EVALUATION OF THE *IN VITRO* ACARICIDAL PROPERTIES OF THE LATEX AND MAJOR COMPOUNDS ISOLATED FROM *THE LEAVES OF ALOE YAVELLANA* (REYNOLDS) AGAINST *AMBLYOMMA VARIEGATUM* (IXODIDAE TICKS)

Tibebu Hailesillassie

A Thesis Submitted to the Department of Pharmaceutical Chemistry and Pharmacognosy in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacognosy

Addis Ababa University

Addis Ababa, Ethiopia

December 2017

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School of Graduate Studies

This is to certify that the thesis prepared by Tibebu Hailesillassie, entitled*:* "Evaluation of the *In Vitro* Acaricidal Properties of the Latex and Major Compounds Isolated from the Leaves of *Aloe yavellina* (Reynolds) Against *Amblyomma variegatum* (Ixodidae Ticks)" was submitted in partial fulfillment of the requirements for the Degree of Master of Pharmacy in Pharmacognosy with the regulation of the university and meets the accepted standards with respect to the originality and quality.

Signed by the Examining Committee:

External examiner Dr. Tadesse Eguale	Signature _____Date_____
Internal examiner Mr. Biniam Paoulos	Signature_____ Date_____
Professor Kalab Asres (Advisor)	Signature_____ Date_____
Dr. Daniel Bisrat (Advisor)	Signature_____ Date_____

ABSTRACT

Evaluation of the *in vitro* acaricidal properties of the latex and major compounds isolated from *the leaves of Aloe yavellana* (Reynolds) against *Amblyomma variegatum* (ixodidae ticks).

Tibebu Hailesillassie

Addis Ababa University, 2017

Aloe yavellana Reynolds is endemic to Ethiopia where its leaf latex is traditionally used for the treatment of various illnesses of humans and domestic animals in Yabelo town and other pastoralist areas of south western part of the country. The latex and isolated compounds were assessed for their acaricidal activities against *Amblyomma varigatum* tick larvae by using larval packet test (LPT). At a concentration of 50 mg/ml, the leaf latex showed acaricidal activity 24 h post exposure, with percentage mortality (E %) of 62.50%. Phytochemical investigation of the leaf latex led to the isolation of two anthrones, identified as microdontin A/B and aloin A/B by means of spectroscopic techniques including ESI-MS, ^{1}H, ^{13}C NMR and DEPT spectral data. Among the isolated compounds, microdontin A/B showed E% of 30.83 at a dose of 50 mg/ml. EC_{50} and EC_{99} values of the latex, microdontin A/B and aloin A/B were estimated to be 35.82 and 83.48 mg/ml; 89.40 and 196.49 mg/ml; 257.69 and 585.98 mg/ml, respectively. On the basis of the above results the latex was found to be more effective in killing larvae ticks than the individual isolated compounds. Dose response data of the latex, microdontin A/B and aloin A/B indicated the gradual increase in mortality pattern with slopes and R^2 values of 1.047 and 0.909; 0.459 and 0.946; 0.164 and 0.988, respectively. In conclusion, the leaf latex of *A. yavellana* and its isolated compounds could have the potential to be used as bioacaricides against ticks and tick born disease (TBDs).

Key words: *Aloe yavellana* latex; Aloaceaae; *Amblyomma varigatum*; anthrones; microdontin A/B; aloin A/B.

Acknowledgements

Above all I thank the almighty GOD and St. Mary for giving me the strength throughout my study. My sincere gratitude goes to my supervisors Professor Kaleab Asres and Dr. Daniel Bisrat for their guidance, cooperation and encouragement throughout the course of this research work. I am indebted to Dr. Solomon Gebre, National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia, for providing me tick rearing facilities in the Acarolgy Laboratories. I would like to sincerely thank the academic and technical staff of the Department of Pharmaceutical Chemistry and Pharmacognosy, School of Pharmacy, Addis Ababa University for the assistance accorded to me whenever I needed it. My profound appreciation is also forwarded to Ato Hailemeskel Meshesha, for his Valuable assistance in Pharmacognosy laboratory. My heartfelt thanks go to all my lovely family who understood the importance of education and always worked hard to educate me. I extend my sincere thanks to my companion Ato Habtamu Kifle for his remarkable support.

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List of abbreviations

^{13}C NMR:	Carbon Thirteen Nuclear Magnetic Resonance
^{1}H NMR:	Proton Nuclear Magnetic Resonance
AAU:	Addis Ababa University
DMSO:	Dimethyl sulphoxide
DEPT:	Distortion Enhancement by Polarization Transfer
DPPH:	2, 2-Diphenyl-1-picrylhydrazyl
EATC:	Ehrlich Ascites Tumor Cells
ESI-MS:	Electro Spray Ionization Mass Spectrometery
FAO:	Food and Agricultural Organization
FEE:	Flora of Ethiopia and Eritrea
GC:	Gas Chromatography
HPLC:	High performance Liquid Chromatography
ICTTD:	Integrated Consortium on Tick and Tick Born Diseases
IR:	Infrared
LC/MS:	Liquid Chromatography/Mass Spectrometry
LPT:	Larval Packet Test
MIC:	Minimum Inhibitory Concentration
MoARD:	Ministry of Agriculture and Rural Development
NAHDIC:	National Animal Health Diagnostic and Investigation Center
PGE_2	Prostaglandin E_2
PTLC:	Preparative Thin Layer Chromatography
SNNPR:	Southern National Nationalities and People Regional state
TBDs:	Tick Born Diseases
TLC:	Thin Layer Chromatography
TMS:	Tetramethylsilane
USDA:	United State Development Agency
UV:	Ultraviolet

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1. INTRODUCTION

1.1 Ticks

Ticks are blood feeding external parasites of animals that belong to the phylum Arthropoda in the order Acari (Rajput *et al.*, 2006; Barker and Walker, 2014). There are two main families of ticks, namely Argasidae (argasids) and Ixodidae (ixodids). There is also the family Nuttalliellidae, represented by the mono specific genus *Nuttalliella*, which lives in Africa (Parola and Raoult, 2001). Argasids are often called "soft ticks" because they do not have hard plates on their bodies, while ixodids are commonly referred to "hard ticks" because they have these hard plates (Walker *et al.*, 2003).

1.2 Economic importance of tick

Although ticks have been known since biblical times, it was not until the second half of the 19th century, when the world cattle population increased rapidly to feed the expanding human population that the importance of disease which they transmit and their serious debilitating effect on cattle become apparent (Gebre and Kaaya, 1998). Among major problems of diseases in livestock, ticks and tick-borne diseases are widely distributed throughout the world particularly in tropical and sub-tropical countries which cause a tremendous economic importance in livestock production. It has been estimated that 80 percent of the world's cattle population is exposed to tick infestation (FAO, 1984).

Ticks cause substantial losses in cattle production, in terms of diseases, reduced productivity and fertility and often death, and are economically the most important ectoparasites of cattle (Rajput *et al.*, 2006). Ticks suck blood; damage hides and skins introduce toxins and predispose cattle to myiasis and dermatophilosis (Wall, 2007). Furthermore, they reduce body weight gains and milk

yield, in addition to creating sites for secondary invasion by pathogenic organisms (Kaufman *et al.,* 2006). More significantly, ticks transmit diseases from infected cattle to healthy ones. Ticks transmit a greater variety of pathogenic micro-organisms than any other arthropod vector group, and are among the most important vectors of diseases affecting animals (Jongejan, 2007). Although species of ticks and tick born diseases (TBDs) differ among ecological regions, their impact on animal production is important wherever they occur. The most economically important genera of tick-borne prokaryotic and eukaryotic haemoparasites infecting cattle are the rickettsiae: *Anaplasma* and *Ehrlichia* (*Cowdria*), and the protozoan parasites: *Babesia* and *Theileria* (Jongejan *et al.,* 1986; Jongejan and Uilenberg, 1994). The problem is severe in developing countries where the resource for control and eradication is very limited.

In Ethiopia, ticks occupy the first place amongst the external parasites, and the economic loss incurred when they infest livestock particularly cattle is enormous (Gebreab, 1983). Moreover the misuse of acaricides and chemicals has induced the development of resistance in ticks (Ali and De-Castro, 1993; Gebre and Kaaya 1998) and has caused poisoning in both human and animals (FAO, 1998). The availability of chemicals and money to purchase such product is also frequently uncertain in remote areas (Latif and Jungian, 2002). Ticks cause substantial losses in cattle production, in terms of diseases, reduced productivity and fertility and often death, and are economically the most important ectoparasites of cattle (Ahmed, 2016). In Ethiopia, the major tick genera recorded are *Amblyomma*, *Boophilus*, *Haemaphysalis*, *Hayalomma*, and *Rhipicephaus*; and the major cattle tick born diseases are anaplasmosis, babesiosis, cowdriosis, and theileriosis (Ahmed, 2016).

1.3 Life cycle of ticks

Depending on the type of host, larvae develop in the eggs until they are ready to hatch, usually from few to several weeks. Larvae feed once on a host, then detach from the host and hide in the physical environment such as soil or vegetation. Larvae then moult to nymphs. Nymphs feed once and moult in the same way as larvae. From nymphal moult, either a female or male emerges. The female feeds once, lay a batch of eggs, then dies. The males may take several small feeds, mate repeatedly, and then die. Ticks that have recently hatched from eggs or from moulting have soft bodies and are inactive for one to two weeks whilst the external body wall hardens. Depending on their varieties of life histories with respect to optimizing their chance of contact with appropriate host to ensure survival, ticks can be one host ticks, two host ticks and three host ticks (Barker and Walker, 2014).

1.3.1 One-host tick

A one-host tick requires only one host to complete life cycle (Figure 1). Eggs are laid on soil. Larvae hatch after several weeks of development and crawl onto vegetation to quest for a host. Moulting between stages that is larvae - nymph and nymph - adult takes place on one host. *Boophilus* species are typical one-host ticks. After the female drops from the host, she seeks a sheltered place for oviposition, where she lays a single batch of several thousand eggs and then dies. In one host ticks, the nymphs also remain on the same host and continue to feed as adults (Hoogstraal, 1956).

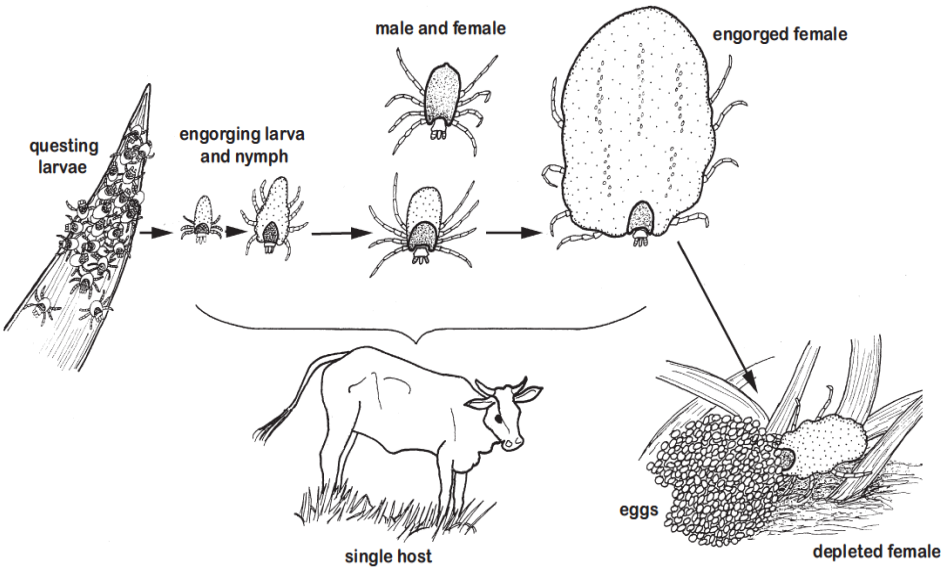

Figure 1. One-host tick life cycle (eg. *Rhipicephalus decoloratus*) (Walker *et al.*, 2003)

1.3.2. Two-host ticks

Two-host ticks require two hosts to complete their life cycle (Figure 2). The larvae and nymphs of a two-host tick feed on the same individual host, and the adults feed on another host. Two-host ticks include *Rhipicephalus bursa* and some *Hyalomma* species.

