2019 SCIENCELINE
Asian Journal of Medical and Pharmaceutical Researches
***Asian J Med Pharm Res,* 9**(1): 1-10, Jan 25, 2019 ISSN 2322-4789

Extraction and Phytochemical Screening of *Culpurina Aurea* Leaves and Their Effect against Malopaagus Ovinus

Ambaye Kenubih[1], Mikru Belay[2] and Nesibu Awol[2], Mastewal Birhan[*1]

[1]*College of Veterinary Medicine and Animal science, Department of veterinary Paraclinical studies, University of Gondar, Gondar, Ethiopia*
[2]*College of Agriculture and Animal science, Department of Veterinary Medicine studies, Wello University, Ethiopia*

[*]Corresponding author's email: maste675@gmail.com; ORCID: 0000-0002-0984-5582

ORGINAL ARTICLE
PII: S2322-4789190001-9
Received: 18 Dec 2018
Accepted: 01 Jan 2019

ABSTRACT: This study investigated the acaricidal effects of methanolic and ethanolic crude extracts of *Calpurnia aurea* against the Melophagus ovinus or sheep ked at different time intervals using in vitro adult immersion test. For this study, Melophagus ovinus was collected from naturally infested sheep from Ambageiorgis district, central Gondar zone, Amhara region and placed into petri dishes (n=10). Both methanolic and ethanolic crude extracts of *Calpurnia aurea* were treated with n-hexane, Dichloromethane, Ethyl acetate and Water for fractionation and tested for their insecticidal activity against M. ovinus. At concentration of 6.25, 12.5, 25, 50 and 100mg/ml, all fractionates of methanol and ethanol crude extracts showed 100% effectiveness against M. ovinus within 6 hours of exposure. However, mortality of M. ovinus increased as concentration and exposure time to methanol and ethanol extracts of different fractionates increased within the first one hour. On qualitative phytochemical investigations, the crude extracts of *Calpurnia aurea* had alkaloids, flavonoides, phenolic compoundes, saponin and and tannins. The results of this study proved the acaricidal effect of all fractionates of methanol and ethanol crude extracts of *Calpurnia aurea* against Melophagus ovinus. Hence, *Calpurnia aurea* can be considered as a potential candidate for biocontrol of Melophagus ovinus and for the discovery of active compounds that substitute commercially available acaricides.

Keywords: *Calpurnia Aurea;* Chromatography; Ectoparasites; Mallophagus Ovinus

INTRODUCTION

Ethiopia has the largest number of livestock in Africa. Sheep represent the most important segment of the Ethiopian livestock system. The national sheep population is estimated at 25.9 million (Central Statistical Agency, 2013). Annually, 16.6 million pieces of skins are produced in the country, based on the off take rates of 33% for sheep (Tadesse, 2005). The exportation of skin and hide is the largest foreign exchange earns in Ethiopia and the leather industry is one of the fourth growing economic sectors (National Bank of Ethiopia, 2000). However, this sector and the country as whole lost revenue due to a decline in quality and fall in export prices (Kassa, 2005). The majority of leather defects in the factory are due to damage at pre slaughter stage of production on the live sheep, a considerable portion of which are due to skin diseases caused by external parasites (Kassa et al., 1998). In Ethiopian tanneries, 35% of sheep skins have been downgraded and rejected due to defects caused by external parasites (Kassa, 2005). External parasites are very common and widely distributed in all agro ecological zones in Ethiopia (Kumsa et al., 2012). External parasites pose serious economic losses to the farmer, the tanning industry and the country as a whole (Berhanu et al., 2011). External parasites cause skin lesions or defects in all layers of the skin. However, the extent and type of gross and microscopic skin lesions may vary with the specific causes of skin diseases (Wall and Shearer, 1997). Mallophagus ovinus is among the most economically important external parasite of sheep (Wall, 2007; Mersha et al., 2010; Berhanu et al., 2011; Kumsa et al., 2012). Heavily M. ovinus infestation in sheep results in weakness, unthritiness, weight loss, anemia, and wool staining and reduced resistance to diseases (Abadi, 2000 and Wall, 2007). Moreover, presence of chemical residues in meat, milk and the environment has also prompted interest in finding new alternatives. In addition to these, the accessibility and affordability to the poor farmers makes them less preferable compared to other alternatives (Robert et al., 2010). The majority of farmers and pastoralists in the developing countries rely on traditional health care practices to keep their livestock healthy. These indigenous practices include the use of medicinal plants or Ethno-Veterinary Medicine (EVM) (Robert et al., 2010). The application of botanicals to livestock to control ectoparasites of veterinary importance is widespread in the developing countries (Robert et al., 2010). In contrast, to

