# *C H A P T E R 3*

## 3. NUTRITIONAL VALUE *OF E. DELEGORGUEI*

### 3.1 Background

In Thoyandou region of Limpopo Province of South Africa; traditions, beliefs, myths, habits and customs are still deeply rooted among the Venda people. The consumption of various insects such as grasshoppers, termites, mopane worms and stinkbugs is a tradition and custom that persists. Edible insects are harvested and sold at the open markets around Thoyandou. The edible stinkbug, *Encosternum delegorguei* Spinola Tessaratomidae (Order Heteropreta) is one of the most important insects in South Africa from a cultural point of view. Thongolifha is consumed by the va-Venda tribe in Thoyandou of the Limpopo Province. The stinkbug, which is popularly known as thongolifha, it is in great demand at the Thoyandou market although it emits a very strong odour. Among the Venda tribe, young and old are fond of the thongolifha and they eat it raw or cooked either with porridge or alone (Faure, 1944). The harvesters travel to other areas such as Modjadji village that is about 200 km from Thoyandou to collect them. The insect is also imported from southern African countries such as Mozambique and Zimbabwe as the local supply can not meet the demand. During our field trips we have observed that, thongolifha feeds on several plants including *Dodonaea viscosa* and *Diospyros mespiliformis.* These two host plants have well-established antimicrobial value (Getie *et al*., 2003; Adzu *et al*., 2002). Thongolifha is probably an important diet supplement and it was therefore important in this study to analyse its chemical composition for nutritional supplement because this has not yet been done before.

#### 3.1.1 Morphology of the thongolifha

The body is c. 25 mm in length, green-yellow or brown (Fig. 1) with lateral margins of abdomen not exposed as in similar *Natalicola* species. Nymphs are circular and green. Their habitat is subtropical and open woodland and bushveld (Picker *et al*., 2000; Faure, 1944).

Thongolifha is mainly harvested during winter season either at sundown or early in the morning or better still on misty, cloudy days. At these times the insects are cold and less active and congregate in clusters, possibly to conserve heat. They fly in swarms on hot days and settle in the evening on evergreen plants such as *D. viscosa* and *D. mespiliformis*. The bug is scarce during summer or wet seasons. It is believed that they congregate and lay their eggs during spring and the development of nymphs will then occur in summer (Toms and Thagwana, 2003).

**Figure 3.1:** *E. delegorguei* settling on *D. viscosa* and on a paper (Photograph- Dr R.Toms).

### 3.2 Results and Discussion

Available literature on nutritional values of insects is scarce; but indicates that insects have high protein, energy, mineral and vitamin content as well as low to high fat content. The results for macro nutrient composition of thongolifha (*E. delegorguei)* are represented in Table 3.1. Thongolifha in this study had a protein content of 35.5%. The fat content of the thongolifha was 50.6%. This is not surprising since during processing of the bug; fat can be seen floating on the water. Calculated carbohydrate content was 7.63 g/100g. The energy value of 2599 kJ/100g is acceptable and comparable with other edible insects (Table 3.6). High energy content based on carbohydrates and fats is very important in foodstuffs because it complements diet and helps in complete utilization of protein thereby increasing nutrition significantly. Vitamins A, $B_1$, $B_2$, and E were detected at reasonable concentrations of 0.23, 0.63, 0.86 and 2.17 mg/100 g respectively (Table 3.2) with recommended daily allowance of 1.7-8 mg/day.

Essential amino acids were also detected, the concentration of essential amino acids varied from 0.82 mg/100 g (threonine) to 1.32 mg/100g (valine). Other essential amino acids were also present in reasonable concentrations (Table 3.4). The percentage of different essential amino acid of thongolifha protein (Table 3.5) was compared to that of beef and chicken meat (Beach *et al*., 1943) The data has indicated that although the percentage of methionine and lysine for thongolifha has scored low values compared to beef and chicken, however the values for phenylalanine, tyrosine, threonine and tryptophan are closer to that of beef and chicken. This implies that thongolifha protein quality is not nearly as good as that of beef and chicken and therefore consumption of thongolifha will provide to a degree for most essential amino acids.