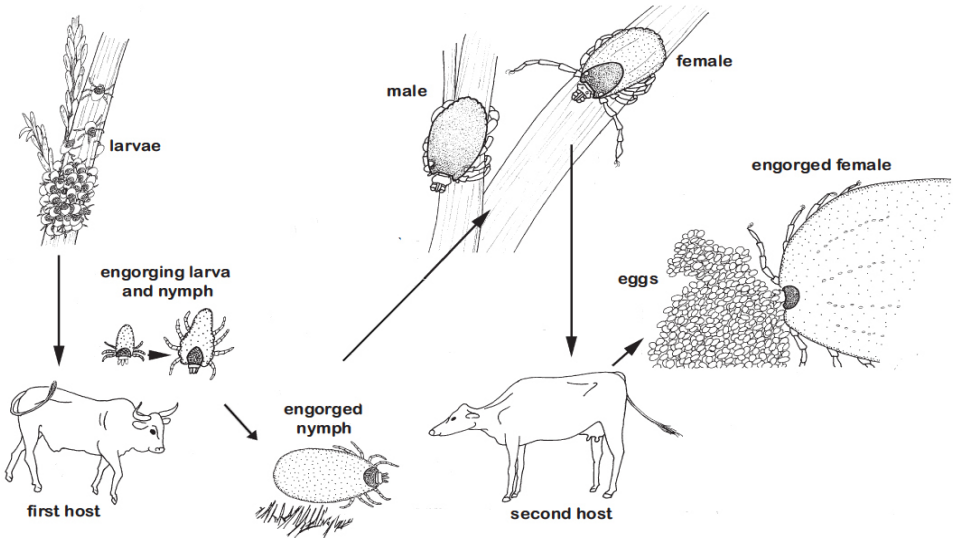

Figure 2. Two-host tick life cycle (eg. *Rhipicephalus bursa*) (Walker *et al.*, 2003)

1.3.3 Three-host ticks

A three-host tick requires three hosts to complete the life cycle (Figure 3) and each instar requires its own single host (Walker *et al.*, 2003). Each instar seeks out of a host, attaches, feeds to full engorgement and then drops off. After dropping off, a three-host ticks such as *Rehipicephalus appendiculatus* and *Amblyomma variegatum* find a resting place where they can digest its blood meal and moult to the next instar or enter diapauses, a state characterized by reduced metabolism and delayed development (Parola and Raoult, 2001).

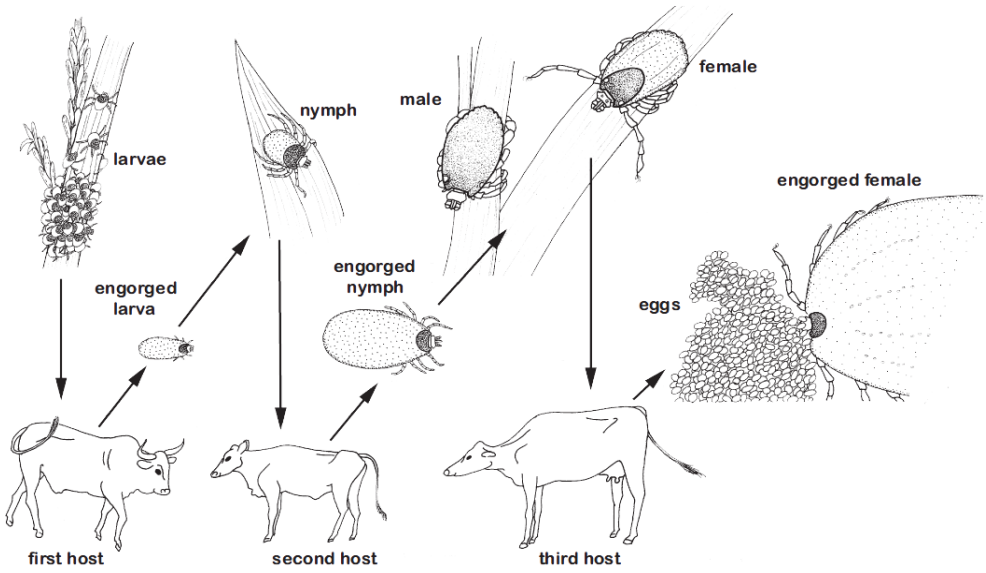

Figure 3. Three-host tick life cycle (eg. *Rhipicephalus appendiculatus*) (Walker *et al.*, 2003)

1.3.4. *Amblyomma variegatum*

Amblyomma variegatum is known as the tropical bont tick ("bont" refers to the colored pattern on the scutum). This species is one of the commonest and most widely distributed ticks on livestock in Africa. It is notorious as the most predominant vector of the causative organism of heartwater and for having spread to the Caribbean on imported cattle (Walker *et al.*, 2003).

A. variegatum (Figure 4) is a member of the family Ixodidae (hard ticks) which have a dorsal shield (scutum) and their mouthparts (capitulum) protrude forward when they are seen from above (Walker *et al*., 2003). *Amblyomma* ticks are large ticks with long, strong mouthparts. The palps are long; the second segment is twice as long as it is wide. Eyes are present and the festoons are well developed. The males have no adanal shields, accessory shields or subanal shield. Female *A. variegatum* is brown, but the males are brightly ornamented with orange. When they are engorged, the adult female ticks are about the size of a nutmeg. Tick identification to the species level can be difficult, and ticks should be submitted to an expert for identification whenever possible (Kelly *et al*., 2010). Adults of *A. variegatum* have long mouthparts and banded legs, with different colour patterns on the conscutum and scutum, the colour pattern on the male conscutum is dark-orange. Their eyes are beady, and the males have uniformly dark festoons (Walker *et al.*, 2003).

A. variegatum is widely distributed through West, Central, North-East and East Africa and in southern Africa extends into Zambia, north-eastern Botswana, the Caprivi Strip of Namibia, north-western Zimbabwe and central and northern Mozambique. Its spread southwards appears to be limited by interspecific competition with *A. hebraeum* with which it shares similar habitats, hosts and sites of attachment and by the drier conditions in the south. It has also been imported onto the Caribbean islands where attempts to eradicate it have cost millions of dollars without success, mainly because of the variety of hosts it infests, particularly the immature stages, and its re-introduction by birds infested with the immature stages flying from one island to the next (Madder *et al*., 2012).

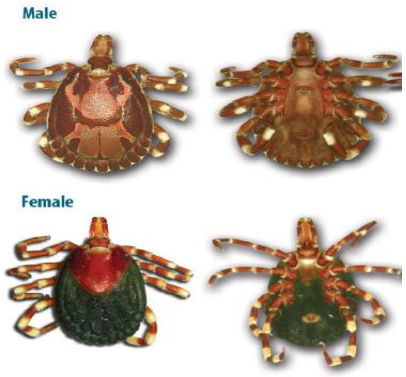

Figure 4. Male and female *Amblyomma variegatum* ticks (Tick website of African ticks: http://www.itg.be/photodatabase/African_ticks_files/index.html)

A. variegatum is a three-host tick. Immature ticks feed on small mammals, ground–feeding birds and reptiles, as well as cattle, sheep and goats. Adult ticks prefer cattle, but can also be found on other livestock including camels, as well as dogs and some wildlife. The adult ticks are usually found on the relatively hairless parts of the body; most are located on the ventral body surface and the genitalia, or under the tail. Adult *A. variegatum* feed mainly in the rainy season, while the immature ticks feed primarily during the dry season (Kahn, 2003).

A. variegatum is a host for a number of microbial pathogens. This tick can transmit *Ehrlichia ruminan-tium* (formerly *Cowdria ruminantium*), the agent of heartwater, and *Dermatophilus congolensis*, agent of bovine dermatophilosis. It is a host for *Rickettsia africae*, the agent of African tick-bite fever, an emerging zoonosis in rural sub-Saharan Africa and the Caribbean. *A. variegatum* can also carry other human or animal pathogens including Crimean-Congo hemorrhagic fever virus, Dugbe virus, Thogoto virus, Bhanja virus, Jos virus and yellow fever virus (Pegram and Eddy, 2002).

1.4 Ticks prevention and control

Ticks are difficult to control because they use multiple hosts and exhibit three developmental stages, making it difficult to thwart them with one method at once (Young *et al.*, 1988). Major advances have been made in the development of novel methods and strategies for the control of ticks in the recent years (Rajput *et al*., 2006). New and easier methods of applying acaricides are available, ear tags, neck bands, tail bands and pour-on. A mechanical applicator has also been developed (Pegram *et al*., 1993). The currently used method for the control of ticks in Ethiopia is mainly the application of acaricides which seems to be regulated primarily by availability in the remote rural area where the densities and distribution of tick's population are usually greater.

Apart from the use of chemical compounds for tick control, certain cultural practices such as hand picking of ticks, burning or piercing of ticks with hot iron and use of plant preparation are widely used by cattle owners in the rural areas (Mekonnen, 2002). The application of some crude plant extracts, some of them have to be encouraged provided that toxicological studies indicate their safety to the user and that there are no residue problems, are recommended and these herbs that have acaricidal properties also need further investigation (Mekonnen, 2001).

1.5 Medicinal plants used for the control of ticks

Several reports are available in the literature on the ethnoveterinary use of medicinal plants. In most of the sources, however, there is only brief description of the plants used, and the purported condition for which they are applied. Moreover, most of these reports do not provide information on the part of the plants used and method of preparation; often no validation of the effect against the disease condition is provided (Mesfin and Obsa, 1994).

In Ethiopia, ethnoveterinary medicinal studies focus mainly on survey and documentation of traditional practices used for various animal health problems. Mesfin *et al.* (2009) documented 155 medicinal plants in the natural vegetation and home gardens in Wonago Woreda, Gedeo Zone in Southern Nations, Nationalities and Peoples Regional State (SNNPR), Ethiopia, of which, 72 plant species were recorded as having medicinal value in veterinary medicine. According to Tamiru *et al.* (2013), 48 medicinal plant species classified into 35 families are claimed to treat 22 animal health constraints in Dabo Hana district of Ilubabor Zone, West Ethiopia. Similarly, Bussmann *et al.* (2011) reported the plants used by the Oromo people in Southern Ethiopia. The Oromo people in Odobulu and Omera, Bale had names/uses for 294 species in comparison to 230 species documented in the lower reaches of the Bale area. Only13 species were used for veterinary purposes. Another study conducted in rural areas of Akaki District, Eastern Shewa, Ethiopia recorded lists of 18 plant species for protection against insect bite and for the treatment of livestock health problems (Bekele *et al*., 2012).

In their notes on ethnoveterinary knowledge in Sanaag region Somaliland, Catley and Mohammed (1995) listed 70 local remedies for animal diseases, including 32 veterinary medicinal plants. It was apparent that the most popular local treatments were burning or cauterization, the use of soups and broths and of salt solutions. In addition to these remedies, pastoralists in Sanaag used various methods for preventing livestock disease. At watering wells there were often specific rules which prevented sick animals mixing with healthy animals and at some wells each family used its own pan for watering stock. Within the homestead the thorn or stone enclosure used to house livestock at night was regularly cleaned to prevent build up of

dung which attracted the ticks, particularly *Rhipicephalus pulchellus* (Catley and Mohammed, 1996).

In spite of these valuable documentations of medicinal plants and ethnoveterinary knowledge in many countries, very few efforts were made to scientifically evaluate these plants for their claimed medicinal properties. Most of the works in these regard are concentrated on medicinal plants against meng mites and sheep keds but little is done on ticks. Zorloni (2010) evaluated acetone and hexane extracts of 28 plant species used in Ethiopia to control ticks on camels, on toxicity and repellency activity against *Rhipicephalus pulchellus.* Similarly, Moyo and Masica (2013) validated the acaricidal properties of *Aloe ferox, Lantana camara, Pateroxylon obliquum, Tagetes minuta* and used engine oil and Jeyes fluid against *Rhipicephalus sanguineus*. Preliminary *in vitro* efficacy tests of *Euphorbia obovalifolia, Ficus brachypoda* and *Solanum incanum* on engorged female *Boophilus decoloratus* showed that the extracts of these plants have 30 – 100 % killing effects (Regassa, 2000). Subsequently, *in vivo* treatment trials of these preparations were conducted using indigenous cattle (*Bos indicus*) naturally infested with ticks. Results indicate that treatments at the rate of once per day for 5 consecutive days with the latexes of *E. obovalifolia* and *F. brachypoda* can reduce tick burdens by up to 70% on cattle.

Mawela (2008) extracted the leaves of *Aloe ferox, Aloe marllothii, Cleodendrum glabrum, Jatropa curcas, Ricinus Communis* and *Strychnos madagascariensis* with methanol, acetone and dichloromethane and evaluated the extracts against the livestock tick *Rhipicephalus appendiculatus*. The results proved that the acetone extracts of *A. ferox, A. marllothii, C. glabrum*; the dichloromethane extracts of *A. marllothii* and *R. communis,* and the methanol

extract of *J. curcas* have the capacity to repel the ticks. Of all the aqueous extracts, only the decoction of *Strychnos madagascariensis* repelled the ticks.

