INSECTICIDAL ACTIVITIES OF THE SISAL PLANT, *AGAVE SISALANA*, AGAVACEAE EXTRACTS AGAINST WHITE TERMITES, *RETICULITERMES FLAVIPES* (KOLLAR) RHINOTERMITIDAE

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ABSTRACT

From 986 g of fresh chopped leaves of the sisal plant, *Agave sisalana,* in a conical flask, methanol was added and left standing for three days. The filtrate obtained was then subjected to partition chromatography using *n*-hexane followed by dichloromethane to afford three soluble fractions. The three fractions were then concentrated separately in vacuum before various samples for anti-termites and anti-brine shrimps bioassay were prepared. The three fractions indicated high toxicity levels, 100% mortality at a concentration of 1000 ppm against both, the white termite, *Reticulitermes flavipes*, Rhinotermitidae, and brine shrimps, *Atemia salina.* The methanol fraction showed relatively higher toxicity levels at lower concentrations compared to *n*-hexane and dichloromethane fractions.

Key words: Sisal plant, *Agave sisalana*, Agavaceae, white termites, *Reticulitermes flavipes*, Rhinotermitidae, brine shrimp, *Atemia salina,* toxicity tests.

1.0 INTRODUCTION

The sisal plant, *Agave sisalana,* is a xerophyte, short-lived, monocarp perennial plant with a short thick stem and a close rosette of leaves. It has fibrous adventitious roots, immature creamy white leaves, packed tightly round the meristem and a leaf phylastaxy of 5/34 clockwise or anticlockwise (Howell and Ehrlich, 1990). *Agave sisalana* has been used since time immemorial in making twines, hammock and mats. Its leaves exudates contain hecogenin, a steroidal sapogenin used as a starting point for the manufacture of cortisone derivatives, oral contraceptives and other steroids (Glove, 1986). It also contains saponins and cardiac glycosides that are highly toxic against insects (Vickery and Vickery, 1990). In Kenya, *A. sisalana* is commonly found in Machackos, Makueni, Kajiado and Thika districts.

The Eastern Subterranean termite, *Reticulitermes flavipes* (Kollar), Rhinotermitidae is widely distributed all over the world, particularly in the US and Africa. In Kenya, it is commonly found in the arid and semi-arid areas of Machakos, Kitui, Makueni, Kajiado, Thika, Taita Taveta and Nairobi. The members of this group always maintain contact with the soil and often construct earthen tubes in woods rather than on the soil. It is the most destructive and difficult to control among the genus *Reticuliterme* (Charles *et al.*, 1990).

The aim of this study was to investigate the toxicity effects of the extracts from *A. sisalana* against the destructive termite, *Reticulitermes flavipes* and brine shrimp, *Atemia salina.*

2.0 MATERIALS AND METHODS

2.1 Collection of the Sisal Plant, *Agave sisalana*

The sisal plant, *A. sisalana* (about 1 kg) was collected from around Embakasi area, South-East of Nairobi and in Thika District, Kenya. Collection was done by plucking the leaves from the point of attachment around the meristem. At each cutting, only the lowest leaves were removed. This exercise was done during the morning hours when the leaves were still fresh and juicy.

Immediately after collection, the leaves were chopped into tiny pieces and placed in a conical flask in which methanol was added to a level just above the surface of the leaves. The content was left standing for five days at room temperature. During this period of soaking, extraction of both polar and non-polar compounds took place. Filtration followed where the filtrate was subjected to partitioning between the methanol/water layer against *n*-hexane and dichloromethane successfully. Three fractions were therefore obtained with the polar compounds in methanol, the medium polar compounds in dichloromethane and the non-polar compounds in *n*-hexane. After concentration, each of these fractions was subjected to bioassay tests against the white termites, *Reticulitermes flavipes* and brine shrimps, *Atemia salina,* to determine their toxicity.

2.2 Anti-termite Toxicity Bioassay

2.2.1 Collection of Termites

Termites belonging to the family *Rhinotermitidae* and the species *Reticulitermes flavipes* were collected from a healthy mould. This was done early in the morning to avoid dehydration of the insects. A sampling bottle, a pair of gloves and a pair of tongs were used. The termites were then starved for 24 hours before starting the assay.

