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The Leaf Extracts of *Dodonaea viscosa* have a Detrimental Impact on Tunneling and Midgut Enzyme Activities of *Odontotermes obesus*

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Abstract

The potential of the leaf extracts of *Dodonaea viscosa* Jacq. as termiticide on tunneling and midgut enzyme activities of *Odontotermes obesus* (Ramb.) (Termitidae: Isoptera) was evaluated in the present studies. In the preliminary bioassays, leaf extracts in polar and non-polar solvents were mixed in the soil at 1, 5 and 10% concentrations and their effect on mortality (LT) and tunnel length (mm) was determined. The toxic fraction following gel filtration was analysed for kaempferol by HPLC. The effect of standard kaempferol, gel fraction, crude leaf extract and chlorpyrifos was ascertained on tunnel length and enzyme systems in the gut of the termite species. n-Hexane crude leaf extract of *D. viscosa* was the most toxic registering minimum LT_{50} (104.41, 95.53 and 66.68 hours) and tunnel length (63.00, 36.33 and 16.67 mm), respectively at 1, 5 and 10% concentrations. Gel filtrated fraction No. 3 had significantly lowest LT_{50} (58.70, 33.96 and 15.94 h) corresponding to 1, 5 and 10% concentrations. The presence of kaempferol in gel filtrated fraction matched with peak of its standard in HPLC system. Interaction of effect of kaempferol, gel filtrated fraction, n-Hexane crude leaf extract and chlorpyrifos at their respective concentrations was non-significant but significantly different from their parallel control treatments. A significant decrease ($p<0.05$) in the activities of esterases, proteases and cellulases in the gut of termite workers after exposure to kaempferol, gel filtrated fraction, n-Hexane crude leaf extract treatments at different concentrations was observed. The activity of esterases in the gut was reduced in chlorpyrifos exposed termite worker, whereas proteases and cellulases in these workers remained non-significant with their control treatments. From these studies, it is concluded that kaempferol in n-Hexane extract of *D. viscosa* can be effective in the development of baiting system against the termites.

Keywords: *Dodonaea viscosa*; Leaf extracts; Termites; Proteases; Esterases; Cellulases

Introduction

Dodonaea viscosa Jacq. is abundantly and widely distributed in diverse ecosystems of the world. The Potohar region of Pakistan has dense populations of this plant and many uses of this plant have been described since long. The leaf powder of *D. viscosa* is traditionally utilized as ayurvedic medicine for the treatment of various fungal skin diseases in Pakistan (Shahani and Memon, 1988). Antimicrobial properties of leaf and seed extracts are also well documented (Khurram *et al*., 2009; Pirzada *et al*., 2010).

Biopesticidal property of *D. viscosa* has been reported on numerous insect species. The hexane extract of *D. viscosa* tested against *Spodoptera littoralis* was the most effective, which gave the highest larval mortality and reduction in hatchability, fecundity and pupation (Abdelaziz and Omer, 1995). The extract of *D. viscosa* was also compatible with those of *Colocynthus* against *S. littoralis* (El-Din and El-Gengaihi, 2000). *D. viscosa* var. *angustifolia* induced significant reduction in adult longevity and also adversely affected the reproductive potential of *Helicoverpa armigera* (Hub.) (Subashini *et al*., 2004). The crude extracts of *D. viscosa* var. *angustifolia* drastically reduced the number of *Earias vittella* larvae similar to a neem product when both were applied as separate treatments on the cotton in a field experiment. (Malarvannan and Subashini, 2007). In addition to insects and microbes, cattle and goats/sheep avoid feeding on leaves of this plant (Colodel *et al*., 2003; Cattani *et al*., 2003), however, *D. viscosa* (Purple hop bush) is also considered a termite resistant shrub (Anonymous, 2001).