1.6 The genus *Aloe*

The aloes are perennial plants that display a wide range of habitats. Members of the genus vary from small herbs; shrubs and climbers to trees. They occur in a wide range of sizes from a dwarf rosette about 30 cm high to the tall trees about 12-15 m in height (Smith and van Wyk, 1991). Over 500 known species including trees, shrubs and perennials of aloe are recognized. The genus is predominantly native to Africa, with species also found on the Arabian Peninsula and Jordan, as well as on several islands including Madagascar and African coast (Reynolds 2004; Cock, 2015).

1.6.1 Ethnobotany

The literature contains numerous references to the traditional uses of *Aloe* spp., but a comprehensive analysis of the biocultural value and documented uses of the genus has been lacking. Consequently, information on the ethnobotanical significance of aloe has remained largely inaccessible, despite this knowledge being of potential importance in biodiversity conservation and ecotourism (Grace *et al.,* 2009). The curative nature of some aloes has been exploited in modern societies as well. For example, *Aloe vera* is known to kill *Mycobacterium tuberculosis*, the organism responsible for tuberculosis, and also the herpes virus responsible for herpes (Steenkamp and Stewart, 2007). A research work by Reynolds and Dweck (1999) has shown that *A. vera* inhibits growth of many common organisms such as yeasts, fungi, and the bacteria associated with wound infection. An insect repellent can be made by drying and burning

aloe leaves and similar preparations are used to protect animals against ticks and stored food against weevils. The sap of *A. lateritia* is used in some communities in Kenya and Ethiopia for treatment of eye aliments (Wabuyele, 2000) and also some species have been used traditionally for treatment of constipation, burns and dermatitis in west Africa (Morton, 1961).

A multitude of *Aloe* species are used throughout Africa in traditional medicine and for other purposes. In South Africa, leaf saps of *A. maculata* are used locally in the tanning of garments made from skins. The exudates from some species such as *A. megalacantha* and *A. confusa* are used to dye cloth and for making ink. The spiny leaves of *A. marlothii* are used for scarping and thinning animal hides to prepare them for making garments (Reynolds, 2004). Some species of *Aloe* have a wide range of commercial uses. For example, leaves of *A. vera*, are used in the production of many cosmetic products. For many years, roots from species in the *A. saponaria* group have been used to make soap. The many kinds on the market include after shaving gel, mouthwash, hair tonic and shampoo, skin-moistening gel, and even a 'health drink'. In South Africa, dried leaf exudates of *A. ferox* are exported (Van der Bank *et al.,* 1995).

Given the high degree of biodiversity in the Southern African region, it is not surprising that numerous traditional healing systems from that region use *Aloe* species in the treatment of many disorders (Cock, 2015). Aloe species occur in different regions and their usage is often widespread and is often associated with specific cultural/ethnic groupings. Thus, a species used by one cultural group for treating a specific disease or disorder may have had different therapeutic uses (or no uses) by the same or other cultures in different regions. Despite such

variation, two South African *Aloe* species (*A. arborescens* and *A. ferox*) stand out as having the widest usage and most commercial potential after *A. vera* (Cock, 2015)

1.6.2 Pharmacological activity

The first somewhat detailed description of the pharmacological effects of aloe is recorded in "The Greek Herbal", written by Dioscorides in the first century AD. According to such literature, *Aloe* has multiple pharmacological effects on healing wounds, burns and frostbite, constipation, insomnia, stomach disease, pain, hemorrhoids, itching, headache, hair loss, gum disease, kidney disease, blisters, sunburn and more (Park and Lee, 2006). *Aloe* has long been used as a remedy in many cultures. *Aloe* products, which include the latex, gel, and whole leaf, are used, among other reasons, as laxatives, in creams for skin ailments, and as a treatment for a wide range of diseases, respectively (Chen *et al.*, 2012). The heterogeneous nature of *Aloe* products may contribute to the diverse biological and therapeutic activities that have been observed (Mascolo *et al*., 2004). *Aloe* can penetrate and anesthetize tissue; it is bactericidal, virucidal, and fungicidal. It possesses anti-inflammatory and immunomodulatory properties and it serves as a stimulant for wound healing, a fuel for proliferating cells and a dressing for open wounds (Mascolo *et al.*, 2004).

1.6.3 Aloes in Ethiopia

About 40 species of *Aloe* have been described in Ethiopia and Eritrea so far. According to the analysis based on the flora account (Demissew and Gilbert, 1997) and later research (Demissew and Doli, 2000; Demissew and Gilbert, 2000), 35 (i.e., 87%) of the 40 species of *Aloe* found in the flora area are endemic or near endemic (i.e. have restricted distribution in one or few neighboring countries). Only four species namely, *A. laterita*, *A. macrocarpa*, *A. secundiflora*

and *A. vituensis* are widely extending to East and West Africa (Demissew *et al.*, 2011). Most species have very restricted distribution and three local centers of endemism are identified each of which has its own set of endemic taxa. These are: (1) Northern and central highlands north and west of the rift valley with 14 endemics; (2) Eastern highlands with 5 endemics; and (3) A southern highlands, lowlands and rift valley with 9 endemic taxa (Demissew *et al.*, 2001).

According to Demissew and Nordal (2010), most *Aloe* species inhabiting in the flora area are highly threatened due to agricultural expansion into marginal lands and habitat destruction due to new development schemes near urban centers and regional centers.

1.6.4 *Aloe yavellana* Reynolds

Aloe yavellana (Figure 5) is a narrow endemic restricted to two localities in Sidamo floristic region, near Yavello town and in the north-eastern slopes of Mega Mountain, Ethiopia, where it occurs in great numbers in forest, in clearings, and on rocks.

Figure 5. Individuals of *Aloe yavellana*; photograph taken 2 km outside the town of Yavello (Source Tibebu 2012)

The specific epithet 'yavellana', refers to the place of growth, Yavello in Sidamo floristic region from where the type collection was made by Reynolds. The species belongs to a group of

caulescent aloes mainly characterised by erect, ascending or sprawling stems (Demissew and Nordal, 2010). Although the species was described in 1954, to the best of our knowledge, there is no chemical or biological study published on this plant.

1.7 Statement of the problem

Tick infestation is one of the major parasitic diseases causing the tick born diseases like babsioses, anaplasmosis, cowdriosis and theilerioses known to exist and cause damage on productivity of cattle production in Ethiopia. A huge amount of foreign currency is also lost annually only through rejection of downgraded hides and skins attributed to tick damage. Besides, the increase in cost of foreign currency to purchase chemical acaricide and medicine for the control of ticks and tick born diseases respectively, makes the country export earnings dis-proportionally low (Moges and Bogale, 2012). On the other hand prolonged and incorrect use of acaricides causes resistance to ticks, and conventional acaricides tend to contaminate the environment and indiscriminately kill beneficial insects (parasitoids), birds (oxpecker) and fishes (USDA, 1967; Laffont *et al*., 2001; Latif and Jogejan, 2002; Uilenberg, 2005). In addition, acaricides are poisonous to the environment and their use is complicated especially for resource-limited farmers. Furthermore, they are also expensive and unaffordable to resource-limited farmers; as a result farmers have resorted to ethnoveterinary practices and remedies (Laffont *et al*., 2001). Thus, the aim of the present study is to assess the acaricidal activity of *A. yavellana*, an endemic species claimed to be effective against *Amblyomma varigatum*.

2. OBJECTIVES

2.1. General objective

- To evaluate the *in vitro* acaricidal activity of the latex of *Aloe yavellana* and the compounds isolated thereof against *Amblyoma varigatum*.

2.2. Specific objectives

- To test the *in vitro* acaricidal activity of the latex of *Aloe yavellana*;
- To isolate and characterize compounds present in the leaf latex; and
- To determine the in vitro acaricidal activity of the isolated compounds using Larval packet test (LPT).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant material

The latex of *A. yavellana* was collected in February 2012; 10 km from Yavello town on the main road from Yavello to Addis Ababa. The plant was authenticated by Professor Sebsebe Demissew, the National Herbarium, Department of Biology, Addis Ababa University, where voucher specimen (collection number: TH/02/03) was deposited.

3.1.2 Chemical, reagent and drug

The following chemicals, reagents and drugs were used for the study: chloroform, ethyl acetate, and methanol (Pharmacos Ltd. Essex, England), silica gel for thin layer chromatography F_{254} (E. Merck, Darmstadt, Germany), Whatman filter paper No 1 (Whatman International Ltd. Maidstone, England), dimethylsulfoxide (DMSO) (Sigma-Aldrich, St. Louis, USA), and Diazinon EC 60 (Kafr El Zayat Pesticides and Chemicals Co. Ltd, Egypt). All the chemicals were analytical grade and most of them were purchased from Micron PLC, Addis Ababa, Ethiopia, while the rest were obtained from the Department of Pharmaceutical Chemistry and Pharmacognosy, School of Pharmacy, Addis Ababa University (AAU).

3.1.3 Instruments

Ultraviolet-visible spectra were obtained on a Shimadzu UV-1800 spectrophotometer (UV-1800, Shimadzu, Japan). Infra red (IR) spectra were determined on a Perkin- Elmer BX spectrometer (400-4000 cm^{1}) (Spectrum BX 11, Perkin-Elmer, USA). Electron spray ionization-mass spectrometry (ESI-MS) was performed using liquid chromatography (LC) coupled with mass

spectrometer (MS) (Varian 910, Agilent Technologies, USA). Nuclear Magnetic resonance (NMR) was run on a Bruker Avance DMX 400 FT-NMR spectrometer (Avance DMX 400, Bruker Analytik, Germany).

3.1.4 *Amblyoma varigatum*

Engorged adult ticks were collected from heavily infested cattle kept for research purpose in National Animal Health Diagnostic and Investigation laboratory animal facility at Sebeta. The collected ticks were processed as per the standard procedure for morphological confirmation of *Amblyoma varigatum*. The processed ticks were examined microscopically and identified as *Amblyoma varigatum*. Engorged adult ticks were selected, cleaned, stored in a petri dish. The female ticks was examined daily until oviposition. The eggs were separated and allowed to hatch in glass vials with cotton plug and kept in optimal conditions. The obtained seed ticks were maintained at an average temperature of 20 ± 5 ºC and average humidity of 80 ± 10% for 14-21 days.

3.2 Methods

3.2.1 Preparation of plant material

The leaf latex of *A. yavellana* was collected by cutting the leaves transversely near the base and allowing the yellow sap to come down into a plate. The water was evaporated by leaving the latex at room temperature for three days to yield a golden coarse powder. The dried latex (42 g) was kept in a closed sample bottle, transported to NAHDIC and stored at 4 °C until used.

3.2.2 Chromatographic techniques

3.2.2.1 Preparative thin layer chromatography (PTLC)

Isolation of compounds was performed by dissolving the latex in methanol and applied directly to PTLC plates (20 × 20 cm) of 0.25 mm thickness. The isolated compounds were purified by repeated PTLC. Purity of the isolated compounds was monitored by pre-coated analytical TLC aluminum sheets (10 x 10 cm) (E. Merck, Darmstadt, Germany).

3.2.2.2 Solvent System

A mixture of ethyl acetate, methanol and water in a ratio of 7.7:1.3:1.0 was used as a solvent system for PTLC. The solvent system used for purification of the isolated compound was chloroform: methanol (4:1).

3.2.2.3 Visualization

The chromatographic zones were visualized first in daylight and then by using ultraviolet light of wave lengths 254 and 366 nm. After visualization the zones were coded as AY-1 and AY-2 based on descending order of Rf values. Then, each band was carefully scrapped off separately from the plate and dissolved in methanol and chloroform (1:1), filtered and concentrated.

3.2.3 Spectroscopic techniques

Ultraviolet (UV) spectra were measured by dissolving the compounds in spectroscopic grade methanol (200 - 400 nm) at room temperature. IR spectra were obtained in the region 400 - 4000 cm^{-1} in KBr pellets. ESI-Mass spectra measurements were carried out by an electrospray ionization method with negative mode. The source voltage and temperature were fixed at 3kV

and 250 ºC. NMR spectra were recorded on a spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C at room temperature using deuterated methanol. A region from 0 to 12 ppm for ^{1}H and 0 to 205 ppm for ^{13}C was employed for scanning. Signals were referred to an internal standard tetramethylsilane (TMS). Chemical shifts are reported in δ units and coupling constants (*J*) in Hz. Multiplicities of ^{1}H NMR signals are indicated as *s* (singlet), *d* (doublet), *dd* (doublet of doublets), *t* (triplet), *m* (multiplate) and *nr* (not resolved).