2.2.2 Bioassay Treatment and Test

Each of the *n*-hexane, dichloromethane and methanol fractions was made into 1000 ppm stock solutions. Dried wood chippings and a source of cellulose were food for the termites. A specific mass was weighed accurately into a glass beaker. The stock solutions of the three portions were diluted to various concentrations: 800 ppm, 600 ppm, 400 ppm 200 ppm, and 100 ppm. A specific volume of the stock concentrations was mixed uniformly with the wood chips. The mixture was evaporated and left for one hour to ensure that all the toxic fumes had escaped. A control was set up containing constant mass of wood chippings, mixed with a specific volume of the respective solvent system. The mixture was evaporated and a known number of termites put into the two beakers and their behavior noted. This was done in triplicate for all the three portions. The number of the dead and surviving termites was recorded after every two hours and their values converted into percentage relative to the control (% mortality).

3.0 RESULTS AND DISCUSSION

3.1 Yield of Crude Extracts

Out of 986 g of fresh chopped leaves of the sisal, *A. sisalana,* the following materials were collected:

Polar (methanol) portion = 0.55 g (0.05% of chopped sample)
Medium-polar (dichloromethane) portion = 0.79g (0.08% of chopped sample)
Non-polar (*n*-hexane) portion = 1.56 g (0.16% of chopped sample)

3.2 Anti-termite Bioassay Results

The results of anti-termite bioassay are given in Table 1.

Table 1: Anti-termite bioassay results

Conc. (ppm)	*Methanol extract* (%) mortality ± sd	*Dichloromethane extract* (%) mortality ± sd	*Hexane extract* (%) mortality ±sd
1000	100 ± 0.00	100 ± 0.00	100 ± 0.00
800	60 ± 4.97	20 ± 4.24	30 ± 4.24
600	60 ± 2.59	10 ± 1.98	30 ± 1.98
400	30 ± 4.24	10 ± 1.98	0 ± 1.98
200	0 ± 0.00	0 ± 0.00	0 ± 0.00

3.3 Brine Shrimp Bioassay

The results of brine shrimps bioassay are given in Table 2.

Table 2: Brine shrimp bioassay

Conc.	*Methanol extract*	*Dichloromethane extract*	*Hexane extract*
(ppm)	(%) mortality ± sd	(%) mortality ± sd	(%) mortality ± sd
1000	100 ± 0.00	100 ± 0.00	100 ± 0.00
800	60 ± 1.53	20 ± 1.41	30 ± 3.74
600	60 ± 0.87	10 ± 0.87	30 ± 0.87
400	30 ± 1.41	10 ± 0.87	0 ± 0.87
200	0 ± 0.00	0 ± 0.00	0 ± 0.00

Fresh leaves of *Agave sisalana* (986 g) were collected. After extraction, separation and concentration, 0.55 g (0.05%) of polar compounds (methanol soluble), 1.56 g (0.16%) of non-polar (*n*-hexane soluble) and 0.79g (0.08%) of medium polar compounds (dichloromethane soluble) were obtained. Bioassay tests indicated that all the three extracts contained toxic compounds. A 1000 ppm portion of each of three fractions showed a percentage mortality of 100%.

The polar extract registered higher toxicity levels compared to other two at 60% (800 ppm) and 30% (400 ppm). The dichloromethane and n-hexane fractions had lower values at the same concentration. The methanol extract was also the most active against brine shrimps at between 100% at 1000 ppm to 30% at 400 ppm. The dichloromethane and *n*-hexane fractions were only active (100%) at high concentrations of 1000 ppm, but lower than 30% at 800 ppm and below.

From the above results, it was observed that the polar extract of *Agave sisalana* was the most potent against both the termites and brine shrimps. The dichloromethane fraction showed the least activity against these target organisms. The results of this study indicate that the extracts of this plant are potent as an insecticide against termites, and can be used to control destructions caused by this pest.

4.0 CONCLUSION

The results of this study show that there is need for further investigation in an effort to isolate and elucidate the structure(s) of the compounds responsible for these observed activities and at the same time try to unravel the mechanism of the reactions behind this observed phenomena.

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