μ g/ml against IVth instar larvae of *Ae. caspius* and 867.63 μ g/ml for IVth instar larvae of *Cx. pipiens* (Table 1). No activity was shown when ethyl acetate fraction of *X. strumarium* crude extract was used against IVth instar larvae of *Cx. pipiens* and *Ae. caspius*.

4. Discussion

In this study, we have shown for the first time that the seed extract of *X. strumarium* exhibited antiproliferative activity against HepG2, Jurkat, L929 and A549 cell lines. It was found that the percentage of growth inhibition was found to be dose dependent for the methanol extract and ethyl acetate fraction. However, ethyl acetate fraction showed higher toxicity to all cell lines tested when compared to the methanol extract. It has been reported that *X. strumarium* fruit extract contains 3,4-dihydroxybenzaldehyde that inhibits human cancer cell U937 (Lee et al., 2008). Two xanthanolate sesquiterpene lactones, 8-epi-xanthatin and 8-epi-xanthatin-5 β -epoxide have been isolated from leaves that inhibit different cancer cell lines such as A549 (lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF498 (CNS) and HCT-15 (colon) (Kim et al., 2003).

The MTT test results of this study revealed that the effect of the ethyl acetate fraction in growth inhibition of all cell lines was in a dose-dependent manner and higher than the crude methanol fraction. The above-mentioned studies indicated that there is a difference in ethyl acetate fraction effects and this can be attributed to the different types of cell lines tested. However, on further purification using Sephadex as stationary phase, all fractions tested lost their activity at the concentration of 400 μ g/ml; this is in accordance with traditional Medicine researchers' belief that the mixture of herbal extracts that contain several compounds is more effective in comparison to the pure active ingredient. Traditional Medicine researchers also believe that the mixture of herbal extracts also has lower toxicity and higher efficacy (1). Wong et al. (2010) and his colleague reported that the crude extract of *Rabdosia rubescens* showed higher synergistic effects at several concentrations than the active ingredient i.e., pure origin against prostate cancer cell lines (1). Also, in another study conducted by Seeram et al. (2004) the total phenolic extract of blueberry significantly inhibited the growth of several oral (CAL27 and KB) and prostate cancer cell lines (22RV1, RWPE-2, and RWPE-1) (2).

As reported above, the treatment by a *X. strumarium* is not related to just one specific chemical compound, but it is the

combination of different compounds that gives such an inhibitory effect. Therefore, further fractionation of *X. strumarium* resulted in the loss of its integrity and therapeutic potential. This further justifies the use of the total extract by herbalist in the treatment of various diseases.

An insecticide need not cause high mortality on target organisms in order to be acceptable but should be eco-friendly (Ghosh et al., 2012). Phytochemicals may serve as eco-friendly insecticides since they are, readily available in many parts of the world, comparatively safe, biodegradable, inexpensive and many of them are selective (Rajeswary and Govindarajan, 2014; Samidurai et al., 2009). Our result showed that the crude methanol extracts of seeds of *X. strumarium* have moderate larvicidal properties against the two vector mosquitoes *Ae. caspius* and *Cx. pipiens*. However, the partially purified crude extract was not active.

It is proved that crude plant extracts are less expensive and highly effective for the control of mosquitoes rather than the purified compounds or extracts (Jang et al., 2002; Cavalcanti et al., 2004; Abutaha and Al-Mekhlafi, 2014). This observation could be supported by some evidence that semi-purified natural mixtures (Ndung'u et al., 2004) or blends of pure compounds (Mokua, 2013), do act synergistically as larvicides.

5. Conclusion

The *X. strumarium* methanol extract and ethyl acetate fraction investigated in this study appears to have a weak larvicidal activity but a promising cytotoxic activity. The future studies will include the purification and investigation in further detail for the action of *X. strumarium* phytochemicals and synthesis of new active derivatives for their biological application.

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