3.2.4 Determination of acaricidal activity

3.2.4.1 Maintenance of ticks

A. varigatum (Reynold) unfed larval ticks disregarding sex were used for the experiments. The ticks were kept in a glass chamber, closed by removable cover, at an average temperature of 20 ± 5 ºC. An average humidity of 80 ± 10% was maintained by a potassium chloride saturated solution placed on the floor of the chamber for each bioassay. The ticks were stored in vials closed with cotton wool in order to allow normal air exchange; the vials were set on square glass plate, placed at the base of the chamber on four small bearings, so that the edges of the plate were at a distance of 1.5 cm from the wall. In this way, the saturated saline solution on the floor could also prevent the ticks from reaching the walls in case of accidentally escaping the vials. After one and half month, the solution was completely renewed to restore the appropriate humidity

3.2.4.2 The larval packet test (LPT)

The Larval Packet Test (LPT) was used to test acaricidal activity of the test substances against unfed larval stages of *A. varigatum* (Garcia *et al*., 2012). The test substances were dissolved in

2% DMSO to obtain a test concentration of 50 mg/ml. Lower concentrations were prepared by further dilution to 25 mg/ml and 12.5 mg/ml with the same solvent. The commercial acaricide diazinol 60% EC 1 ml/l was used as a positive control, while the negative control employed was 2% DMSO.

In the LPT test, a filter paper Whatman No. 1 (12.5 cm diameter) was folded through the edge in the form of rectangular packet in such a way that ticks could move freely inside the packet. Forty larval ticks were introduced into each folded filter paper and secured with clips. Three ml of each of the test substances having different concentrations were applied on the folded surface of the filter paper and placed inside a plastic Petri dish. After drying in an open air, the lids were fitted, and the Petri dishes incubated at 27 - 28 ºC and an average humidity of 70 - 80% for 24 h. The entire test was conducted in triplicate. The packets were opened and the numbers of live and dead larvae were recorded to assess percent mortality. Ticks were considered alive if they exhibited normal behavior (movement looking for the host) when breathed upon or physically stimulated with a wooden stick. For each time point, if ticks were incapable of moving, maintaining a normal posture, leg coordination, being upright themselves, or not showing any sign of life, they were considered moribund or dead.

3.2.5. Data analysis

Results were presented as percent mortality (E %), which was calculated according to the following equation:

$$E\,\% = \frac{\text{Dead Larvae}}{\text{Total Larvae}} \times 100$$

The ED_{50} and ED_{99} value of percent mortality (E %), were determined applying regression equation analysis to the Probit transformed data of mortality.

4. RESULTS AND DISCUSSION

4.1 Isolation and structural elucidation

Repeated preparative-TLC of the leaf latex of *A. yavellana* over silica gel using chloroform: methanol (4:1) as a solvent system, afforded two major compounds with R*f* values of 0.58 and 0.35. These compounds were designated as AY-1 and AY-2, respectively, as shown in (Fig. 7). When the leaf latex constituents were viewed under 254 nm UV light (A), absorbing compounds quenched UV light and appeared as dark spots on a bright green background, while the constituents appeared as deep orange spots under UV light of 366 nm (B). Structural elucidation of the isolated compounds was based on their spectroscopic data and by comparison of their spectroscopic characteristics with those reported in the literature.

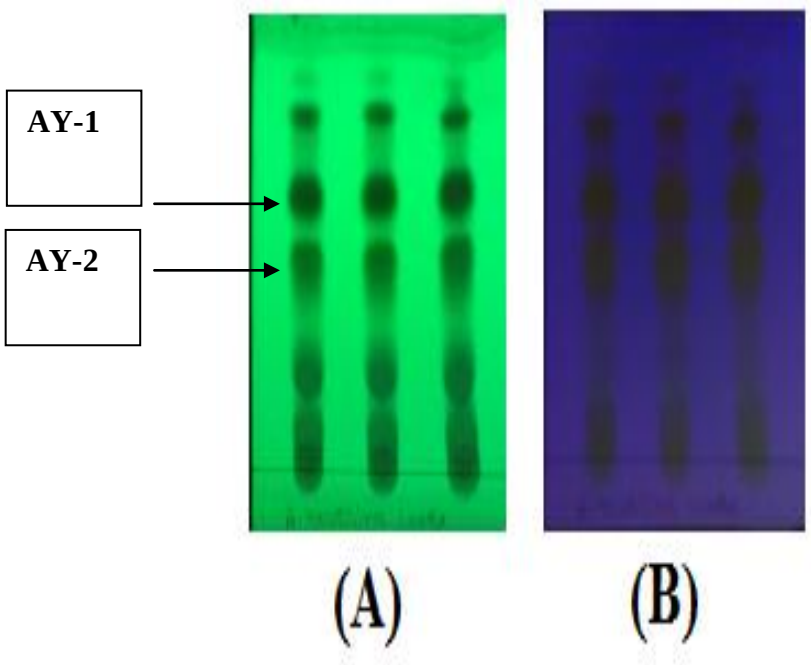

Figure 6. Adsorption TLC of the leaf latex of *Aloe yavellana* when viewed under UV light of (A): wavelength 254 nm and (B): wavelength 366 nm [Solvent system: $CHCl_3$/MeOH (4:1)].

4.1.1. AY-1

AY-1 was isolated as a yellow solid with R_f value of 0.58 in $CHCl_3$: MeOH (4:1). The negative-ion ESI–Mass spectrum (Fig. 7) of AY-1 gave a pseudomolecular ion at *m/z* 563 $[M-H]^-$, indicating a relative molecular weight (M_r) of 564. A molecular formula of $C_{30}H_{28}O_{11}$ was deduced for compound AY-1, which was consistent with ^1H and ^{13}C-NMR spectral data (Table 2). The UV spectrum of AY-1 in MeOH (Fig. 8), displayed an absorption band at λ_{max} 302 and 311 nm indicating the presence of an anthrone moiety (Dagne *et al*., 2000).

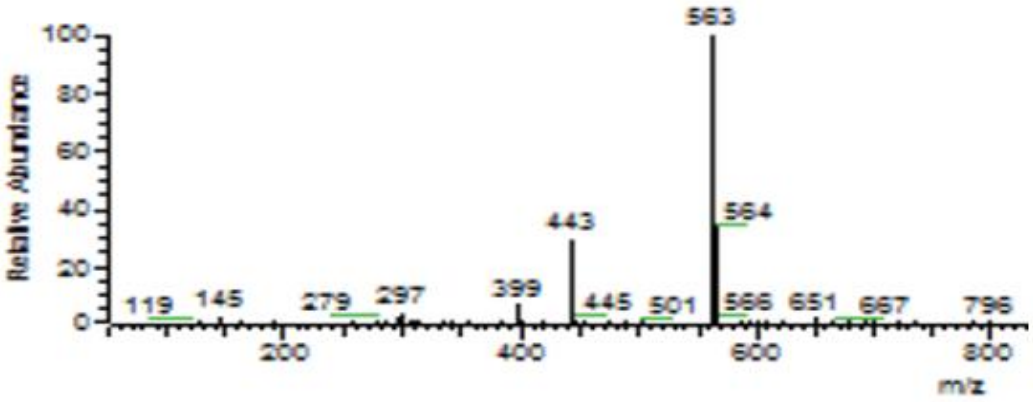

Figure 7. Electron spray ionization mass spectrum of AY-1

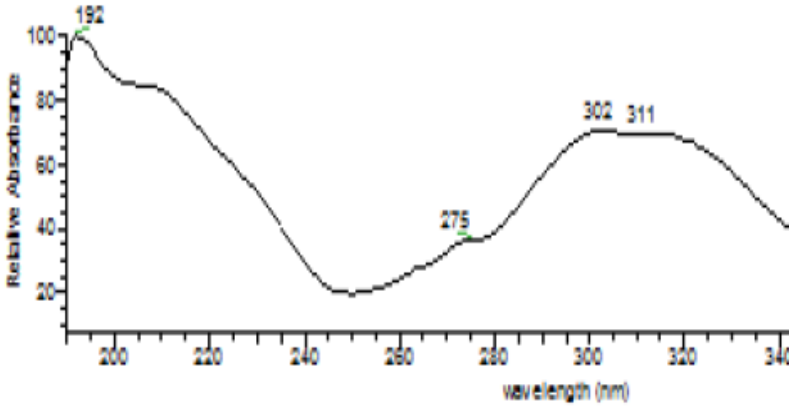

Figure 8. UV spectrum of AY-1

In the IR spectrum (Fig. 9), a broad absorption band at 3415 cm^{-1} and a strong absorption band at 1709 cm^{-1} indicated the presence of OH and carbonyl groups, respectively. Two medium peaks at 1297 cm^{-1} and 1168 cm^{-1} indicated the presence of C-O group. Peaks at 1603 cm^{-1} and 1453 cm^{-1} are indicative of C=C bond of aromatic ring .

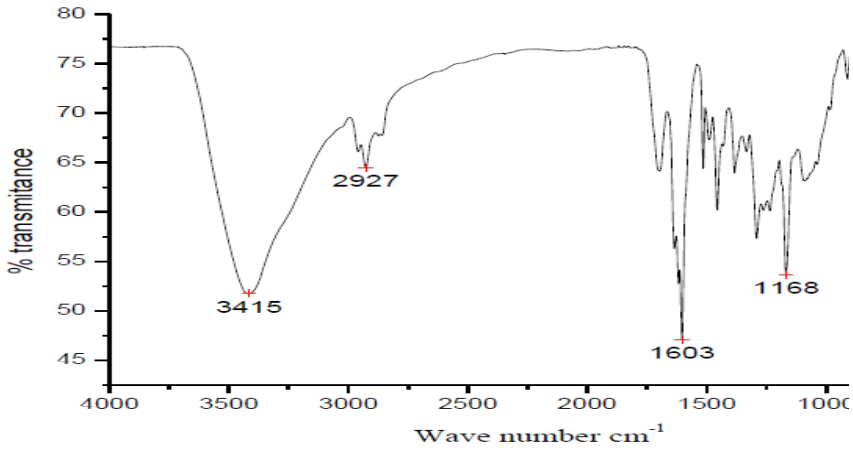

Figure 9. IR spectrum of AY-1

The presence of two chelated hydroxyl groups was supported by the ^{1}H NMR spectrum (Fig. 10 and Table 1), which showed two singlets at δ 12.01 (1H, *s*, H-1) and δ 12.17 (1H, *s*, H-8). In addition, five aromatic protons assignable to H-2 (δ 6.91), H-4 (δ 7.28), H-5 (δ 7.09), H-6 (δ 7.5) and H-7 (δ 6.91) were observed. Furthermore two olefinic protons which were assignable to *trans*-vinyl H-8" (δ 5.92, *d*, *J* = 16 Hz) and H-7" (δ 7.24, *d*, *J* = 16 Hz) and four aromatic protons to H-2" & H-6" (δ 7.36, *d*, J = 8.2 Hz, 2H) and H-3" & H-5" (δ 6.82, *d*, J = 8.2 Hz, 2H) led to a *trans*-cumaroyl residue as part of AY-1.

Table 1. ^{1}H and ^{13}C NMR data of AY-1 compared with microdontin (Farah *et al*., 1992)

Assignment	^{1}H (δ, ppm)		^{13}C (δ, ppm)	
	AY-1	Microdontin A/B	AY-1	Microdontin A/B
1	12.01	-	163.78	163.7
2	6.91	6.88	114.21	114.5
3	-	-	152.43	152.3
4	7.28	7.25	118.8	118.8
5	7.09	7.07	121.1	121.0
6	7.5	7.49	137.1	137.0
7	6.91	6.87	117.3	117.3
8	12.17	-	163.67	163.6
9	-	-	194.8	194.72
10	4.55-4.59	4.5	46.5	46.5
1a	-	-	116.68	116.6
4a	-	-	143.81	143.8
5a	-	-	143.53	143.5
8a	-	-	117.4	117.4
11	4.69-4.74	4.63-4.72	64.5	64.5
1'	3.92	4.01	85.18	85.1
2'	4.36	4.38	72.9	72.9
3'	4.05	3.36	78.3	78.28
4'	3.11	3.08	71.5	71.5
5'	3.33	3.3	81.8	81.8
$6'_a$ $6'_b$	3.67- 3.87	3.64 3.86	63.15	63.1
1"	-	-	127.24	127.12
2"	7.36	7.35	131.31	131.2
3"	6.82	6.81	116.81	116.7
4"	-	-	161.25	161.2
5"	6.82	6.81	116.91	116.7
6"	7.36	7.35	131.31	131.2
7"	7.24	7.23	146.81	146.7
8"	5.92	5.89	114.67	114.7
9"	-	-	167.73	167.57

