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Insecticidal activity of chicory (*Cichorium intybus* L.) extracts against two dipterous insect-disease vectors: Mosquito and housefly

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ABSTRACT

The plant chicory, *Cichorium intybus* L. (Asteraceae), was subjected to a bioassay-guided fractionation scheme, starting with successive extraction of the whole plant powder with petroleum ether, chloroform, ethyl acetate and methanol. Toxicity screening against larvae and adults of the mosquito (*Anopheles pharoensis*) and the housefly (*Musca domestica*), revealed high potency for petroleum ether and chloroform extracts, with LC50 of 15.3 mg kg^{-1} and 0.023 mg/cm^2 against larvae and adults of mosquitoes, respectively. The LC50 for housefly larvae equaled 65.8 mg kg^{-1} and the LD50 for adults equaled 0.112 μg/insect. Saponification of petroleum ether extract resulted in saponifiable and unsaponifiable fractions, the latter was highly toxic than the former. Their activity was referred to the presence of fatty acid methyl esters in the saponifiable fraction, and sterols and hydrocarbons in the unsaponifiable fraction. Successive TLC fractionation to the chloroformic extract resulted in isolation and identification of two biologically active compounds, e.g., lactucopicrin-15-oxalate (compound I) and chicoralexin (compound II); both showed high toxicity towards the two tested insects. Compound II was more toxic than compound I to the mosquito larvae, while the opposite was obtained for the housefly larvae. The two compounds possessed equitoxic values against the adult stages of both insects. It was concluded that fractionation of the chicory plant (*C. intybus*) was in favor of toxicity increase towards the two insect pests used in the present study. Moreover, the obtained results provide new data on the insecticidal efficacy of this native plant against pests of medical importance such as mosquitoes and housefly.

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1. Introduction

The order Diptera presents an array of insects which more than any other group poses the greatest challenge to human and veterinary health as vectors of diseases. One such insect, which share a close ecological niche with man is the housefly, *Musca domestica* Linnaeus (Diptera: Muscidae). Apart from disease transmission, *M. domestica* soils man's food and usually constitutes a nuisance, particularly the adult stage (Ande, 2001). Houseflies occur throughout the tropics and are also found in warm temperate regions and some cooler areas. It is recognized as a serious public health pest to human beings and livestock by transmitting many infectious diseases (Khan and Ahmed, 2000). It acts as important mechanical carriers of pathogenic bacteria, such as *Shigella* sp., *Vibrio cholerae*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* sp. (Greenberg, 1973).

Also, mosquitoes are responsible for the biological transmission of several diseases like filariasis, dengue fever, Japanese encephalitis and malaria. Despite an array of control measures taken to suppress mosquito populations, they continue to flourish and contribute to high human mortalities, particularly in developing countries (Sagar and Schgal, 1997).

Indeed, the use of insecticides remains the first line of defense against herbivorous insects, nematodes, plant pathogens and insect vectors of disease. Control measure against these insects in the short-term still depends on the use of conventional insecticides (Malik et al., 2007). The indiscriminate use of chemical insecticides has given rise to many well-known and serious problems, such as the risk of developing insect resistance and insecticidal residual for humans and the environment (Ahmed et al., 1981). Insecticide resistance in housefly and mosquitoes is a global problem and several surveys have shown that such resistance is wide spread and increasing (Georghiou and Mellon, 1983). These problems coupled with the high cost of chemical pesticides have stimulated the search for biologically based alternatives. Accordingly, botanical insecticides based on natural compounds from plants, are expected to be a possible alternative to traditionally chemical pesticides. They tend

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to have broad-spectrum activity, relative specificity in their mode of action and easy to process and use. They also tend to be safe for animals and the environment (Belmain et al., 2001). Specifically, many studies have drawn attention to the toxic effects of plant extracts on related Diptera (Promsiri et al., 2006; Malik et al., 2007).

Our concern here is directed to one candidate plant of the Asteraceae family, chicory (*Cichorium intybus* L.); a perennial herb with blue, lavender, or occasionally white flowers. It is also known as blue sailors, endive, succory, and coffee weed, Kashen'na or Kasini (Uigur) (New Medical College of Jiangsu, 1977), and is native to the Mediterranean region, mid Asia and northern Africa. Historically, chicory was grown by the ancient Egyptians as a medicinal plant, coffee substitute, vegetable crop, and occasionally for animal forage (Munoz, 2004). Greeks and Romans also began to grow chicory as a vegetable crop 4000 years ago (Plmuier, 1972). Today, it is cultivated in Europe and North America with many commercial uses (Wang and Cui, 2011).

The chicory, as a crop, is one of the earliest known and most widely used raw materials for manufacturing of coffee substitutes (Pazola, 1987). The leaves can be used as salad as they are rich source of vitamin A and C. and also micronutrients (Bremness, 1998). Chicory root is reputed to be a blood detoxifier, tonic, and decongestant of the internal organs. Chicory rhizome contains many useful compounds (Nishmura et al., 1999). The boiled leaves and flowers have anti-inflammatory properties. Dried chicory roots are being extensively used by the beverage industries. It has also bifidogenic property as it is rich in inulin (Roberfroid et al., 1999; Niness, 1999). Chicory root extract has the free radical scavenging activity and liver protecting property. It has tumor inhibitory property also (Hazra et al., 2002). Thus this plant can provide industry with products of several importances.

