CHAPTER 5

5. MEDICINAL VALUE OF HOST PLANT

5.1 Introduction

Based on the results obtained in Chapter 4, it now makes sense to investigate the host plant rather than thongolifha. The main reasons been that, the plant material were easy to obtain, the methods to analyse those plant material have been already developed and the facilities to perform the analysis in our laboratory are in place. At the end we will be able to determine if any antibacterial compounds isolated from the plant could be present in the insect.

5.2 Results and Discussion

5.2.1 Thin Layer Chromatography analysis

The leaves of *D. viscosa* extracted separately with various solvents (acetone, EtOH, hexane, DCM, and EtOAc). To examine the chemical composition 100 μg of each extract were spotted on TLC plates and developed in different solvent systems. The yields achieved in mg/ml were the following: hexane = 17, dichloromethane = 66, ethyl acetate = 114, acetone = 150 and ethanol =187 (Table 5.1). This indicates that *D. viscosa* contains many polar compounds as there was an increase in the yield with the more polar extractants. The TLC chromatograms revealed that the extracts contain various constituents (Fig 5.1). The CEF solvent system separated more components followed by EMW whereas BEA separated least constituents. This implies that the extracts of *D. viscosa* have more components that are moderately polar (CEF) and polar (EMW) and fewer compounds that are non polar (BEA).

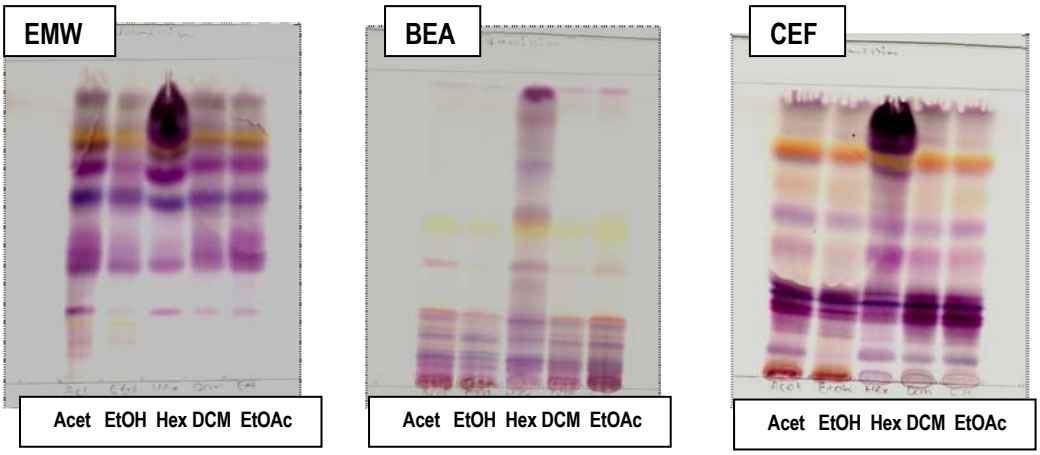

Figure 5.1: TLC chromatograms of *D. viscosa* crude leaf extracts developed in EMW, BEA, and CEF solvent systems. The solvents used for extraction are acetone, EtOH, hexane, DCM and EtOAc.

5.2.2 Bioautography assay

The results in Fig. 5.2 show that, EMW eluent system separated components that are active against *S. aureus*, *E. faecalis* and *E. coli*. On the other hand, *P. aeruginosa* was not growing well on TLC plates however, one component at Rf of 0.79 showed activity (data not shown). A different number of antibacterial compounds were separated by EMW, with acetone (5) >>>>ethyl acetate (4)>>>dichloromethane (3) >>ethanol (4)>hexane (1). Hexane extracts contained few active compounds, thus less activity was observed. Four components were active against *S. aureus* at R_f values of 0.79, 0.75, 0.37 and 0.25 respectively. Two components showed activity against *E. faecalis* at R_f values of 0.78 for compound 1 and 0.74 for compound 2. There was only one compound that was active against *E.coli* at R_f value of 0.85. The TLC chromatogram in Fig. 5.3 shows that the BEA eluent system was also able to separate 4 components that are active against *E.coli* at R_f values of 0.08, 0.15, 0.22 and 0.15 respectively.