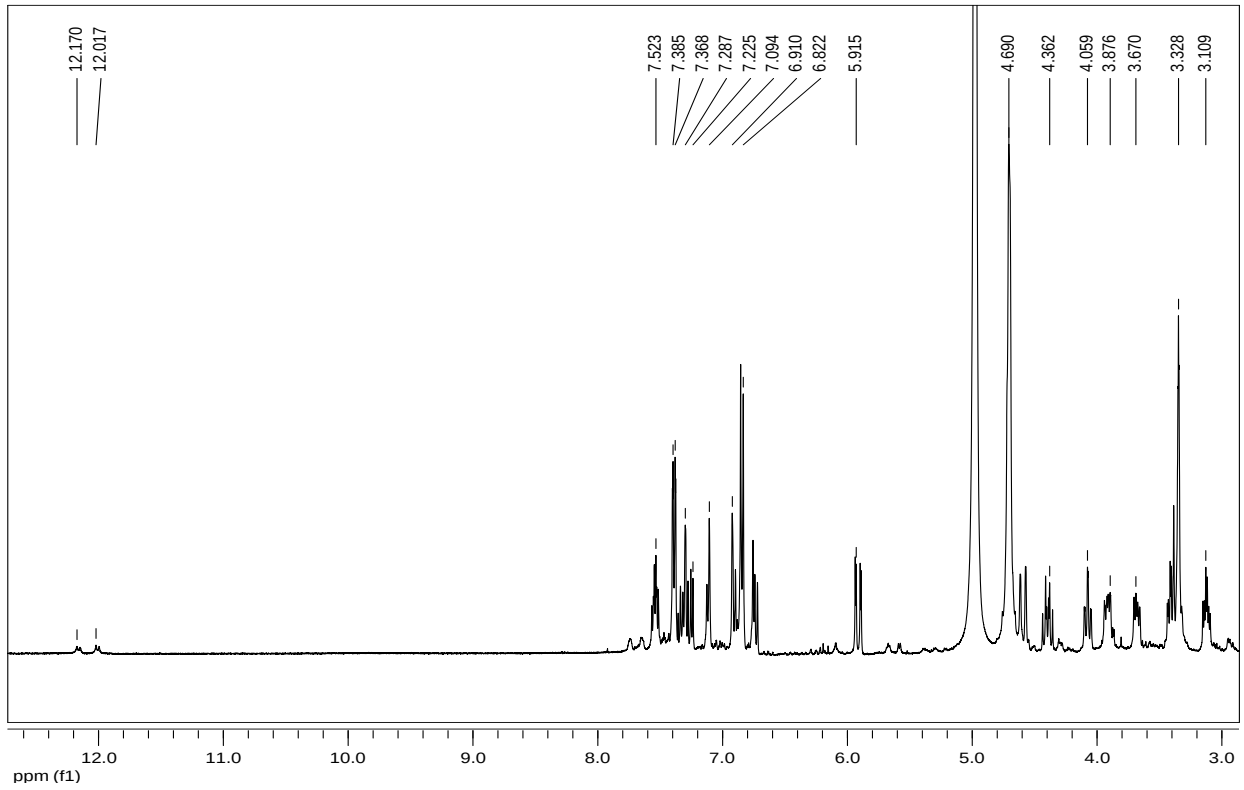

Figure 10. ^{1}H NMR spectrum of AY-1

The ^{13}C NMR and DEPT-135 spectra (Figs. 11 and 12), showed signals for 30 carbon atoms corresponding to 2 oxymethylenes, 16 methines and 12 quaternary carbon atoms including a chelated carbonyl (δ 194.80) and an ester carbonyl carbon (δ 167.73). In addition, the DEPT-135 spectrum displayed two downward peaks at δ 63.15 and δ 64.5, which showed the presence of two methylene groups attached to oxygen.

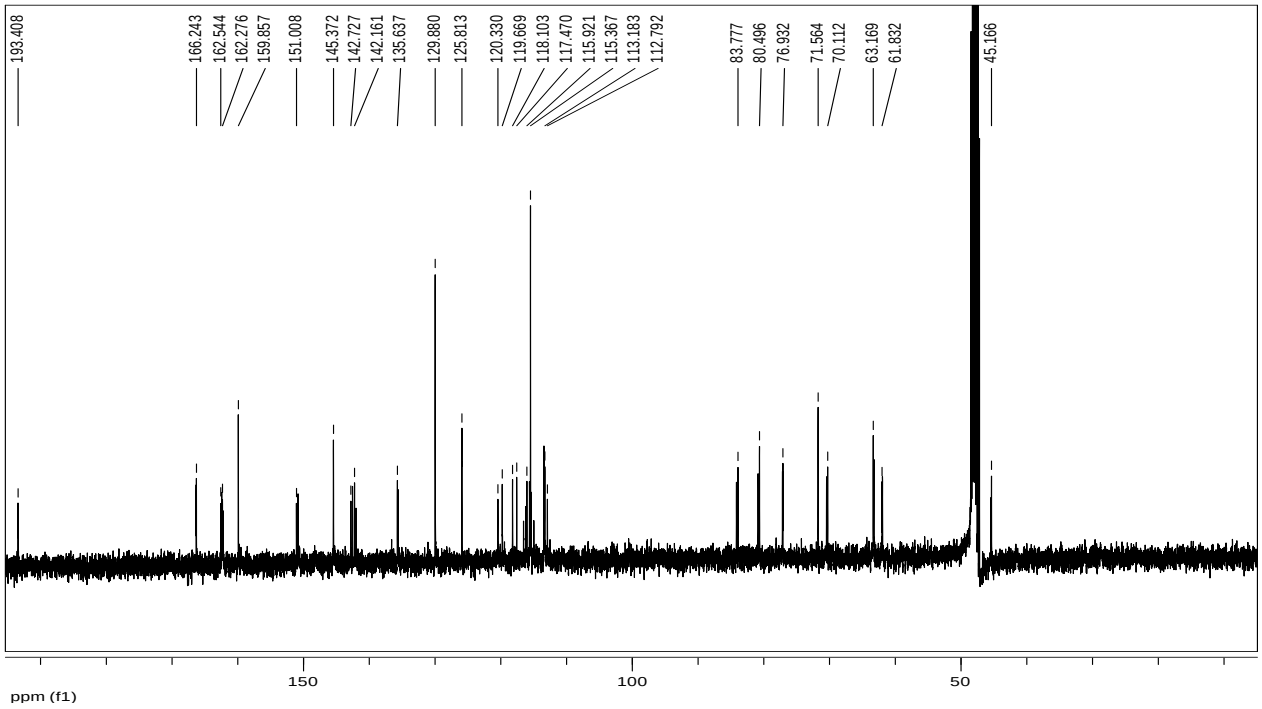

Figure 11. ^{13}C NMR spectrum of AY-1

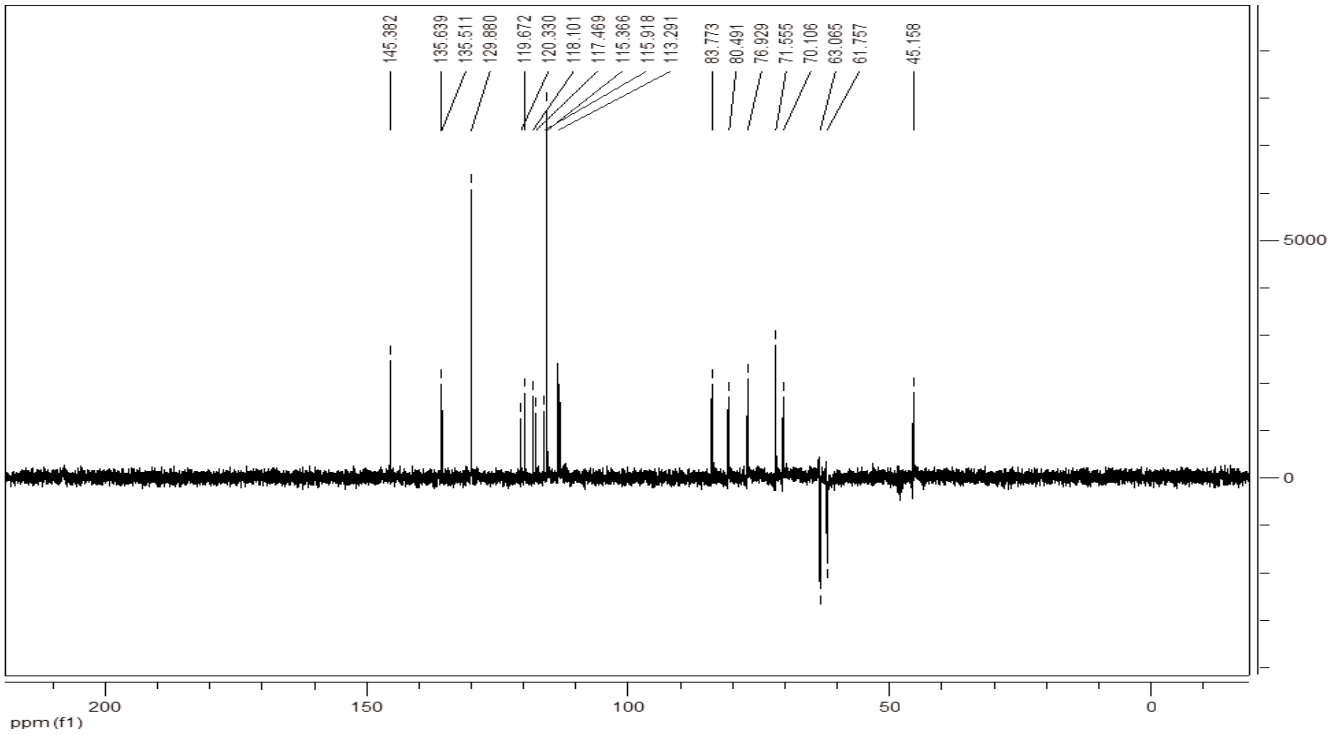

Figure 12. DEPT-135 spectrum of AY-1

From the data presented above and by comparison of the ^{1}H and ^{13}C NMR data reported for a similar compound (Farah *et al*., 1992), AY-1 was identified as microdontin A/B (Fig.13).

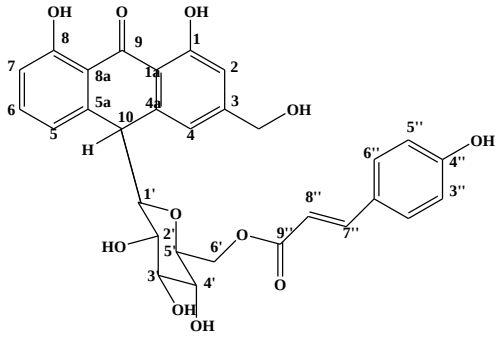

Figure 13. Structure of microdontin A/B

4.1.2. AY-2

AY-2, with R_f value of 0.35 in $CHCl_3$: MeOH (4:1), was obtained as a yellow amorphous solid. The negative-ion ESI-MS data (Fig. 14) of AY_2 showed a pseudomolecular ion at *m/z* 417, corresponding to M_r of 418. The IR spectrum (Fig. 15) of AY-2 exhibited absorptions consistent with a hydroxyl (3447 cm^{-1}), C=O stretching of unsaturated carbonyl group (1631 cm^{-1}) and C=C stretching of aromatic ring (1618 cm^{-1}). AY-2 exhibited absorption maxima (208, 299 and 357 nm) in its UV spectrum (Fig. 16) that are typical of anthrone moiety (Dagne *et al*., 2000). This together with ^{1}H and ^{13}C NMR data including DEPT was in agreement with the molecular formula $C_{21}H_{22}O_9$.