Important minerals diet supplements such as iron, potassium, phosphorus and selenium were also found at high concentrations (Table 3.3). Total minerals content was at 1.2 g/100g which is at comparable levels to other bugs such as *Acantocephala declivis* (1.0 g/100g). The data about the nutritional content of thongolifha has confirmed that consumption of these bugs by the vhaVenda tribe is not just a traditional delicacy but it provides them with good source of protein, various minerals and essential amino acids

The nutritional contents of the most widely consumed insects and other animal food has been determined and their values are compared in Table 3.6. The protein content varied from as low as 31% (wasp) to as high as 63.5% for mopane worm. Because of mopane worm's high protein content, it is used in health care whereby it is crushed and mixed with porridge to cure children with kwashiorkor. Termite and thongolifha have high fat content compared to other insects and they present with high energy content when consumed. From the data in Table 3.6, it is evident that insects have higher protein content than animal food. Insects are comparable with expensive fish in their protein content. Insects can be a cheaper sources of protein, so people should be encouraged to accept them as a respectable food item based on their nutritional and economic implications.

Some of the insects that are regarded as food are sometimes available in abundance and could be regarded as pests by farmers. Widespread harvesting and consumption of insect might serve as a form of biological control of these pests. Harvesting of insects for consumption will results in reduction in the use of pesticides as well as creating new economic opportunities for indigenous people. However, public education is vital if we need to preserve safe insects foods, the subject can also be incorporated into school curriculum for young people to learn and appreciate insects as food as they grow up. Harvesters must also be taught to look after the forest in order to preserve insects. Communities must be encouraged to manage the forest very wisely thereby enhancing the sustainable harvesting of plants and insects.

Not all insects are edible, some are toxic and may create allergy problems but the ones that are edible should be looked after and their host plants must be preserved. Some toxic insects may be used in medicine and thongolifha may have some toxic elements as it was mentioned earlier in chapter 1 that its host plant *D. viscosa* contain some toxic substances. Traditional preparation of insect should also be improved to avoid contamination and wastage, thereby providing for a high quality and acceptable product. As people are encouraged to eat insects, the subject of food safety should be looked into. Food contamination is now regarded as a serious public health problem worldwide. Insect are mostly collected by rural people and in rural areas, there is problem of food hazard, which is linked to poor sanitation, lack of water supply food storage, and marketing of food that includes mainly vending site. At Modjadji, an important village were thongolifha is harvested, all these health hazards exist and one cannot guarantee that the end product is always safe. In reality, many insects are far cleaner than other creatures, for example, grasshoppers and crickets eat fresh clean plants whereas crabs, lobster and pigs (that many people enjoy eating) eat any kind of decomposing material as a scavenger. The other important factor about insects is that, by weight insects such as termites, grasshoppers, caterpillars are a better source of protein than beef, chicken, pork or lamb (Lyon, 2005).

If one evaluates the number of thongolifha that have to be consumed per day to provide in the daily required minimum for essential amino acid (Table 3.4) the number varies from 680 for phenylalanine to 3 400 for methionine.

**Table 3.1:** Macro Nutrient composition of *E delegorguei* (g/100 g edible weight)

| Protein | Fat | Energy (kJ/100 g) | Carbohydrate (g/100 g) | Moisture | Dry matter | Ash |
|---|---|---|---|---|---|---|
| 35.2% | 50.5% | 2599 | 7.63 | 4.9% | 95.1% | 1.7% |

**Table 3.2:** Vitamin content of *E. delegorguei* (mg/100g)

| Vit A mg/100 g | Vit $B_1$ mg/100 g | Vit B2 mg/100 g | Vit C mg/100 g | Vit E mg/100 g |
|---|---|---|---|---|
| 0.23 | 0.63 | 0.86 | Not detected | 2.17 |