A number of plants are inedible to insects and animals owing to possession of phenolic compounds. Several studies have shown that phenolics including flavonoids are active agents to protect various plants from termite attack due to their antitermite activities. These plants include *Diospyros sylvatica*, *Capparis deciduas* and *Parkia biglobosa* in the

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most recent publications (Ganapaty *et al*., 2004; Upadhyay *et al*., 2010; Olugbemi, 2012). The phenolics having medicinal value have also been reported from leaf and seed extracts of *D. viscosa* (Carter *et al*., 1978; Scheffrahn, 1991; Boué and Raina, 2003; Prakash *et al*., 2012), however, potential of such phenolics in *D. viscosa* as termiticide is not known. The leaf and seed extracts of *Jatropha curcas, Calotropis procera, Ricinus communis, Parthenium hysterophorus* and *Prosopis juliflora* in organic solvents were not only toxic to termites but also prevented tunnel formation in treated soils (Vasant and Narasimhacharya, 2008).

The digestive enzymes in termites especially cellulases have been target of many synthetic and natural inhibitors (Zhu *et al*., 2005); AChE stimulating effects were highly correlated with toxicity of tetrahydronootkatone (Ibrahim *et al*., 2007). Flavonoids are reported as the inhibitors of a number of catabolic glycohydrolases, starch and cellulose hydrolyzing enzyme (Todera *et al*., 2006). One of naturally occurring flavonoids, kaempferol, was reported to inhibit hydrolase (fatty acid amide, aceylcholinesterases) (Thors *et al*., 2008; Priya, 2012). Fractionation of dichloromethane and acetone fractions obtained by serial extraction from the leaf powder of *D. viscosa* Jacq. var. *angustifolia* (Sapindaceae) resulted in the isolation of four kaempferol methyl ethers, which exhibited antibacterial and antioxidant activities (Teffo *et al*., 2010).

The current investigations were designed 1) to extract and explore the antitermatic potential of *D. viscosa* leaves by calculating LT_{50} values, 2) to detect the presence of kaempferol, an important flavoniode from the leaves of *D. viscosa*, 3) to explore the tunneling behavior of *O. obesus* (Ramb.) in the presence of kaempferol by design bioassays, 4) to explore the physiological impacts of kaempferol by calculating proteases esterases and cellulases activities of mid gut homogenates of *O. obesus* (Ramb.) (Isoptera: Termitidae).

Materials and Methods

Collection of Termites

The assorted workers and soldiers of the termite species, *O. obesus* were collected from the corrugated cardboard baits in PVC monitors installed in the sugarcane fields at various places around the campus and at Post-Graduate Agriculture Research Station, University of Agriculture, Faisalabad (Ahmed *et al*., 2006). This termite species was abundant and exclusively present at these places.

Preparation of Crude Leaf Extracts

For crude leaf extracts, different non polar to polar solvents viz., petroleum ether, n-hexane, chloroform, methanol, ethanol, acetone and water were used. The leaves of *D. viscosa* were collected from the Botanical Garden, University of Agriculture, Faisalabad, Pakistan, where no pesticide or any other chemical has ever been applied. The leaves were taken from mid peripheral portion of the plants and washed with distilled water, air dried in a room for 24 h before freeze drying. The dried leaves were reduced to a powder form by grinding in an electrical grinder (Monilex Australia Pvt. Ltd) for 45 seconds. One hundred gram (100 g) of the leaf powder was extracted in 200 mL of the solvent in the ratio of 1:2 (w/v) in a conical flask. The plant material was soaked in each solvent for 24 h and then shaken in an electrical shaker for 72 h. The supernatant was filtered with two layers of What-man Filter Paper No. 42. The above procedure was repeated thrice to obtain maximum extractable. All the filtrates were pooled and evaporated under vacuum in a rotary evaporator. The crude extracts were weighed to measure the yield and then used at a desired concentration for the bioassay.

Bioassays

The soil to carry out the bioassays was collected from sugar cane fields and other places of termites collection at University of Agriculture, Faisalabad, and its physical properties and composition were noted. There was no application of pesticides of any sort in this soil. The soil was sieved through a 30-mesh screen and moisture was determined with the help of a moisture meter. Soil was sterilized in a vacuum oven. Moisture of the soil in the field was also determined in order to obtain uniformity in the moisture content in bioassays. Antitermitic bioassays were done in Petri dishes of 10 cm diameter × 1.5 cm in height containing 20 g sifted sterilized soil and a strip of sugarcane (1.5 cm × 6 cm) in each. The sugar cane strips were used to keep the termites unstarved. Each leaf extract at 1%, 5% and 10% concentration and a control (without extract) were repeated thrice with a different set of termite workers and soldiers. 100 active workers and 10 soldiers were released in the Petri dishes having treated and untreated soils and then placed in a growth chamber at 28±2°C and 80%±5% relative humidity. Data for the mortality of the termite workers and soldiers were recorded after an interval of 2 h up to 12 h and then after every 12 h until the mortality of 100 workers and 10 soldiers was occurred. Kaplan Meiyer Survival test was used to obtain LT_{50} of different plant extracts at various concentrations in different treatments (Minitab 15).