The chemical composition of the chicory plant had been extensively investigated by many workers (e.g., He et al., 2002; Ablimit et al., 2008; Wu and Luo, 2009; Yang et al., 2009), and its biological activity was referred to certain phytochemicals such as: inulin, flavonoids and coumarins (Dem'yanenko and Dranik, 1971), sesquiterpene lactones (Zidorn, 2008), tannins and phenolic acids (Sareedenchai and Zidorn, 2010). It has been reported by Jurgonbski et al. (2011) that different parts of chicory and its by-products might be good sources of functional compounds (inulin, chicoric acid, quercetin glucuronide, chlorogenic acid and other caffeoylquinic acids). The root and peel extracts were characterized by large mass fractions of inulin and phenolics, determined as caffeoylquinic acids. The leaf and seed extracts had decidedly lower mass fractions of inulin and higher mass fractions of phenolics recognized as caffeoylquinic acids, chicoric acid and quercetin glucuronide.

From an industrial point of view, the US imports more than 2.3 million kilograms of chicons and 1.9 million kilograms roasted chicory roots for coffee (Schmidt et al., 2007). Chicory was originally used as a Uighurian and Mongolian traditional medicinal material or herb (Chinese Leechdom and Bioproduce Test Office, 1990). In China, new broad leaf varieties of chicory are improvedly bred. They are prospective forages and cash crops with high production, multiple function and good quality; therefore, they can be used as both health care food and medicine, authorized by the Ministry of Health of the People's Republic of China in 1998. Moreover, the chicory forage is highly palatable to livestock (Wang and Cui, 2010).

Over the past decades, a number of publications have reported on chicory and its extractives with respect to the biochemical composition, the biological activities and utilization technologies (Wang and Cui, 2011). Indeed, the literature offers data on the insecticidal activity of this plant against the stored grain pest *Triboilum castaneum* Herbst (Pascual-Villalobos and Robledo, 1998). Also, recent studies were conducted in our laboratory (Mansour et al., 2010, 2011, 2012) to screen the insecticidal activities against the mosquito, *Anopheles pharoensis* and the housefly, *Musca domestica* for ethanolic crude extracts of some plant species including *C. intybus*. To the best of our knowledge, except our previous studies, the literature offers no data on the insecticidal activity of *C. intybus* against the above mentioned insects. Thus, the current work was undertaken to subject this plant to a bioassay-guided fractionation scheme and to estimate toxicity of the different fractions and isolates against larval and adult stages of the mosquito *Anopheles pharoensis* and the housefly, *Musca domestica*.

2. Materials and methods

2.1. Plant

Chicory (*Cichorium intybus* L.) plant was collected from clover field and authenticated by Prof. M. El-Gebaly, Flora Dept., National Research Centre, Cairo, Egypt. The fresh herb was washed, air dried, powdered and kept in a dark glass bottle until used in the extraction processes.

2.2. Test insects

Anopheles pharoensis: *A. pharoensis* were reared under standard conditions at 27 °C, 70% relative humidity and a 12L:12D photoperiod. Eggs were placed in plastic trays filled with distilled water. Larvae were reared at a fixed density of 100 larvae per tray and fed upon tetramine, which is recommended for larval development and female fecundity (Kasap and Demirhan, 1992). Pupae were collected from day 11 to 15, placed in emergence cages of 50 cm × 50 cm and provided with a piece of sponge supplied with 10% glucose solution that was suspended by a wire thread from the roof of the cage. Adult females were fed on pigeons. The eggs laid were transferred to small plastic containers filled with distilled water and a small amount of tetramine. After breeding for several generations, 4th instar larvae as well as 5-day-old female adults were selected for running bioassay tests.

Musca domestica: *M. domestica* (MD) houseflies were reared in the insect rearing room of our laboratory at 25–27 °C, and 55–60% relative humidity. A standard rearing method (Sawicki, 1964) was adapted to provide 3rd larval instars and adult flies of 0–24 h old for running bioassay tests.

2.3. Systematic fractionation of Cichorium intybus

2.3.1. Successive solvent extraction

Samples of 300 g of the dried plant powder were extracted in a Soxhlet apparatus, using different solvents of increasing polarity; e.g., petroleum ether, chloroform, ethyl acetate and methanol at a rate of 3 ml/g plant material and for 8 h extraction period. The solvents were evaporated to dryness under vacuum using a Rotavapor with a water bath adjusted at 50 °C maximum.

The residue was obtained and bioassayed against larvae and adult stages of the tested insects (*A. pharoensis* and *M. domestica*) at the values corresponding to LC50 & LD50 as previously reported for the ethanolic crude extract of *C. intybus* (Mansour et al., 2010, 2011, 2012).

As shown from the diagram illustrated by Fig. 1, the most effective extracts were obtained from petroleum ether and chloroform which gave more than 50% mortality against the tested insects. Therefore, they were subjected to detailed toxicity screening and further fractionation of their phytochemical constituents.

2.3.2. Extraction of lipids (separation of saponifiable and unsaponifiable constituents)

A quantity of 150 g from chicory herb was exhaustively extracted in Soxhlet by petroleum ether at a ratio of (1:3; w/v). The petroleum ether extract was saponified to separate the lipoidal

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