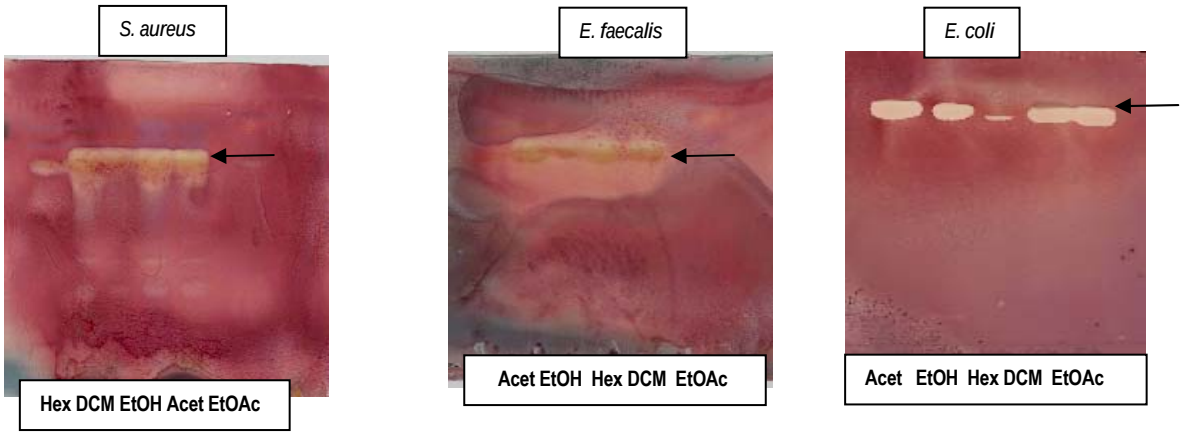

Figure 5. 2: The plant extracts were separately extracted in acetone, EtOH, Hex, DCM and EtOAc. The TLC chromatograms of *D. viscosa* extracts were all developed in EMW solvent system. The extracts (TLC plates from left to right) were tested against *S. aureus*, *E. faecalis* and *E. coli* and clear zones on the chromatograms indicated inhibition of growth.

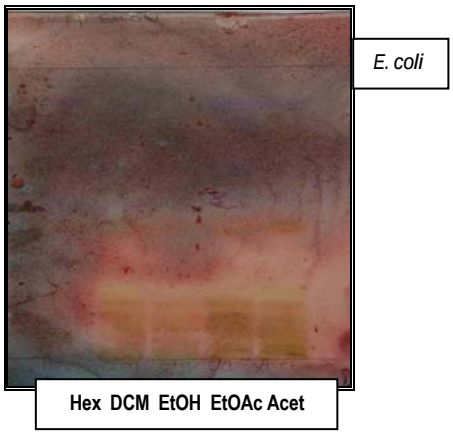

Figure 5.3: The extracts of *D. viscosa* were separately extracted with Hex, DCM, EtOH, EtOAc, and acetone. Bioautography chromatograms of *D. viscosa* extracts were developed in BEA eluent system and components tested for activity against *E. coli.* Clear zone indicate inhibition of growth.

5.2.3 Microdilution assay

The MIC results in Table 5.1 reveal the MIC value (i.e. the lowest concentration of the extract that inhibited growth of bacteria) of hexane extract tested on *E. coli* to be at 0.31 mg/ml. This value of 0.31 mg/ml is the highest inhibitory concentration compared to other values obtained from other four solvents (DCM, EtOH, EtOAc and acetone) that exhibited a lower MIC value of 0.08 mg/ml. This indicates that hexane extracted fewer components that are active against *E. coli*. The most sensitive microorganism tested was *E. faecalis* with the lowest MIC of them all, which is 0.02 mg/ml with acetone extract. Other solvents, that is, DCM, EtOH and EtOAc had similar MIC values of 0.04 mg/ml. Although hexane extract had an MIC value of 0.08mg/ml, the data still indicates that *E. faecalis* is the most sensitive strain among the organisms tested. On average, all solvent extracts of the leaves of *D. viscosa* inhibited growth of both *S. aureus* and *P. aeruginosa* at concentration of 0.28 mg/ml, with *E. coli* at 0.13 mg/ml and the lowest being 0.04 mg/ml against *E. faecalis*.

The results calculated for total activity (TA) are represented in Table 5.2. Eloff, (2000) describes total activity as the volume to which the bioactive compounds that are present in 1 g of the extract can be diluted and still inhibit growth of bacteria. The data for total activity indicates that acetone extract exhibited the highest total activity for all organisms tested ranging from 48.4-750 ml/g (Table 5.2). Hexane extracts showed the lowest total activity for all the bacteria tested ranging from 5.5 -21.3 ml/g (Table 5.2). The highest TA of 750 ml/g was obtained against *E. faecalis* which makes it to be the most sensitive microorganism of them all. This means that 1 g of *D. viscosa* leaf extract can be dissolved in the highest volume (750 ml) of acetone and still inhibit the growth of *E. faecalis*. These results agree with MIC results, whereby hexane has demonstrated higher MIC values of 0.31 mg/ml (Table 5.1) for *E. coli* and *S. aureus* (with TA of 5.5 ml/g, Table 5.2) and the lowest MIC value of 0.08 mg/ml (Table 5.1) for *E. faecalis* (with TA of 21.3 ml/g, Table 5.2).