Galleries Formation

Members of family *Termitidae* make galleries during foraging. This shows the activity of the termites in the soil. The termites started making tunnel along the bottom of each Petri dish around the sugarcane strip. Termites response towards galleries formation for each leaf extract at each concentration after 5, 10 and 15 h were determined by plotting the tunnels on the cellophane paper and measured the length in mm with the help of planimeter. Control

treatment in this case was respective solvent. Fraction No. 3 (from the fractionation procedure explained below), Kaempferol, crude leaf extract of n-Hexane, chlorpyrifos (mock control) and a control (only n-Hexane) were treatments for determining effect on tunneling activities of *O. obesus* in the soil. The length of tunnel was determined in the same manner as described above. The standard kaempferol, n-hexane and reagents for enzyme assays were purchased from Sigma-Aldrich (MS Traders, Lahore, Pakistan). The mean tunnel lengths in various treatments of solvents and concentrations were compared by ANOVA in MSTATC through Least Significant Difference (LSD) test.

Isolation of Active Agent from the Plant Extract

n-Hexane leaf extract of *D. viscosa* was chosen for fractionation on account of its toxicity (Table 1) and impact on gallery formation (Table 2).

Fractionation Procedure for n-Hexane Leaf Extract by Gel Filtration

The crude leaf extract of *D. viscosa* was fractionated by gel filtration. The column (30 × 2.5 cm) packed with silica gel was equilibrated with hexane. 5 mL of hexane extract was loaded and fractionated in size of 1 mL. Four fractions were isolated with pure hexane. These four fractions were applied at three concentrations, i.e., 1%, 5%, 10% along with a control, on three groups of *O. obesus* following same bioassay procedure as stated above. The control treatment was hexane only. In this way, most toxic fraction was isolated.

Analysis of Toxic Fraction by HPLC

The most active fraction was then analyzed by an HPLC system equipped with a Shim-pack CLC-ODS (C-18) column (15 cm×4.6 mm, 5 µm). The mobile phase was acetonitrile (100%, isocratic) and the flow rate 2 mL/min for the detection of Kaempferol. 50 ppm of a standard sample of kaempferol was injected for the detection of standard peak. The eluent was detected by UV at 370 nm. The kaempferol peak was detected at a retention time of 0.987 min.

Enzyme Assays

Twenty grams of the soil previously used for the bioassays were added to each Petri dish to obtain termite workers for the determination of enzyme activity in the midgut of these workers. The different concentrations, i.e. 1%, 5%, and 10% of Fraction No. 3 (gel filtration), n-Hexane crude leaf extract, Kaempferol and chlorpyrifos in acetone were mixed with the soil in the Petri dishes with a glass spatula. The solvent was allowed to evaporate for up to 6 h. A few drops of distilled water were added to the Petri dishes to maintain wetness of the soil. One hundred termite workers (3^{rd} instar) and 10 soldiers were released in Petri dishes containing treated and untreated soils. The experiment was carried out in a completely randomized design with three replications. The control treatments were hexane and acetone in their respective case. A new set of termite workers were used for each replicate. The surviving workers after 24 h of exposure were collected by sieving through 60 mesh screen. The workers were put in 80% ethanol. The midguts of the workers were dissected in an insect saline solution, put in 80% ethanol and stored at -20°C until used for enzymes assay. The protein concentration of the sample (10 µL) was determined by Bradford assay (1976). Proteases activity was determined by the casein digestion assay described by Drapeau *et al*. (1974). The determination of cellulases activity was carried out according to the procedure recommended by IUPAC (International Union of Pure and Applied Chemistry) (1987), using carboxymethyl cellulose as a substrate. Esterases were determined following method of van Asperen (1962). Differences in enzymes activity in different treatments was determined by ANOVA and means were separated by Tukey's test at 5% level of significance.