To cite this paper: Kenubih A, Belay M and Awol N, Birhan M (2019). Extraction and Phytochemical Screening of *Culpurina Aurea* Leaves and Their Effect against Malopaagus Ovinus. *Asian J. Med. Pharm. Res.*, 9 (1): 1-10.

chemical acaricides, botanical acaricides have many advantageous features of being degraded in the environment, not remaining in livestock, not prone to resistance, and are relatively safe for humans, animals, the environment (Alawa et al., 2003). They have also greater accessibility with lower costs and apparent effectiveness (Mwale et al., 2005). *C. aurea* is one of the most commonly used medicinal plants to control external parasite infestation. However, limited research work is conducted on the effectiveness of *C. aurea* against the most common external parasite of sheep, sheep ked.

MATERIALS AND METHODS

Study Area

This experimental study was conducted from October to April in Gondar, Amhara region Ethiopia. Gondar is located 740 km northwest of Addis Ababa and 175 km north of Bahir Dar the capital city of Amhara region. The climatic condition of the area is 'weyna dega' with an altitude of 2220 meter above sea level. Its annual temperature is 19.3 and annual rain fall is 1153mm. The study plant *C. aurea* was collected. From Azezo district, central Gondar zone of Amhara region. Azezo district is, located 738km northwest of Addis Ababa and 173 km north of Bahir Dar. The climatic condition of the area is 'weyna dega' with an altitude of 2220 meter above sea level. Its annual temperature is 19.3 and annual rain fall is 1153mm.

Study Design

The experimental study was conducted from October 2017 to May 2018 to evaluate accaricidal efficacy of crude methanol and ethanol extracts of *C. aurea* leaf extract of against *M. ovinus* by using in vitro adult immersion test.

Plant identification and Preparation

Collection and grinding

C. aurea leaf was collected from its natural habitat Azezo district, central Gondar zone of Amhara region and transported to University of Gondar; Collage of computational and natural science, department of botany at the morning. The plant leaves were selected on the basis of reports on their traditional uses against various ectoparasites (Bekele et al., 2012). The plant sample was authenticated at university of Gondar, Collage of computational and natural science, department of botany and the specimen was preserved for further reference, after that the collected leaves were washed with distilled water to remove dirt and soil particles and kept for two weeks under shed to be dried within pharmacology laboratory and after completely desiccating, the leave were transported to chemistry laboratory for grinding by electrical grinder.

Plant Processing and Extraction

Four hundred gram finely grinded *C. aurea* leaf powder were soaked in to 80% methanol and 80% ethanol and vigorously shacked to mix the powder with the alcohol. The level of alcohol was added until it covered the powder. In order to prevent settling, repeated agitation and shaking was done within six hour interval. After three days the extract was filtered with funnel filtration with gauze and resoaked with similar alcohol. These activities were repeated for three times and after nine days; the settled mixture was squeezed by placing in the gauze to obtained active ingredients which are not filtered during these three filtrations. These methanol and ethanol extracts were kept in different bottles. These bottles were labeled and stored at +4^{o}C until further processing. Then, the extracts were evaporated by rotary vaporizer, at 60^{o}C for methanol extract and 68^{o}C for ethanol extract. After complete evaporation of alcohol, the crude was lyophilized for complete removal of the remaining water in the crude.

Phytochemical screening

The qualitative phytochemical investigations of the crude extract of ethanol and methanol fractions of *C. aurea* leaves were carried out using standard tests performed according to Ayoola et al. (2008) and Farhan et al. (2012).

Test for terpenoids (Salkowski test)

To 0.25 g of each solvent extracts of *C. aurea* leaves, 2 ml of chloroform was added. Then, 3 ml concentrated sulfuric acid was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids.

Test for saponins

To 0.25 g of, each solvent extracts 5 ml of distilled water was added in a test tube. Then, the solution was shaken vigorously and observed for a stable persistent froth. Formation of froth indicated the presence of saponins

Test for tannins

About 0.25 g of each solvent extract was boiled in 10 ml of water in a test tube and then filtered. Then, a few drops of 0.1% ferric chloride was added to the filtrate. The formation of blue, blue-black, green or blue-green coloration or precipitation was taken as evidence for the presence of tannins.

Test for flavonoids

About 10ml of ethyl acetate was added to 0.25 g of each solvent extracts and heated on a water bath for 3 min. The mixture was cooled and filtered. Then, about 4 ml of the filtrate was taken and shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and the formation of yellow color in the ammonia layer indicated the presence of flavonoids.

Test for anthraquinones (Borntrager's Test)

About 0.5 g of sample of each solvent extract was shaken with 5 ml of chloroform and filtered. A 10% ammonium hydroxide solution (5ml) was added to the filtrate, and the mixture was shaken. The presence of a pink, red or violet color in the ammoniac phase was taken as an indication of the presence of anthraquinones.