**Table 3.3:** Mineral content of E. *delegorguei* (mg/100 g)

| Chloride | Sodium | Copper | Sulphate | Calcium | Magnesium | Potassium | Phosphorus | Iron | Selenium | Manganese | Zinc |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 85.4 | 55.3 | 4.4 | 66.7 | 91 | 109 | 275 | 575 | 20.2 | 0.2 | 0.8 | 4.6 |

**Table 3.4:** Essential amino acid profile of *E. delegorguei* and daily requirement

| Essential Amino Acids | Amino acid content of *E.delegorguei* (mg/100g) | Daily minimum requirement (mg) | Mass (g) required for daily minimum |
|---|---|---|---|
| Isoleucine | 830 | 450-700 | 54 |
| Leucine | 1050 | 620-1100 | 59 |
| Methionine | 400 | 550-1100 | 137 |
| Phenylalanin | 810 | 220-1100 | 27 |
| Tryrosine | 1260 | 900-1100 | 71 |
| Threonine | 820 | 310-500 | 37 |
| Tryptophan | 160 | 160-250 | 100 |
| Valine | 1320 | 650-800 | 49 |
| Lysine | 850 | 500-800 | 58 |

**Table 3.5:** Percentage of protein of different essential amino acid of *E. delegorguei* compared to that of beef and chicken.

| **Essential amino acids** | **Methionine** | **Phenylalanin** | **Tyrosine** | **Threonine** | **Tryptophan** | **Lysine** |
|---|---|---|---|---|---|---|
| **E. delegorguei** | 1.1 | 2.3 | 3.5 | 2.3 | 0.4 | 2.4 |
| **Beef** (Beach *et al*., 1943) | 3.1 | 4.9 | 4.3 | 4.5 | 1.4 | 8.1 |
| **Chicken** (Beach *et al*., 1943) | 3.2 | 3.8 | 4.2 | 4.6 | 1.3 | 8.4 |

**Table 3.6:** Nutritional components of some of the commonly consumed insects and other mammals based on 100g serving**.**

| **Species** | **Protein (%)** | **Energy K.cal/100 g** | **Minerals (g/100 g)** | **Carbohydrates (g/100 g)** | **Fat (%)** | **Reference** |
|---|---|---|---|---|---|---|
| Thongolifha (*E. delegorguei*) | 35.2 | 2599 | 1.2 | 7.63 | 50.5 | Chapter 3 results |
| Termite (*Macrotermes falcigen)* | 41.8 | 7611 | 0.75 | N/A | 44.3 | Phelps *et al*., 1975 |
| Beetles (*Callipogon barbatus*) | 41.5 | 474 | 2.1 | 23.2 | 34.8 | Ramos-Elorduy, 1997 |
| Mopane worm (*Imbrasia belina*) | 63.5 | 543 | 3.5 | 11.4 | 18 | Dreyer and Wehmeyer, 1982 |
| Wasp (*Polistes instabilis*) | 31.0 | 655 | 2.1 | 3.0 | 62 | Ramos-Elorduy, 1997 |
| Larvae *(Apis mellifera)* | 42.0 | 475 | 3.1 | 1.0 | 19.1 | Ramos-Elorduy, 1997 |
| Bug (*Acantocephala declivis*) | 35.1 | 547 | 1.0 | 18 | 45.3 | Ramos-Elorduy, 1997 |
| Grasshoppers/locust (*Sphenarium histrio*) | 77.2 | 363 | 2.1 | 12.4 | 12.0 | Ramos-Elorduy, 1997 |
| Beef | 27.4 | 219 | 3.5 | *N/A | *N/A | Lyon, 2005 |
| Fish | 28.5 | 170 | 1.0 | *N/A | *N/A | Lyon, 2005 |

*N/A- results not available.

### 3.3 Conclusion

Thongolifha is not just a traditional food; it has high protein content. It is therefore, recommended as good source of protein and people should be encouraged to consume it. However, sustainable harvesting is recommended in order to maintain availability of stink-bugs. The harvesters from Venda are already traveling as far as Modjadji area to collect this traditional delicacy. Thongolifha is not only a traditional food but many families rely on the sale of the insect to maintain their standard of living. For these reasons sustainable harvesting and preservation is vital. Most widely consumed insects have been proven to have high protein content. This will ultimately encourage people to consume insects, especially if they know that they are acquiring high quality protein for less cost.