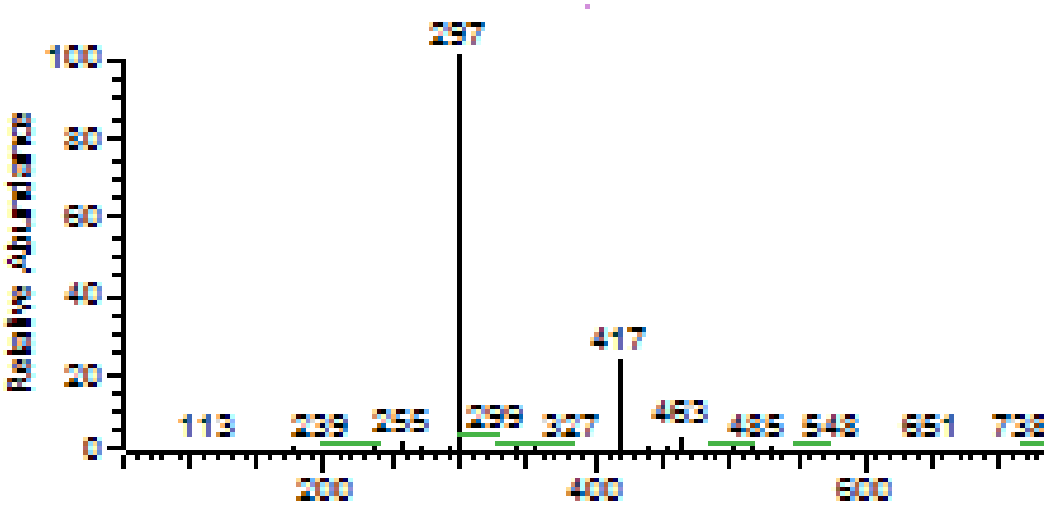

Figure 14. Electron spray ionization mass spectrum of AY-2

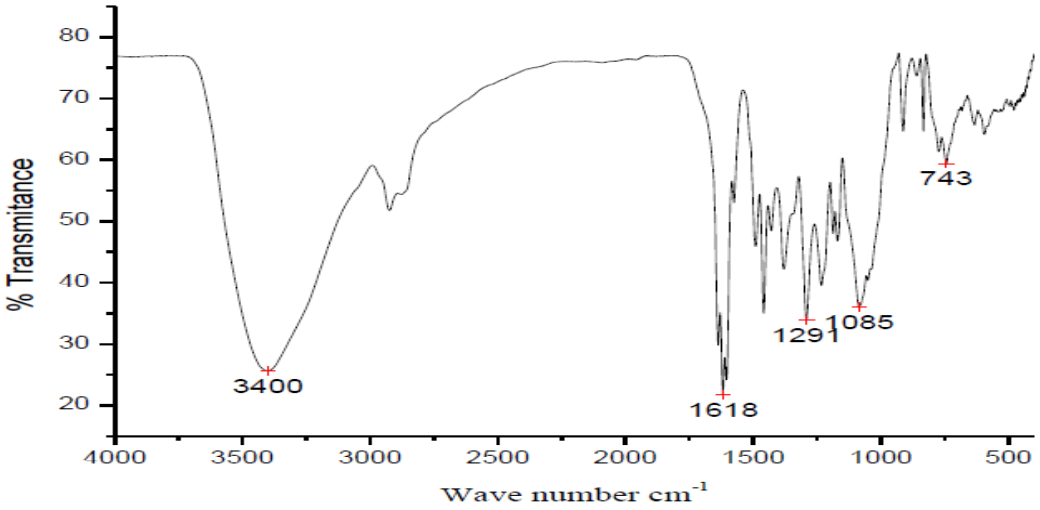

Figure 15. IR spectrum of AY-2

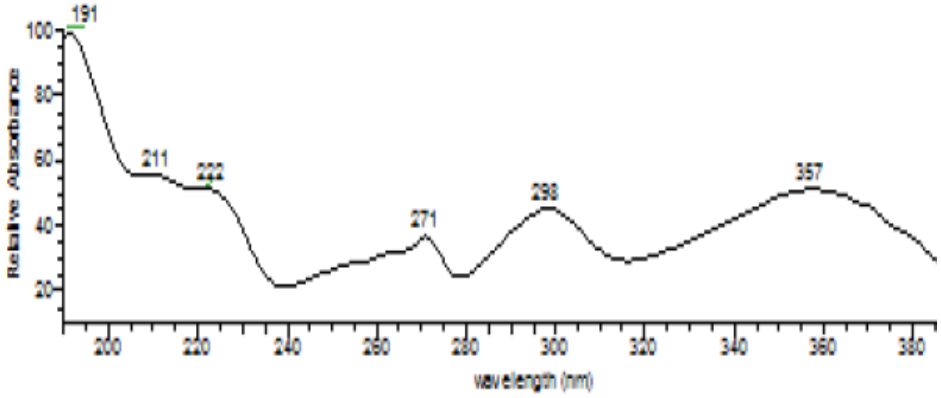

Figure 16. UV spectrum of AY-2

The ^{1}H NMR spectral data (Table 2 and Fig. 17) of AY-2 revealed the presence of two H-bonded phenolic OH singlets at 11.96 and 11.97 ppm. Moreover five aromatic signals were assigned to H-2 (δ 6.88, 1H, *s*), H-4 (δ 7.04, 1H, *nr*), H-5 (δ 7.06 1H, *d*), H-6 (δ 7.5 1H, *dd*) and H-7 (δ 6.87, 1H, *dd*). A singlet at δ 4.67 was also observed in the ^{1}H NMR due to the presence of a methylene group attached to oxygen (2H, *s*, H-11).

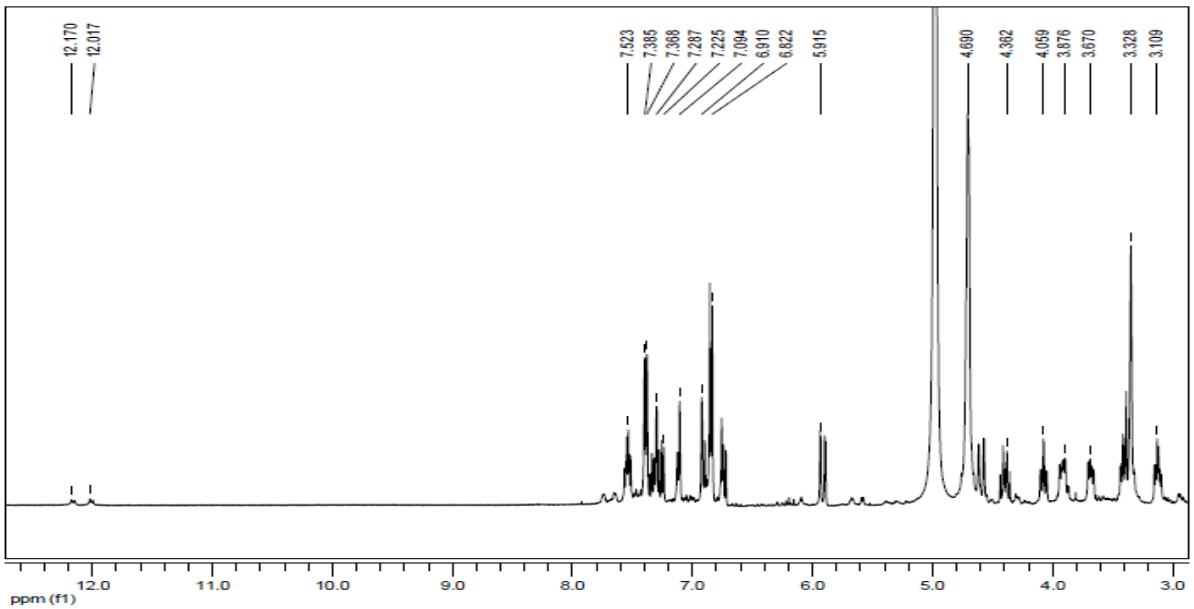

Figure 17. ^{1}H NMR spectrum of AY-2

The ^{13}C NMR and DEPT-135 spectra (Fig. 18 and 19) revealed 21 carbon atoms, which were identified as two oxymethylene (δ 63.12 and 61.82), five aromatic methine (δ 113.02, 115.42, 117.75, 118.89 and 135.66) and five oxymethine (δ 70.45, 70.56, 78.56, 85.21 and 78.56). Eight quaternary carbons were also identified in AY-2 from ^{13}C-NMR and DEPT-135 spectra, including signal due to a carbonyl ketone (δ 194.1).

Table 2. ^{1}H and ^{13}C NMR data of AY-2 compared with aloin A/B (Dange *et al.*, 2000)

Assignment	^{1}H (δ, ppm)		^{13}C (δ, ppm)	
	AY-2	Aloin A/B	AY-2	Aloin A/B
1	11.97	-	161.95	163.4
2	6.88	6.88	113.02	114.4
3	-	-	150.11	151.6
4	7.04	7.06	117.75	119.1
5	7.06	7.07	118.59	120.0
6	7.5	7.5	135.66	137.0
7	6.87	6.86	115.42	116.8
8	11.96	-	161.49	163.0
9	-	-	194.1	195.4
10	4.58	4.62	44.48	46.0
1a	-	-	117.75	117.7
4a	-	-	141.84	143.3
5a	-	-	145.15	146.6
8a	-	-	117.23	118.7
11	4.67	4.61-4.69	63.12	64.5
1'	3.41	3.42	85.21	86.7
2'	3.02	3.01	70.56	71.9
3'	3.28	3.25	78.56	81.7
4'	2.91	2.9	70.45	72.0
5'	2.93	2.92	80.26	80.0
6'a 6'b	3.36 3.56	3.38 3.57	61.82	63.30

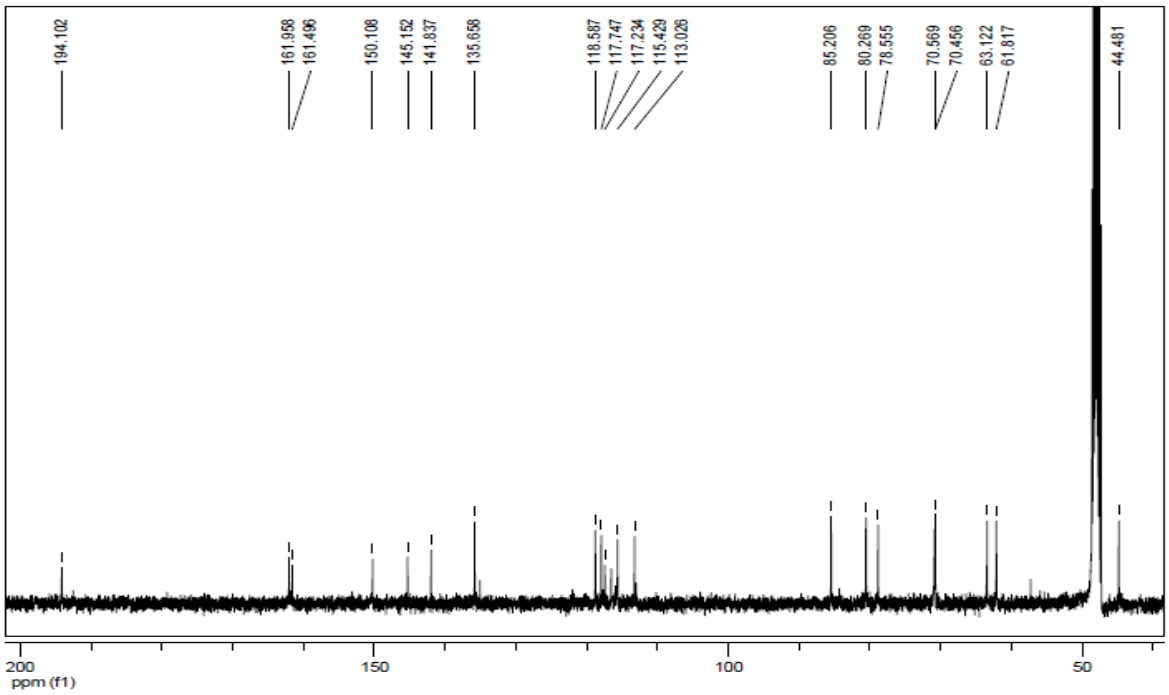

Figure 18: ^{13}C NMR spectrum of AY-2

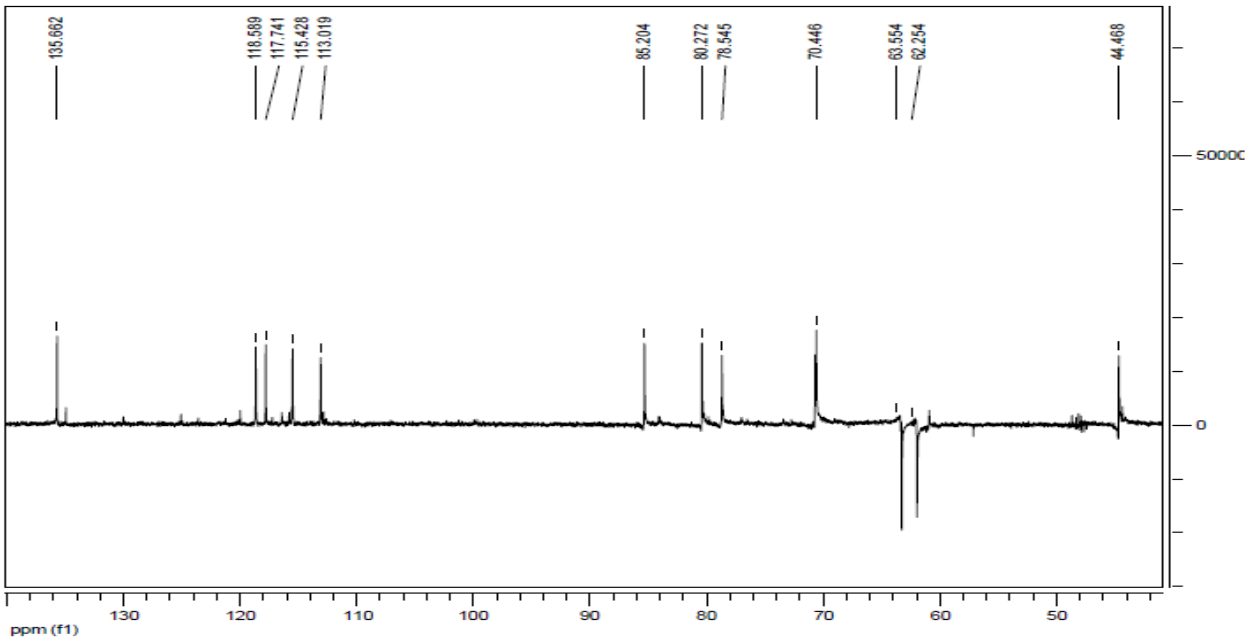

Figure 19. DEPT-135 spectrum of AY-2

The structure of this compound was further confirmed as aloin A/B (10-C-β-D-glucopyranosyl-1,8-dihydroxymethyl-9-anthracenone) Fig. 20 by comparing the NMR data with those reported in the literature for the same compound (Farah *et al*., 1992).