From the literature, Getie *et al*., (2003) used only methanol extracts to determine the activity of *S. aureus* and *E. coli* by using the agar diffusion technique. Antibacterial activity was observed only with *S. aureus* (8 mm) whereas *E. coli* was resistant. Rojas et al., (1992) also detected antifungal and antibacterial activity of *D. viscosa* extracts from Mexican species with MIC less than or equal to 100 µg/ml. These results differs completely with our quantitative data obtained from MIC and TA which explains a lot about the strength of activity of *D. viscosa* leaf extracts. We have obtained antibacterial activity from all different solvent extracts but low activity was observed from hexane extracts. Furthermore, we have also observed strong activity against *E. faecalis* and *E. coli*, whereas the above authors did not obtain any activity against *E. coli*. The reason for them not obtaining activity from *E. coli* could be that methanol extracted more polar components that did not exhibit activity against *E. coli* as it is a Gram negative microorganism and is prone to resistant. Our data have demonstrated *E. faecalis* to be the most sensitive bacteria followed by *E. coli* then *S. aureus*. Although we were not successful to determine activity with *P. aeruginosa* by using bioautography, the MIC and TA gave us better results. The level of activity of *P. aeruginosa* was at the same level with that of *S. aureus*.

Table 5. 1: MIC values of *D. viscosa* leaf extracts

Solvents	**Amount Extracted (mg/g)**	% Yield	**Minimum Inhibitory Concentration (mg/ml)**				
			E. coli	*S. aureus*	*E. faecalis*	*P. aeruginosa*	**Average**
Hexane	17	1.7	0.31	0.31	0.08	0.16	0.21
DCM	66.2	6.62	0.08	0.16	0.04	0.31	0.14
EtOAc	114.0	11.4	0.08	0.31	0.04	0.31	0.18
Acetone	150	15	0.08	0.31	0.02	0.31	0.18
EtOH	186.5	18.65	0.08	0.31	0.04	0.31	0.18
Average	**106.7**	**10.67**	**0.13**	**0.28**	**0.04**	**0.28**	
Gentamicin			0.8 µg/mL	0.2 µg/mL	6.3 µg/mL	0.8 µg/mL	**2.0 µg/mL**

Table 5. 2: Total activity in ml/g of leaf extracts of *D. viscosa*

Solvent	**TA (ml/g)** *E. coli*	**TA (ml/g)** *S. aureus*	**TA (ml/g)** *E. faecalis*	**TA (ml/g)** *P. aeruginosa*	**Average**
Hexane	5.5	5.5	21.3	10.6	10.7
DCM	82.8	41.4	165.5	21.4	78.1
Ethyl Acetate	142.5	36.8	285	36.8	125.2
Acetone	187.5	48.4	750	48.4	258.5
Ethanol	233	60.2	466	60.2	204.8
Average	**130.3**	**38.4**	**338**	**35.4**	**135.5**

5.2.4 Antioxidant assays

Qualitative assay

The three solvent systems EMW, CEF and BEA separated the antioxidant constituents contained in the extracts (Fig. 5.4). DPPH, a stable purple free radical can be reduced to yellow diphenylpicryl hydrazine. Separated constituents of the *D. viscosa* extracts, turned yellow after spraying with 0.2% DPPH in methanol. The yellow bands on the purple TLC chromatogram background indicated that extracts from *D. viscosa* contains components that have antioxidant activity. All extracts were best separated by BEA solvent system. Most of the components separated by BEA eluent system reacted rapidly and bleached the purple colour background when sprayed with 0.2% DPPH in methanol (Fig. 5.4). The major antioxidant compounds in the extracts appeared at R_f of 0.14 and at the baseline in BEA and 0.81 in EMW eluents. DCM extracted another major component at R_f 0.48.

The results demonstrated that antioxidant compounds of various polarities can be isolated from the leaf extracts especially from ethyl acetate and acetone crude extracts. Analyses of the nature of the active compounds as revealed by vanillin-sulphuric acid spraying reagent demonstrated the presence of phenolic compounds (Stahl, 1969). Several flavonoids such as santin, aharin, penduletin, pinocembrin, 5-hydroxy-3, 6, 7, 4'-tetramethoxyflavone were reported from the genus *Dodonaea* (Sachdev and Kulshreshtha, 1983; Payne and Jefferies, 1973; Dawson *et al.,* 1966). This is in line with the previous study that linked antioxidant activity in higher plants to the presence of polyphenolic compounds (Wangensteen *et al.*, 2004).