The large number of insects required to satisfy the daily required essential amino acids indicates that insects can only be an additional food source and would not easily provide all the protein requirements.

CHAPTER 4

# 4. MEDICINAL VALUES OF INSECTS

## 4.1 Background to use of insects

### 4.1.1 Use of insects in Folk Medicine

Entemotherapy, which is the use of Insects and insects' derived products in medical systems have been used by different human cultures throughout the world for centuries. Insects are used live, cooked, ground, in infusions, in plasters, and as ointments (Costa-Neto, 2002). For example the Maya were already using maggots for therapeutic purposes a thousand years ago (Zimmer, 1993). In Brazil, about 42 insects species were reported to have been used in folk medicine (Costa-Neto, 2002) and in India, the use of insects in folk medicine is also common (Hitchcock, 1962). Different species of insects are used as remedies. The mud wasps, leaf-cutting ants, cockroaches, termites and crickets are mainly used to treat asthma, bronchitis and sore throat. They are usually crushed and made into tea which is drunk three times a day and the patient is not supposed to know what they are drinking (Costa-Neto, 2002) In order to treat stroke, insects such as dung beetle, coconut borer, stingless bee, grasshoppers are burned and patient must inhale the smoke (Costa-Neto, 1994, Lima, 2000). The larvae of coconut borer are fried in their own fat in order to extract oil that is used as a treatment for dandruff (Costa-Neto, 1994).

Herbalists from Feira de Santana prescribe the sting of honey bee, *Apis melifera scutellata* for the treatment of rheumatism, backache and joint problems. In India the Pankarare people use honey bee to cure whooping cough and tuberculosis, and the Ichu people eat it for curing ulcers. The Remanso community use honey bee to cure cough and diabetes (Costa-Neto, 2002). The mud wasp is used by the Pankarare community to treat mumps, whereby the nest is melted in water and the mixture is applied on mumps, they also use honey of stingless bee as an antidote against snakebites (Costa-Neto, 1994, Lenko and Papavero, 1979). Honey is used as an eye drop to treat cataract and glaucoma by the rural people of Tanquinho and Serrinha. In the city of Itaberaba, tea of toasted ant is given to a child to make the child to stop wetting the bed (Costa-Neto, 2002).

In the city of Feira de Santana, the cockroach is cooked and its tea is drunk in order to treat heartburn, on the other hand the people from Matinha dos Pretos put the exoskeleton of a cockroach over wounds to promote their scarring. The sting bug, *Tritoma sp*. is used mainly to treat toothache and earache (Costa-Neto, 1994). The bumble bee, (*Bombus sp*), is used as love charms (Lenko and Papavero, 1979).

Furthermore, insects and other anthropods are used in Korean traditional medicine. The centipede (*Scolopendra sp.*) is used primarily to treat arthritis because centipedes have so many legs it is believed to cure leg problems. The silk moth fungus (*Beauveria bassiana*, which infects silk moth larvae) is used mostly to treat stroke (Pemberton, 1999). In Australia, the Aborigine community use insects such as bush cockroach (Blattidae) for local anaesthetic, they use the green tea ant (Formicidae) to prepare a refreshing drink, cure headaches, as a cold remedy, as an antiseptic and expectorant. The caterpillar is used for wound dressing and honey bees are used to treat sore throat (Cherry, 1993).

#### 4.1.2 Insects as source of new drugs

Insects are known to sequester compounds from host plants and store them for their defense mechanism (Harborne, 1993). This phenomenon has developed much interest in searching for novel drugs derived from insect components. Insects have thus far proven to be promising new source of drugs in modern medicine since they possess immunological, analgesic, antibacterial, diuretic, anaesthetic and antirheumatic properties (Yamakawa, 1998). Chemical screening of 14 species of insects has confirmed the presence of proteins, terpenoids, sugars, polyols and mucilages, saponins, polyphenolic glycosides, quinones, glycosides and alkaloids (Andary *et al*., 1996).