Results

LT_{50}s of Leaf Extracts of *D. viscosa* on *O. obesus*

Table 1 shows comparison of LT_{50} (hours) of leaf extracts of *D. viscosa* in various solvents at different concentrations exposed along with a control treatment (respective solvent only). The concentration dependent LTs (lethal time) were recorded in various treatments. Maximum LT_{50} (167.83 h) was observed with chloroform extract at 1% concentration whereas minimum LT_{50} (66.68 hours) was observed with n-Hexane extract at 10% concentration. LT_{50}s in leaf extracts/solvents were significant lower than their respective controls. LT_{50}s of n-Hexane at three concentrations were lower than rest of leaf extracts in various other solvents. The difference among four groups of termites (Control, R1, R2, and R3) exposed to 0, 1, 5 and 10% concentration of *D. viscosa* leaf extract in different solvents showed that all the three groups had non-significant difference among themselves ($p \geq 0.05$) (data not shown).

Tunneling Activities by *O. obesus* in Soil Treated with *D. viscosa* Leaf Extract with Different Solvents

The tunnel length formed by *O. obesus* at 0%, 1%, 5%, 10% concentrations of leaf extracts of *D. viscosa* in different solvents is shown in Table 2. All solvents induced a significant decrease of the tunnel length when compared to 1% of water extracts (102.33 mm), except for 1% chloroform. The minimum tunnel length (16.67 mm) took place in n-Hexane extract at 10% concentration; 10% of ethanol, petroleum ether and acetone induced a similar pattern of tunneling ($p>0.05$), while the tunnel length was significantly different from all the solvents at 1%, 5% and methanol, chloroform, water at 10% concentration. Control treatments (respective solvents only) having longer tunnels

Table 1: Comparison of LT_{50} values (h) in different concentration of leaf extracts of *D. viscosa*

Treatments	Concentrations			
	Control	1%	5%	10%
Methanol	246.50±5.21a	129.15±4.25ef	111.07±1.91ghi	98.98±2.23ij
Ethanol	241.66±5.65a	128.34±1.93ef	110.31±1.64ghi	93.28±2.24j
Acetone	238.97±3.12a	108.24±3.33g-j	99.15±2.19ij	75.97±1.87k
Ether	215.05±3.40b	120.07±3.59fgh	109.16±3.31g-j	93.29±1.75j
n-Hexane	238.97±3.12a	104.41±5.07hij	95.53±1.75j	66.68±1.84k
Chloroform	254.14±4.07a	167.83±3.67c	136.48±1.52def	124.21±1.80efg
Water	248.80±1.64a	150.38±3.05d	138.00±3,35de	128.35±3.85ef

Table 2: Tunneling length (mm) made by termites at different concentrations in leaf extracts of *D. viscosa* in various solvents

Treatments	Concentrations			
	Control	1%	5%	10%
Methanol	290.67 c	85.67fg	61.33ij	33.33 l
Ethanol	313.33 ab	85.00 fg	54.67j	27.67 lm
Pet.ether	294.33 c	76.33 gh	55.67j	26.33 lm
Acetone	319.00 a	87.67 fg	50.33jk	27.33 lm
N-hexane	295.00 c	63.00 hij	36.33kl	16.67 m
Chloroform	274.67 d	91.00 ef	52.67j	32.33 l
Water	303.00 bc	102.33e	73.67ghi	35.33 l

Table 3: Comparison of four fractions from gel filtration of n-Hexane crude leaf extract on the mortality (LT_{50} in h) of termites

Fractions	Concentrations			
	Control	1%	5%	10%
Fraction 1	127.15±10.83a	82.34±6.23 b-f	69.79±4.94def	55.05±4.80fg
Fraction 2	125.18±3.36a	105.88±8.38abc	88.64±5.03b-e	79.15±3.27c-f
Fraction 3	115.52±8.43ab	58.70±4.86efg	33.96±4.42gh	15.94±4.94h
Fraction 4	127.15±10.83a	100.10±5.07a-d	75.43±6.75c-f	62.35±3.20efg

Means sharing similar letter(s) in a row or in a column are statistically non-significant (P>0.05). Small letter(s) represent comparison among interaction means

were significantly different from three concentrations of leaf extracts of *D. viscosa*.