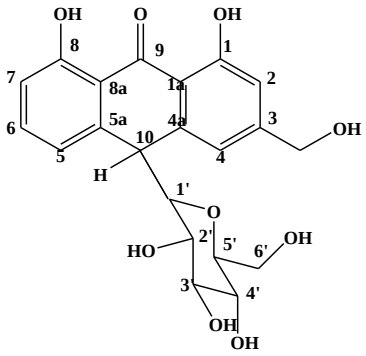

Figure 20. Structure of aloin A/B

4.2 Acaricidal activity of the leaf latex of *A. yavellana*

As shown in Table 3, the latex exhibited acaricidal activity against *A. varigatum* larvae in a dose-dependent manner, with the highest percent mortality of 62.50% at a concentration of 50 mg/ml.

Table 3. Percent mortality (E%) of the latex of *Aloe yavellana* against *Amblyomma variegatum* larvae

Test sample (mg/ml)	E% (Percent mortality)				
	12.50	25	50	2% DMSO (Dimethyl sulphoxide)	Diazinol 60% EC 1 ml/l.
Aloe yavellina (latex)	20.83	45.83	62.50	9.16	100.00

EC_{50} and EC_{99} values which were determined by plotting the percent mortality (E%) against concentration were found to be 35.82 and 83.48 mg/ml, respectively, with regression coefficient (R^2) of 0.909.

4.3. Acaricidal activity of the isolated compounds

The isolated compounds, namely microdontin A/B and aloin A/B were evaluated for their acaricidal activity against *A. varigatum* ticks larvae by using the larval packet test (LPT) method and were found to possess a dose-dependent activity. As shown in Table 5, 50, 25 and 12.5 mg/ml doses were tested for both compounds, and 50 mg/ml of both compounds was found cause at percent mortality of 30.83% (microdontin A/B) and 18.33% (aloin A/B).

Table 4: Percent mortality (E%) of microdontin and aloin A/B against *Amblyomma varigatum* larvae

Test samples	E% (Percent mortality)				
	12.5 mg/ml	25 mg/ml	50 mg/ml	2% DMSO (Dimethyl sulphoxide)	Diazinol 60% EC 1 ml/l.
AY-1	12.8	22.50	30.83	9.16	100.00
AY-2	12.2	14.00	18.33	9.16	100.00

Development of drug resistance, high cost of acaricides, environmental pollution and human health risk of acaricides are the major challenges that the Ethiopian livestock sector is facing today (Abdella, 2016). These harms had encouraged a great deal of research to look for alternative treatments using natural products. Application of plant extracts for the treatment of both human and animal diseases is one of the strategies followed for hundreds of years by the community in general and traditional medicine practitioners in particular (Yarnell *et al*., 2003). The use of natural products mainly of plant origin for the control of ticks has a proven record in many countries. Substances from *Artemisia annua* (Zhang *et al*., 2008), *Phytolacca americana* (Ding *et al*., 2010), and *Piper longum* (Park *et al*., 2002) are just few examples. Various studies have shown the acaricidal properties of plant extracts against phytophagous pests, ticks and mites (Grønvold *et al.,* 1966).

Compounds from members of the genus *Aloe* have been widely reported to have a variety of bioactivities. However, there appears to be very few reports on the acaricidal activity of extracts or pure compounds isolated from *Aloe* species. Previous studies carried out by Wei *et al.* (2011) on *Aloe vera* leaf extract against *Tetrachnus cinnabarinus* (carmine spider mite) by slide deep bioassay revealed that the extract possesses good acaricidal activity with EC_{50} value of 90 ppm.

Further investigation of this extract over silica gel-CC led to the isolation of 22 fractions, of which fractions 10 and 11 showed potent activities with EC_{50} values of 40 and 33 ppm, respectively. Taking this into account, our study focused on the acaricidal effect of the leaf latex of *A. yavellana* and its constituents against *A. varigatum* tick.

Results of the study revealed that both the latex and its constituents possess moderate acaricidal activity. Highest acaricidal activity was recorded for the leaf latex, which showed EC_{50} and EC_{99} of 35.82 mg/ml and 83.48 mg/ml, respectively. These results are consistent with literature reports which indicate that some aloe extracts possess acaricidal properties (Lam, 2012; Hossian *et al.*, 2013). For example, extracts of *A. ferox* have been reported to have insect repellent properties and are used to treat poultry diseases, sheep scab and control ticks in cattle (Moyo and Masika, 2009).

Leaves of six plant species *(Aloe ferox, aloe marllothii, Clerodendrum glabrum, Jatropa curcas, Recinus communis* and *Strynos madagascarienesi* were extracted with organic solvents ranging from polar to non polar (methanol, aceton and dichloromethane). Infusions (soap-water-paraffin) and decoctions traditionally used were also prepared the tick toxicity activities of extracts were evaluated against the livestock tick, *Rehipicephalus appendiculatus*. Organic extracts were not effective. Infusion of *A. ferox* and *S. madegascariensis* had strong topical and dipping application toxicity, infusion of *A.forex* and *J. curcas* had a strong dipping toxicity effect (Mawela, 2008). The infusion of *Strychnos madagascariensis* had strong toxic effect against ticks. Since *S. madegascariensis* bears edible fruits, some of toxic strychnine related alkaloids may be found spread in the leaves and played a role in giving the plant an activity (Moyo and

Masika, 2013). Hence, the possible toxicity of both the latex and isolated compounds *A. yavellana* must be investigated thus; results of toxicity assay provide evidence for efficacy of the traditional use of plants to control ticks.

The isolated active plant constituents and the commercial compounds were discussed according to acaricidal properties against ticks. Cadina-4, 10 (15)-dien-3one was isolated from the leaves and stems of *Hyptis verticillata* collected from Jamaica. The compound disrupted the oviposition and hatching of *Bophilus microplus* egg (Porter *et al.*, 1995). Moreover, Martin *et al.*, 2001 isolated 5 sesquiterpenoids, 4 flavonoids and *P*-comarate ethyl from plants of *Polygonum pimctalnm*. They also assayed acaricidal activity against *B. microplus.* In the present study, both isolated compounds , microdontin A/B and aloin A/B were found to have moderate acaricidal activity although microdontin A/B (EC_{50} = 89.40 mg/ml) showed a higher acaricidal activity than aloin A/B (EC_{50} = 257.69 mg/ml) (Table 5 and Fig. 21). Though both compounds have the anthrone moiety, microdontin A/B is less polar than aloin A/B due to the presence of *P*-coumaric ester as the part of its structure. The higher activity of microdontin A/B might be due to its less polarity than aloin A/B, and this can help the compound to penetrate the cell membrane and enter the cell much easily.

However, both compounds showed weaker acaricidal activity than the latex or Diazinon, a commercially used acaricide. This could be attributed to the existence of synergy between the isolated compounds or there may exist minor compound(s) in the latex with potent acaricidal activity. Though microdontin A/B and aloin A/B were reported to have many biological activities, including antimicrobial (Minale *et al.*, 2014), antimalarial (Geremedhin *et al*., 2014)

and antileishmanial (Abeje *et al*., 2014), this is the first report on the acaricidal activity of the two compounds.

5. CONCLUSION

The present study revealed that the leaf latex of *A. yavellana* possesses genuine acaricidal activity against *A. varigatum*, one of the most widely distributed tick species which causes tick-borne diseases with tremendous economic importance in livestock production in Ethiopia. The investigation also showed that the two major compounds of the latex namely, microdontin A/B and aloin A/B exhibit acaricidal activity against the larvae of *A. varigatum*. However, the activity of the pure compounds was found to be less than that of the latex indicating the possible existence of synergy between the isolated compounds or the presence of other minor compound(s) in the latex with potent acaricidal activity. The preliminary results reported here illustrate that the leaf latex of *A. yavellana* has the potential in the fight against ticks and tick-borne diseases.

6. RECOMMENDATIONS

Based on the results of present study, the following recommendations are drawn:

(1) Only the major compounds were identified from the leaf latex of *A. yavellan*. It is, therefore, worth isolating the other minor constituents, which might have better acaricidal properties.

(2) Other *In vitro* acaricidal test like Adult Immersion Test (AIT) and *In vivo* acaricidal studies on other ticks species have to be carried out

(3) An attempt should be made to establish the mode of action of the latex and isolated compounds.

(4) The possible toxicity of both the latex and isolated compounds must be investigated.

References:

Abdella N (2016) Important Cattle Ticks and Tick Born Haemoparasitic Disease in Ethiopia: A Review *Acta Parasitologica Globalis* **7 (1):** 12-20

Abeje F, Bisrat D, Hailu A, Asres K (2014). Phytochemistry and antileshmanial activity of the leaf latex of *Aloe calidophila* Reynolds. *Phytotherapy research* **28(12):** 1801-1805

Ahmed AN (2016). Review of economically Important Cattle Tick and its Control in Ethiopia. *Advances in Life Science and Technology* **42:** 51–64.

Ali M, De Castro JJ (1993). Host resistance to ticks (Acari Ixodidae) in different breed of cattle at Bako, Ethiopia. *Tropical Animal Health Production* **25:** 215–222.

Barker CS, Walker RA (2014). Ticks of Australia: The species that infest domestic animals and humans. Zootax **3816(1):**1-144 Magnolia Press Auckland, New Zealand pp 19 – 24.

Bekele D, Asfaw Z , Petros B, Tekie H (2012). Ethnobotanical study of plants used for protection against insect bite and for the treatment of livestock health problems in rural areas of Akaki District, Eastern Shewa, Ethiopia. *Journal of Herbal Medicine* **1(2)**: 12–24.

Bussmann WR, Swartzinsky P, Worede A, Evangelista P (2011). Plant use in Odo-Bulu and Demaro, Bale region, Ethiopia. *Journal of Ethnobiology and Ethnomedicine* **7:**28

Catley A, Mohammed AA (1995a). Ethnoveterinary knowledge in Sanaag region, Somaliland (Part I): notes on local descriptions of livestock diseases and parasites. *Nomadic Peoples* **36(37):** 3–16.

Catley A, Mohammed AA (1995b). Ethnoveterinary knowledge in Sanaag region, Somaliland (Part II): Notes on local descriptions of livestock diseases and parasites. *Nomadic Peoples* **39:** 135–145.

Catley A, Ahmed AA (1996). Use of participatory rural appraisal (PRA) tools for investigating tick ecology and tick-borne disease in Somaliland. *Tropical Animal Health and Production*, **28:** 91-98.

Chen W, Ben-Erik VW, Ilze V, Alvaro MV (2012). Cape aloes—A review of the phytochemistry, pharmacology and commercialisation of *Aloe ferox*. *Phytochemistry Letters* **(5):**1-12

Cock IE (2015). The genus Aloe: Phytochemistry and therapeutic uses including treatments for gastrointestinal conditions and chronic inflammation. Progress in Drug Research. pp 179-–235.

Dagne E, Bisrat D, Viljoen A, Wyk B (2000). Chemistry of *Aloe* Species. *Current Organic Chemistry* **4**: 1055–1078.

Demissew S, Gilbert MG (1997). The family Aloaceae. In: Edwards, Demissew S and Hedberg, I. (ed.), *Flora of Ethiopia and Eritrea*. Vol. 6, Addis Ababa and Uppsala.

Demissew D, Dioli M (2000). A new *Aloe* (Aloaceae) species from Ogaden (Southeastern Ethiopia). *Kew Bulletin* **55**: 679–682.

Demissew S, Nordal I (2010). Flora of Ethiopia and Eritrea. Aloes and Lilies of Ethiopia and Eritrea, pp 102

Demissew S, Gilbert MG. (2000). A new *Aloe* (Aloaceae) species from (Southwest Ethiopia). *Kew Bulletin* **55**: 683–686.