Chitosan which is a compound derived from chitin has been used for various purposes: as an anticoagulant, for lowering serum cholesterol levels; to repair tissues and even used to fabricate contact lenses (Goodman, 1989). Chitosan is used widely for various purposes such as pesticides (Cohen, 1993; Watkins et al., 2002), and can also be used for decreasing skin irritation caused by shaving (Lazarowitz and Morris, 2004). Chitosan and chitosan derivatives were found to posses high insecticidal and fungicidal activity tested against cotton leaf worm *Spodoptera littoralis*, the grey mould *Botrytis cinerea* and rice leaf blast *Pyricularia grisea* Cavara (Rabea *et al*., 2005; Badaway *et al*., 2005). Ethanolic extracts of propolis of *Apis mellifera* collected from Brazil have shown anticancer and anti-HIV properties (Park 2000). Kunin and Lawton, (1996) have developed promising anticancer drugs, isoxanthopterin and dichostatin which were derived from the wings of Asian sulphur butterflies (*Catopsilia crocale*, Pieridae) and from the legs of Taiwanese stag beetles (*Allomyrina dichotomus*, Scarabaeidae) respectively.

Because our group has expertise in antibacterial natural products, I decided to evaluate thongolifha and *D. viscosa* for antibacterial activity against the four most important nosocomial pathogens (*S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa*).

### 4.2 Results and Discussion

#### 4.2.1 Thin Layer Chromatography analysis

Serial extraction using hexane, DCM, acetone and methanol was performed as outlined in Chapter 2 section 2.3.1. Various extracts were loaded on TLC plates (10 μl of 10 mg/ml) and separated using EMW, CEF and BEA eluent systems. After spraying with vanillin-sulphuric acid, various separated components were observed. Various components of different Rf values both from the plant (*D. viscosa*) and the insect *(E. delegorguei)* were separated (Fig. 4.1). When duplicate chromatograms were sprayed with ninhydrin- ethanol in 0.2% acetic acid only at the origin was any positive bands for amino acid detected (results not shown). The results demonstrated no similarity in components extracted from the insect and the plant. Developed TLC plates by EMW system revealed major plant components of Rf 0.49, 0.59 and 0.68 whereas the major components of the insect had Rf 0.46 and 0.61. CEF eluent system was able to separate major components of the plant at Rf 0.52, 0.59, and 0.69 and for insect separated components were at Rf 0.79 and 0.66. For non-polar eluent system, BEA, major components of the plant had Rf of 0.3, 0.46 and 0.46 whereas the insect had Rf 0.57 and 0.44. These Rf values obtained from both the plant and insect indicate that the major components separated by the three eluent system are completely different from each other. Although a completely different matrix was present in the insect extracts, it was satisfying that the methods developed for plants extracts gave reasonable results with the insect extracts. If the insect had sequestered any components from the plant, it may be present in lower quantities that were not possible to detect by our TLC method. Alternatively the insect could have metabolized the structure of the compounds resulting into new compounds.