The crux of data on tunneling activities in plant extracts treated and untreated soils revealed that the presence of extracts in soil posed hindrance for the termites to move freely in treated soil.

Fractions from Gel Filtration on Mortality of *O. Obesus*

The comparison of the four fractions from gel filtration in three replications along with a control (only solvent) with regard to mortality of termite workers is given in Table 3. Fraction 3 at 10% concentration had the lowest LT_{50} (15.9 h), which was non-significantly different from LT_{50} at 5% concentration but significantly different from all other concentrations of the fractions 1, 2 and 4.

Analysis of the Most Effective Fraction by HPLC for Kaempferol

After the isolation of the most effective fraction it was analyzed for the detection of Kaempferol by HPLC. The kaempferol peak was detected at the retention time 0.987. Fig. 2 shows the peak of kaempferol in hexane leaf extract of *D. viscosa*. The kaempferol peak was detected in hexane leaf extract of *D. viscosa* at the retention time of 1.007.

The interaction of treatments of kaempferol, gel filtration fraction No. 3, n-Hexane crude extract and chlorpyrifos with their concentrations was non-significant (p>0.05), At 10% concentration kaempferol (30.56 mm), gel filtration fraction No 3 (51.40 mm), and n-Hexane crude leaf extract (47.82 mm) induced a decrease of tunnel formation than chlorpyrifos (55.05mm) (p= 0.05) (Table 4).

Enzyme Activities in Termite Midgut Workers after Exposure to Different Concentrations of Gel Filtration

The data given in Tables 5-7 exhibited that the four treatments, their concentrations and interaction between treatments and concentrations had significant difference for enzyme activities. All four treatments caused decline in the esterases, proteases and cellulases enzymes activities except chlorpyrifos, which had no effect on proteases and cellulases (Tables 6 and 7). Fraction No. 3 and kaempferol were statistically similar in their action on enzymes. The esterases appeared to be affected to a large extent by these treatments compared to cellulases and proteases.

Discussion

The crude extracts of *D. viscosa* in various solvents were lethal to the workers of *O. obesus* as these showed significantly less LT_{50}s when compared with respective controls. These extracts had also affected tunnel formation by workers of termite species in the soil mixed with them. The extract of *D. viscosa* could be used as potential insecticide against termites. These results are in conformity with studies in which mortality in termites and reduction of tunnel length by them have been taken as criterion for effects of leaf extracts (Oyedokun *et al*., 2011; Yuan and

Table 4: Comparison of tunnel (in mm) formation by *O. obesus* in kaempferol, Gel Filtration fraction No. 3, n-Hexane crude leaf extract and chlorpyrifos treated soil

Treatments	Concentrations			
	Control	1%	5%	10%
Gel filtration fraction No.3	134.91±7.02	73.54±5.64	59.68±2.65	51.40±3.13
n-Hexane crude leaf extract of *D. viscosa*	127.15±10.83	62.35±3.20	55.01±1.82	47.82±3.55
Kaempferol	129.09±10.29	60.53±4.85	44.30±4.65	30.56±2.86
Chlorpyrifos	131.03±8.92	82.34±6.23	69.79±4.94	55.05±4.80

Table 5: Mean esterases activities (µmoles/min/mg protein) of *O. obesus* exposed to gel filtration fraction No. 3, n-Hexane crude leaf extracts of *D. viscosa*, Kaempferol and chlorpyrifos

Treatments	Concentrations			
	Control	1%	5%	10%
Gel filtration fraction No.3	38.63±0.38ab	2.9±0.66 fg	1.04±0.45hi	0.19±0.01i
n-Hexane crude leaf extract of *D. viscosa*	39.07±1.09ab	3.8±0.26ef	2.73±0.27fg	1.95±0.10gh
Kaempferol	39.43±0.88a	2.6±0.66fgh	1.00±0.43hi	0.17±0.008i
Chlorpyrifos	37.56±0.54b	11.9±0.60c	8.36±0.29d	4.7±0.70e

Means having same letters in column and row are not significantly different at 5% level of probability

Hu, 2012). The termite workers were continuously in contact with treated soils so leaf extract had direct toxic action on the termite workers.