Demissew S, Friis IB, Awas T, Wilkin P, Weber O, Bachman S, Nordal I (2011) Four new species of Aloe (Aloaceae) from Ethiopia, with notes on the ethics of describing new taxa from foreign countries *Kew Bulletin* **66(1):** 111-121

Demissew S, Nordal I, Stabbetorp OE (2001). Patterns of endemism of the genus Aloe in the Flora of Ethiopia and Eritrea. *Biologiske Skrifter* **54:** 233-246

Ding L, Ding W, Zhang Y, Luo J (2010). Bioguided fractionation and isolation of esculentoside P from *Phytolacca Americana*. *Indiana Crop Production.* **44:** 534–541.

Farah MH, Andersson R, Samuelsson G (1992). Microdontin A and B: two new aloin derivatives from *Aloe microdonta. Planta Medica* **58**: 88–93.

Food and Agricultural Organization (1998). Regional Coordination for integrated tick Born disease control program in eastern and central Africa(Ed)

Food and Agricultural Organization. (1984). Ticks and tick-borne disease control: a practical field manual. Vol. 1: Chapter 5: Acaricide resistance: 264–297, FAO, Rome.

Gebre S , Kaaya GP (1998). Comparisons of resistance in three breeds of cattle against African Ixodida ticks. *Experimental and Applied Acarology* **20:**223–230.

Gebreab F (1983). Notes on ticks species and tick birn disease of domestic animals in Ethiopia Faculty of Veterinary Medicine Addis Ababa University, Debrezeiet Ethiopia

Gebremedihn G, Bisrat D, Asres K (2014). Isolation, Characterization and *In –vivo* antimalarial evaluation of anthrons from latex of *Aloe percrassa* Todaro *Journal of Natural Remedies* **14(2):** 119–126.

Garcia MV, Matias J, Barros JC, de Lima DP, Lopes RD, Andreotti R (2012) Chemical identification of *Tagetes minuta* Linnaeus (Asteraceae) essential oil and its acaricidal effect on ticks. *Revista Brasileira De Parasitologia Veterinaria* 21(4): 405-411

Grace MO, Simmonds SJM, Smith FG, VanWyk EA (2009). Documented Utility and Bicultural Value of Aloe L. (Asphodelaceae): A review. *Economic Botany* **63(2):** 167–178.

Grønvold J, Henriksen SA, Larsen M, Nansen P, Wolstrup J (1996) Biological control. Aspects of biological control: with special reference to arthropods, protozoans and helminths of domesticated animals, *Veterinary Parasitology*, **64(1-2)**: 47–64.

Hoogstraal H (1956). African Ixodidae, Vol. **1**. Ticks of the Sudan with special reference to Equatoria Province and with preliminary reviews of the genera *Boophilus,Margaropus* and *Hyalomma.* Department of the Navy, Bureau of Medicine and Surgery, United States Government Printing Office, Washington D.C., pp1–101.

Hossain SMD, Mamun-Or-Rashid ANM, Towfique N, Sen KM (2013) Areview on Ethnopharmacological Potential of *Aloe Vera L. Journal of Intercultural Ethnopharmacology* 2**(2):** 113-120

Jongejan F, Lemche L, Mwase ET, Kafunda MM (1986). Bovine babesiosis (Babesia bovis infection) in Zambia. *Veterinary Quarterly* **8(2):** 168–171.

Jongejan F (2007) The Global Importance of Tick. Integrated Consortium on Ticks and Tick born diseases ICTTD http://www.icttd.nl/index.php?id=2

Jongejan F, Uilenberg (1994). Ticks and Control Methods *Revue Scientifique et Technique Office International des Epizooties* **13(4):** 1201–1226.

Kahn CM, (2003). Ticks: Introduction The Merck veterinary manual (Ed) Whitehouse Station, NJ: Merck and Co USA.

Kaufman P E, Koehler P G, Butler J F (2006). External Parasites on Beef Cattle. Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida http://edis.ifas.ufl.edu/IG130

Kelly P, Lucas H, Beati L, Yowell C, Mahan S, Dame J (2010). *Rrickettsia Africae* in *Amblyomma variegatum* and domestic ruminants on eight Caribbean Islands. *Journal of Parasitology* **96 (6):** 1086–1088.

Laffont CM, Alvinerie M, Bousquet-Melou A, Toutain PL (2001). Licking behavior and environmental contamination arising from pour-on ivermectin for cattle. *International Journal of Parasitology* **31(14):** 1687–1692.

Lam Y (2012) Research on the Pharmacological Development of Aloe *Traditional Chines Medicine* **1:** 30-36

Latif A, Jongejan F (2002). The wide use of acaricide for the control of livestock diseases in Africa needs a reappraisal. Proceedings of the joint ICTTD-2/ICPTV workshop on integrated vector control including synergistic use of drugs and bait technologies for the control of trypanasomosis and tick borne diseases, held on 10- 12th April 2002 at the institute of tropical medicine, Anturerp, Belgium.

Madder M, Horak H, Stoltsz H (2012). Ticks: Tick identification Licensed under a Creative Commons Attribution license. www.oie.int/doc/ged/D8941.PDFaccecses d 6/12/2016

Mascolo N, Izzp AA, Borrelli F, Capasso R, DI Carlo G, Sautebin L, Capasso F (2004). Healing powers of aloes: in Aloes in habitat; Aloes: the genus Aloe / edited by Tom Reynolds, Medicinal and Aromatic plants – Industrial profile, 38**:** pp 232 by CRC Press Boca Raton London New York Washington, D.C. ISBN 0-415-30672-8.

Martin CJ, Torres F, Quinomes W, Cheverri F (2001) Phytochemistry and evaluation of biological action of *Polygonum punctatum. Revista Eantinoamericana de Quimica* **29(2):** 100- 107

Mawela KG (2008). The toxicity and repellent properties of plant extracts used in ethnoveterinary medicine to control ticks M.Sc. Thesis, University of Pretoria.

Mekonnen S, Hussein I, Bedane B (2001). The distribution of ixodidea ticks in Ethiopia. *Onderstepoort Journal of veterinary Research* **68:**243– 251.

Mekonnen S (2002). Acaricide resistance profiles of single and multi-host ticks in commercial and communal farming areas in the Eastern Cape and North-West Provinces of South

Africa MSc thesis Department of Veterinary Tropical Diseases, faculty of veterinary science, University of Pretoria, South Africa.

Mesfin F, Demissew S, Teklehaymanot T (2009). An ethnobotanical study of medicinal plants in Wonago Woreda,SNNPR, Ethiopia. *Journal of Ethnobiology and Ethnomedicine* **5:**28.

Mesfin T, Obsa T (1994). Ethiopian traditional veterinary practices and their possible contribution to animal production and management. *Review Science and technology of International Epiztoqu*. **13(2):** 417–424.

Minale G, Bisrat D, Asres K Mazumder A (2014). *In vitro* antimicrobial activities of anthrones from the leaf latex of *Aloe sinana* Reynolds. *International Journal of Green Pharmacy* **8(1):**7–12.

Moges N, Bogale B (2012). Assessment of Major Animal Production and Health Problems of Livestock Development in Lay-Armacheho District, Northwestern Ethiopia. *American-Eurasian Journal of Scientific Research* **7(3):** 136–141.

Morton FJ (1961). Folk Uses and Commercial Exploitation of Aloe Leaf Pulp. *Economic botany* **15(4):**311-319.

Moyo B, Masika PJ (2009) Tick control methods used by resource-limited farmers and the effect of ticks on cattle in rural areas of the Eastern Cape Province, South Africa. *Tropical Animal Health and Production,* **41:** 517-523.

Moyo B, Masika PJ (2013). Validation of the acaricidal properties of materials used in ethno-veterinary control of cattle ticks. *African Journal of Microbiological Research* **7(39):** 4701–4706.

Park IY, Lee Seung (2006). Overview of aloe studies, Historic background of Aloe as folk medicine In: New Perspectives on Aloe (Ed) pp.1–10.

Park BS, Lee SE, Choi WS, Jeong CY, Song C, Cho KY. (2002) Insecticidal and acaricidal activity of pipernonaline and piperoctadecalidine derived from dried fruits of *Piper longum* L. *Crop Protection* **21:** 249–251.

Parola, P, Raoult, D (2001). Ticks and tick-born bacterial diseases in humans: An emerging infectious threat. *Clinical infectious Diseases* **32:** 897–928.

Pegram RG, Tatchell RJ, de Castro JJ, Chizyuka HGB, Creek MJ, McCosker PJ, Moran MC, Nigarura G. (1993). Tick control: new concepts. *World Animal Review,* **75(2):** 2-11.

Pegram G, Eddy C (2002). Progress towards the eradication of *Amblyomma varigatum* from Caribbean. *Experimental and Applied Acarology* **28:** 273–281.

Porter RB, Reese PB, Williams LA, Williams DJ (1995) Acaricidal and Insecticidal Activities of Cadina-4, 10(15)- dien- 3one. *Phytochemistry* **40(3):** 735-738

Rajput RI, Hu S, Chen W, Arijo A, Xiao C (2006). Importance of ticks in their chemical and immunological control in livestock. *Journal of Zheiiang University,* **7:** 912–921

Regassa A (2000). The use of herbal preparations for tick control in western Ethiopia. *Journal of the South African Veterinary Association* **71(4):** 240–243

Reynolds T (2004) Aloe. In: Reynolds T (ed) Aloes the genus Aloe. CRC Press, Boca Raton, pp 39–74

Reynolds T, Dweck AC (1999). Aloe vera leaf gel: A review Update. *Journal of Ethnopharmacology* **68:** 3–37.

Smith GF, Wyk BE (1991) Generic relationships in the Alooideae (Asphodelaceae). *Taxon* **40:** 557–582.

Steenkamp V, Stewart MJ (2008). Medicinal Applications and Toxicological Activities of *Aloe* Products. *Article in Pharmaceutical Biology* **(45):** 411–420.

Tamiru F, Terfa W, Kebede E, Dabessa D, Roy RK, Sorsa M (2013). Ethnoknowledge of plants used in veterinary practices in Dabo Hana District, West Ethiopia. *Journal of Medicinal Plant Research* **7(40):**2960–2971.

Uilenberg G (2005). Integrated control of tropical animal parasites. *Tropical Animal Health and Production Journal* **28:** 257–265.

United States Department of Agriculture (1967). Safe use of Agricultural and house hold Pesticide. *In:* Agriculture hand book, No. 321 Washington, D.C., p. 65.

Van der Bank FH, Van Wyk B E, van Der Bank M (1995): Genetic variation in two economically important *Aloe* species (Aloaceae). *Biochemcal System and Ecology* **23:** 251–256.

Wabuyele E (2000). A systematic assessment of the relationships between *Aloe macrocarpa* Tod. and *A. lateritia* Engl. M.Sc. Thesis, Addis Ababa University.

Walker AR, Bouattour JL, Camicas A, Estrand-Pena A, Horak IG, Latif AA, Pegram RG, Preton PM (2003). Ticks of domestic animals in Africa: A guide to identification of species, Bioscience Report, The University of Edinburgh, Scotland, UK.

Wall R (2007). "Ectoparasites: future challenges in a changing world. *Veterinary Parasitology* **148:** 62–74.

Wei J, Ding W, Zhao YG, Vanichpakorn P (2011). Acaricidal activity of *Aloe vera L.* leaf extracts against *Tetranychus cinnabarinus* (Biosduval)(acarina: Tetranychidae). *Journal of Asia-pacific Entomology* **14:**353–356

Yarnell E, Abascal K, Hooper GC (2003) Clinical Botanical Medicine Revised and Expanded 2nd ed, Mary Ann Liebert publisher,USA

Young AS, Groocock CM, Kariuki DP. (1988). Integrated control of ticks and tick borne diseases of cattle in Africa. *Parasitology* **96:** 403-432.

Zhang Y, Ding W, Zhao Z, Wu J, Fan Y. (2008) Studies on acaricidal bioactivities of *Artemisia annua L*. extracts against *Tetranychu scinnabarinus* Bois. (Acari: Tetranychidae). *Agricultural Science China* **7:**577–584.

Zorloni A, Penzhorn BL, Eloff JN (2010). Extracts of *Calpurnia aurea* leaves from southern Ethiopia attract and immobilize or kill ticks. *Veterinary Parasitology* **168:** 160–164.

Appendix 1 Collection, drying and preparation of Latex from *Aloe Yavellana*

Appendix 2 Rearing, incubation and maintenance of *Amblyomma varigatum*

Appendix 3 Conducting larval picket test (LPT) bioassay *Aloe yavellana* againest *Amblyomma Varigatum*