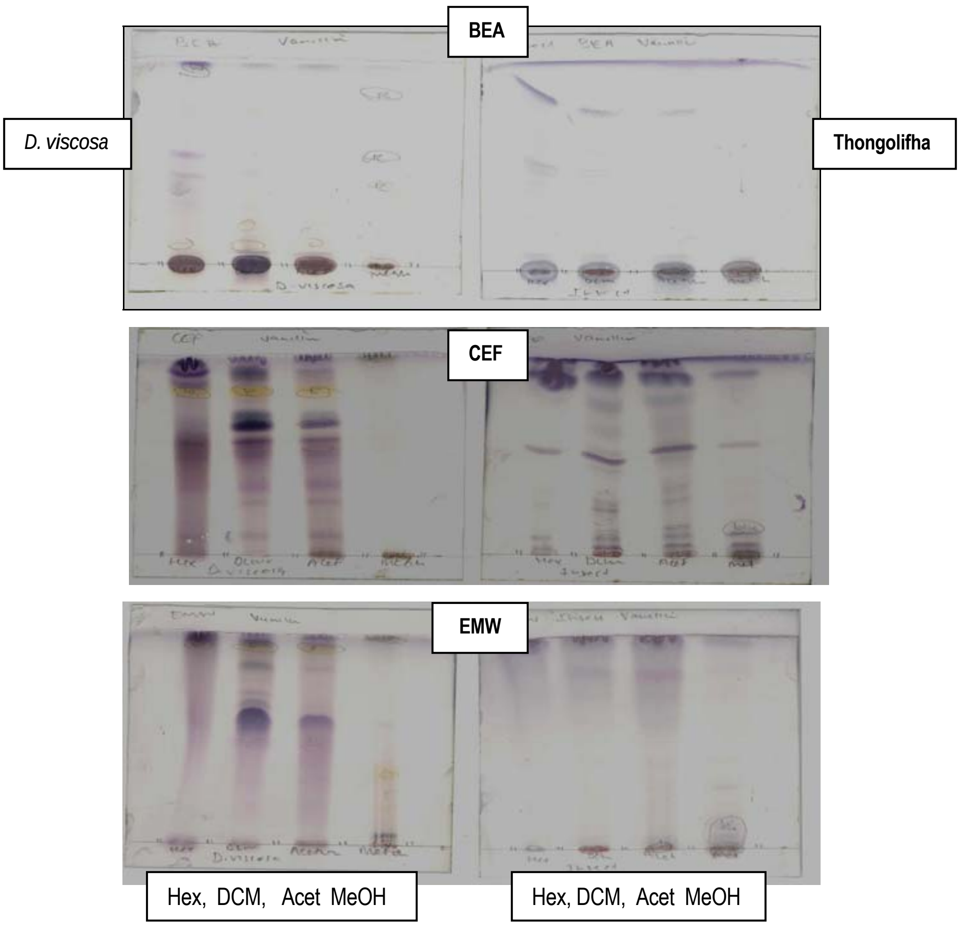


**Figure 4.1:** TLC chromatogram of *D. viscosa* (L) and thongolifha (R) serially extracted with hexane, DCM, acetone and MeOH followed by spraying with vanillin in sulphuric acid. The TLC plates were developed in BEA, CEF and EMW.

### 4.2.2 Bioautography assay

The component at Rf of 0.82 exhibited a bactericidal effect against *E. coli* (Fig. 4.2). The EMW, CEF and BEA solvent systems separated insect extracts components that are susceptible to S. *aureus* at Rf 0.79 and 0.67 (EMW); Rf 0.79 and 0.66 (CEF) and Rf 0.72 and 0.37 (BEA). It is possible that the two major components exhibiting antibacterial activity against *S. aureus* are the same in all the three eluent system. Furthermore, the most active components against *E. faecalis* were only separated by CEF solvent system at Rf 0.66, and the same component was also active against *S. aureus*. The two Gram negative bacteria, *E. coli* and *P. aeruginosa* were resistant to the insect extract.