Among the four fractions of n-Hexane leaf extract of *D. viscosa* by gel filtration, fraction No. 3 was found to be the most toxic to termite workers. Further HPLC analysis of this fraction has demonstrated the presence of kaempferol as major constituent in that fraction. Kaempferol (and some glycosides of kaempferol) is a flavonoid found in many edible plants and in traditional medicines such as *Ginkgo biloba*, *Tilia* spp, *Equisetum* spp., *Moringa oleifera*, *Sophora japonica* and Propolis and are shown to have a wide range of pharmacological activities (Calderón-Montaño *et al*., 2011).

The phenolics (flavonoids) have been isolated from leaves of *D. viscosa* (Khan *et al*., 2012; Riaz *et al*., 2012) and three to four types of kaempferol were identified (Narayana *et al*., 2001; Teffo *et al*., 2010). Erstwhile, *D. viscosa* has shown anti-insect attributes (Abdelaziz and Omer, 1995; El-Din and El-Gengaihi, 2000; Anonymous, 2001; Colodel *et al*., 2003; Cattani *et al*., 2004; Subashini *et al*., 2004; Malarvannan and Subashini, 2007).

The toxic fraction of *D. viscosa* leaf extract (Fraction No 3) also allowed shorter tunneling in the soil as compared to other solvent extracts. Considering that kaempferol was the major compound of this fraction and that the shortest tunnel length was observed in the presence of the highest concentration of kaempferol. These results coincide with our earlier results in which presence of plant extracts limit length of galleries by *Microtermes obesi* (Ahmed *et al*., 2006). The short tunnel length may be expected from effects of plant extracts on locomotors sensillae or in semiochemical, hormone, and lignocellulose processing in the termite gut where esterases can play a major role. Failure to carry out these functions may disrupt orientation in termites. This seems possible as termites were not absolutely prevented in penetrating the leaf extract-treated soils at all tested concentrations.

n-Hexane crude extract of *D. viscosa*, gel fraction No. 3 and kaempferol have been shown to bring about a change in esterases, proteases and cellulases activities of *O. obesus*. Significantly less activities of these enzymes were recorded in gut of termites exposed to plant extract-treated soils. It is likely that they act as inhibitors of these enzymes; the ability of phenolic compounds to inhibit the variety of enzymes like hydrolases, hyalouronidase, alkaline phosphatise, arylsulphatase, cAMP phosphodiesterase, lipase, α-glucosidase, kinase have already been reported (Rani *et al*., 2009).

Kaempferol from different plants have been found to inhibit the *in vitro* and *in vivo* (insects) enzyme activities. For example, kaempferol from *Morus alba* leaf extracts showed a significant acetylcholine esterase inhibitory activity when these extracts were tested individually (Priya, 2012). The extracts from three *Kalanchoe* species (*K. brasiliensis*, *K. pinnata* and *K. gastonis-bornieri*) containing kaempferol showed acetylcholine esterase inhibitory effects on *Aedes aegypti* larvae (Trevisan *et al*., 2006). The esterases activities were reduced to great extent compared with those of proteases and cellulases, which indicate a disturbance in lipid metabolism. Cellulases inhibitors studied for termites belonged to class carbohydrates (Boué and Raina, 2003; Zhu *et al*., 2005) and this difference in chemical nature between kaempferol and carbohydrates has led to present results of no influence of crude extract and kaempferol on cellulases in termites. The non-significant effect of kaempferol on gut proteases can be predicted from the fact that many protease inhibitors are basically proteins.

An earlier report has demonstrated the isolation of kaempferol from *D. viscosa* with antibacterial and antioxidant properties (Teffo *et al*., 2010), yet no reference so far has been found to explain the effect of kaempferol on the biology of the termites.