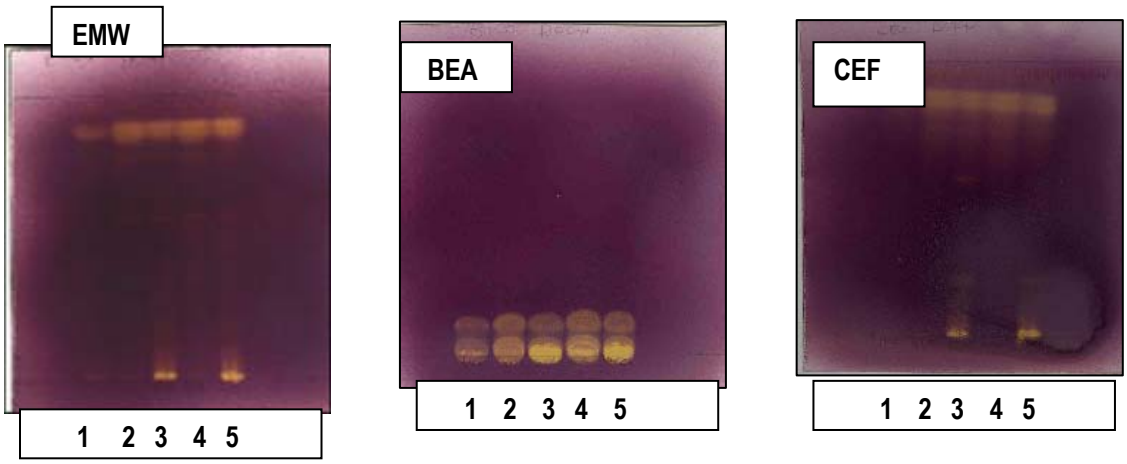

Figure 5.4: The crude extract of *D. viscosa* was separately extracted with Hex, DCM, EtOH, EtOAc and acetone (Lanes 1-5). The extracts were developed in EMW, BEA and CEF solvent systems and the yellow spot indicates components with antioxidant activities.

Quantitative assay

In the quantitative assay, there were big differences in the antioxidant activity of the selected crude extracts. The extracts had moderate activity compared to L-ascorbic acid used as standard which is a pure compound. As expected the more polar solvent extracts (EtOAc and acetone) were the most active (Table 5.3). The EC_{50} indicates extracts that are suitable as sources of potent antioxidant compounds. This is useful for selection of extracts for bioassay-guided fractionation to isolate the antioxidant compounds.

Table 5.3: EC_{50} of antioxidant activity of *D. viscosa* leaf extracts based on DPPH Spectrophotometric assay

Sample	**EC_{50} ± SEM (µg/ml)**
Hexane	NA
Dichloromethane	469 ± 8.8
Ethyl acetate	240 ± 3.4
Acetone	213 ± 4.5
L-ascorbic acid	1.96 ± 0.01

NA- No activity, hexane extract did not inhibit 50% of DPPH free radical even at the highest concentration (500 µg/ml) of the extract tested.

5.3 Conclusion

The leaf extracts of *D. viscosa* possess medicinal properties; the data are comparable with that of Getie *et al*., (2003) where they have reported antibacterial activity against various microorganisms. In our study, *D. viscosa* extracts showed antibacterial activity against E*. faecalis,* E*. coli*, *S. aureus* and *P. aeruginosa*. The antibacterial activity is supported by data of the microdilution assay and the bioautography. The minimum inhibitory concentration value as low as 0.02 mg/ml was obtained. Our findings suggest that the agar diffusion method can not be used alone to make conclusive results about the level of activity of plant extracts. Other method such as the microdilution assay and TA must be employed in order to have conclusive quantitative data. The extracts also had some antioxidative activities, as demonstrated by free radicals scavenging effect on DPPH. The fact that the plant also possesses both antioxidant and antibacterial activities make it a very useful plant, because the same compounds that had antibacterial activity have tested positive for antioxidant activity. This implies that the active constituents possess both antibacterial and antioxidant activity which may lead to an increase of activity by direct or indirect effects. Our results suggest that *D. viscosa* extracts could be useful in therapeutic treatment, but this has to be substantiated by *in vivo* experiments. Both antibacterial and antioxidant components were isolated using bioassay guided fractionation, details in chapter 6.