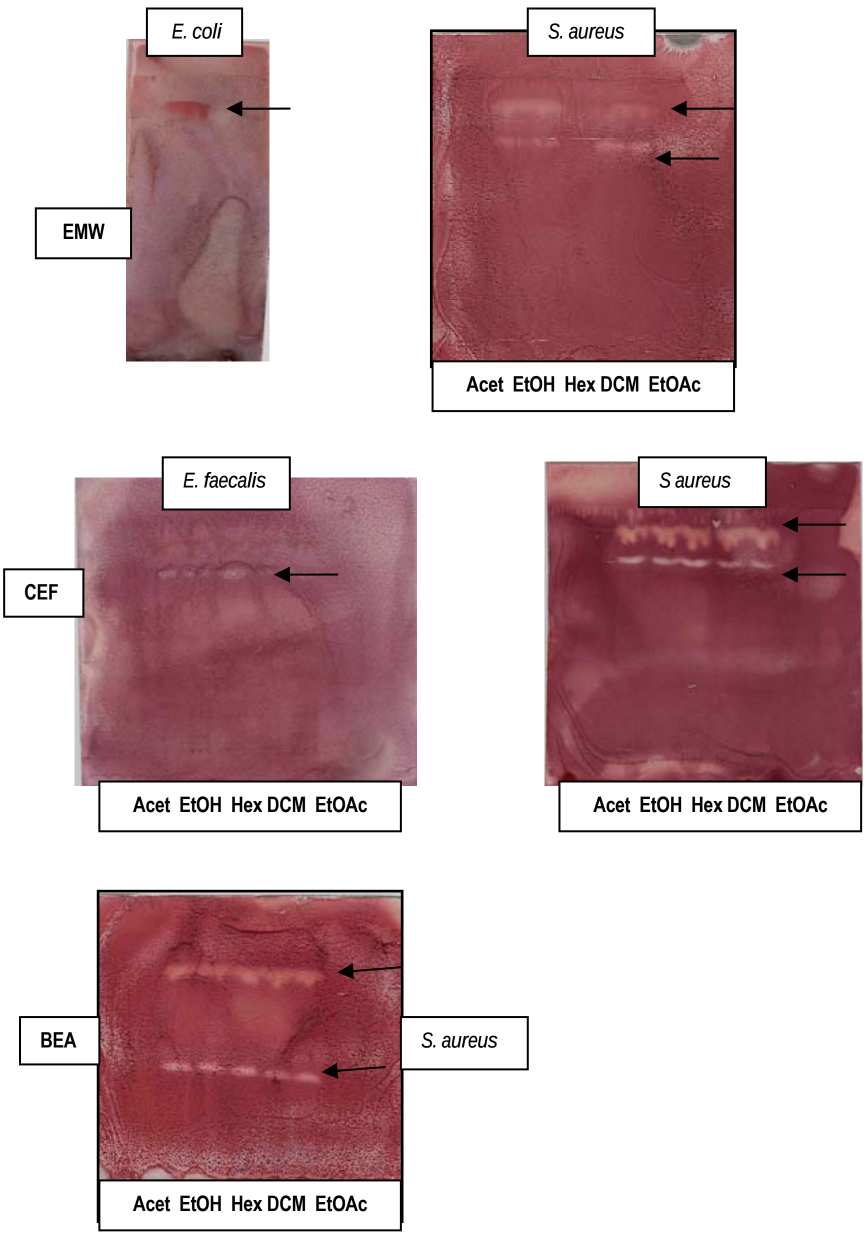


**Figure 4.2:** Bioautograms of *E. delegorguei* extracts against *E. coli, S. aureus* and *E faecalis*. The clear bands on the chromatograms show an area where the compound is active against the test microorganism.

### 4.2.3 Microdilution assay

All the extracts have showed the MIC value of >2.5 mg/ml against all four test microorganisms, *S. aureus, E. faecalis*, *E. coli* and. *P. aeruginosa,* indicating that the insects extracts have no antibacterial activity.

## 4.3 Conclusion

Based on the bioautography results there were at least three compounds active against one or more of the nosocomial pathogens in the thongolifha extracts (Fig.4.2). The MIC values indicated that the antibacterial activity was very low. This may be an artefact of the serial dilution microplate method used, because this method has not yet been tested using insects or animal extracts. Based on the relatively high Rf values found with the different solvent systems used, it is likely that the antibacterial compounds are not peptides, which would probably have been retained much stronger on silica gel due to its polar characteristics. This conclusion was confirmed when only compounds at the origin reacted positively with the amino acid/peptide ninhydrin-ethanol spray reagent.

It appears that the antibacterial compounds separated by CEF (Fig. 4.2) reacted positively with vanillin-sulphuric acid spray reagent (Fig. 4.1) again indicating that the antibacterial compound is possibly a flavonoid or a terpenoid (Fig. 4.1) and not a peptide.