Conclusion

The isolated kaempferol from n-Hexane leaf extract of *D.*

Table 6: Mean proteases activities (I.U/mg/10 µL) of *O. obesus* exposed to gel filtration fraction No. 3, n-Hexane crude leaf extracts of *D. viscosa*

Treatments	Concentrations			
	Control	1%	5%	10%
Gel filtartion fraction No.3	38.73±1.00abc	24.13±1.03e	20.63±0.40f	16.00±0.95g
n-Hexane crude leaf extracts of *D. viscosa*	40.27±0.63ab	28.67±0.88d	26.77±0.65d	18.93±0.63f
Kaempferol	40.83±0.74abc	23.87±1.13e	20.17±0.57f	15.66±0.81g
Chlorpyrifos	39.87±0.14ab	38.63±0.29bc	38.33±0.80bc	38.11±0.57b

Table 7: Mean cellulases activities (I.U./mg/10 µL) of *O. obesus* exposed to gel filtration fraction No. 3, n-Hexane crude leaf extract of *D. viscosa*, Kaempferol and chlorpyrifos

Treatments	Concentrations			
	Control	1%	5%	10%
Gel fraction No. 3	40.86±0.8a	20.03±0.52de	18.4±1.17ef	12.2±0.62g
n-Hexane crude leaf extract of *D. viscosa*	39.86±1.1ab	27.06±0.95c	21.0±0.90d	16.8±0.32f
Kaempferol	40.56±0.82ab	19.6±0.45de	17.9±1.13ef	11.93±0.89g
Chlorpyrifos	39.56±0.37ab	39.03±0.52ab	39.1±1.29ab	38.16±0.57b

Means having same letters in columns and rows are not significantly different at 5% level of probability

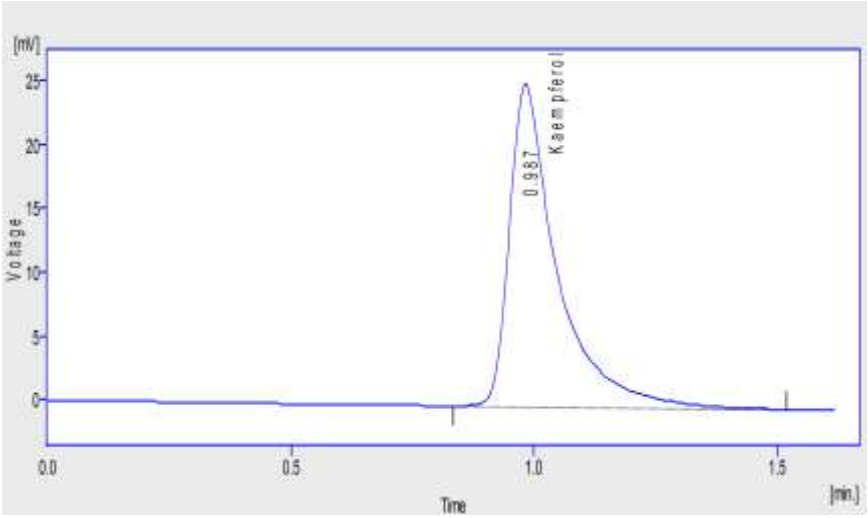

Fig. 1: Chromatogram of standard kaempferol

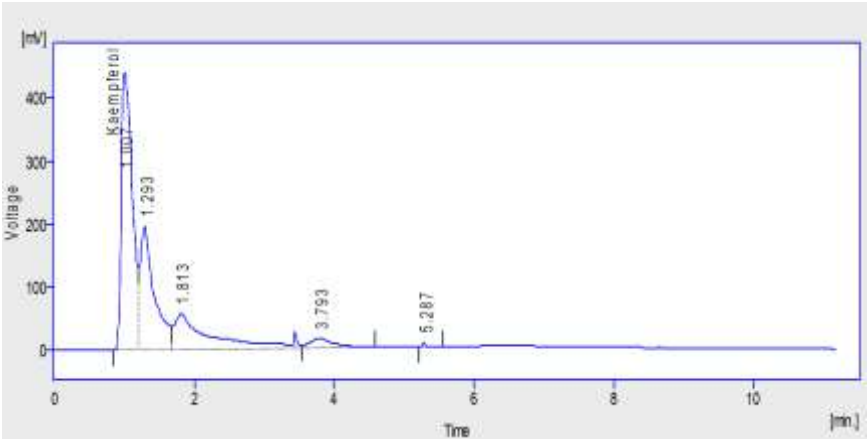

Fig. 2: Chromatogram obtained from *Dodonaea viscosa* n-hexane crude leaf extract

viscosa has significantly caused a reduction in tunnel length and enzyme activities especially mid gut esterases. This property of kaempferol from leaves of *D. viscosa* can be included in termite baiting system.

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