Test for polyphenols

To 5 ml of the aqueous solution of the each solvent extracts, 1 ml of FeCl3 (1%) and 1 ml K3 (Fe (CN) 6) (1%) were added. The appearance of fresh reddish blue color indicated the presence of polyphenols.

Thin- layer chromatography

Thin Layer Chromatography (TLC) is a useful technique to determine bio active compounds in the plant Extract. TLC combines chromatography separation and in situ activity determination facilitating localizes and target directed isolation of active consistence in a mixture. Extracts obtained from four fractionates of ethanol and methanol crude were chromatographed on 0.2-mm silica layer, with a mixture of benzene, ethanol and ammonia (16:2:0.2 v/v) as a mobile phase. Samples were applied with automatic TLC applicator with solvent evaporation. The samples were kept for one and half hour and removed from the mobile solvent and the spots formed on the TLC plates was visualized under Ultra Violet (UV) irradiation light at 254 nm and 360 nm and measured. The plats which were not visualized were stained by iodine and examined.

Parasite collection, area and identification

M. ovinus were collected from naturally infested sheep in Ambageiorgis district, 30 km in the north east direction to Gondar town. After collection it was transported to University of Gondar microbiology laboratory by petridish covered with gauze in sample box.

Adult Immersion Test

In vitro acaricidal efficacy test

In vitro adult immersion tests were carried out to evaluate the efficacy of *C. aurea* against *M,ovinus*. The in vitro tests were started within 60 min after sheep ked collection (Heukelbach et al, 2006). Within one Petri dish, ten active *M. ovinus* parasites were counted and placed (Levot, 2000). One milliliter of each fractionated extract was added to five different petri dishs containing ten active *M. ovinus* each. 1ml of 2% tween 80m were used as negative control. 1ml of (0.1% of ivermectin) was used for positive control (Heukelbach et al., 2006). The experiment was performed in five serial dilutions for each fractionation per treatment petri dish (Gemeda et al., 2014). After 2 minutes of contact time, the extracts were filtered using what man filter paper (Khater et al, 2013). All plates exposed to each concentration of the plant extract and control plates were incubated at 27 ^{o}C and 80% humidity. The total incubation period was 6 hr (Levot, 2000). Immersed *M. ovinus* were examined, under a stereoscope, after 30 min, 1hr and 6hr and death of *M. ovinus* were recorded in each time interval. Death of *M. ovinus* was defined as the lack of limb movement, and failure to respond when the legs were stroked with a needle (Khater et al, 2013). The percentage mortality was calculated by using a formula given (Krishnaveni and Venkatalakshmi, 2014)

Mortality%=number of death ked/total number of ked*100.

Data Analysis

Collected raw data were stored in Microsoft Excel database system used for data management. SPSS windows version 20 was used for data analysis. Results of the study were expressed as a mean of mortality percentage ± standard error (Mean ± SE). Statistical significance was determined by one-way analysis of variance (ANOVA) with multiple comparison tests (Post Hoc/Tukey test) to compare parameter within and between groups. Insecticidal activities were considered to significantly difference when 95% confidence limit levels failed to overlap or All significant levels set at $P<0.05$.

RESULTS

The insecticidal activity of methanol and ethanol extracts of *C. aurea* at concentration of 6.25, 12.5,25,50 and 100mg/ml against adult *M. ovinus* of sheep is displayed in table 1 and 2. The results indicate that *C. aurea* with different concentrations in different time exposures have different insecticidal effect. Mortality of sheep ked increased as concentration and exposure time to methanol and ethanol extracts of different fractionates increased within the first hour. However, 100% mortality was recorded with different concentration in 6 hr exposure. Statistical results (one way ANOVA) have demonstrated

significantly higher insecticidal activity of different concentration for different fractionates of methanol extracts ($P<0.05$). After 30 min exposure of 100mg/ml ethylacetate, n- hexane, dichloromethane and water have ascending order of efficacy. For 50 mg/ml n-hexane and dichloromethane have greater effect than ethyl acetate and water. At 25mg/ml dichloromethane and n- hexane have better effect than ethyl acetate and water. At 12.5mg/ml n-hexane, dichloromethane and water have good effect than ethylacetate. At 6.25 mg/ml n-hexane, dichloromethane and water are more effective than ethylacetate. After one hr exposure for 100mg/ml dichloromethane and ethyl acetate have advanced effect than n- hexane and water. At 50mg/ml dichloromethane, n-hexane, ethylacetate water have descending effect. At 25mg/ml dichloromethane have better effect to n-hexane, ethyl acetate and water. At 12.5mg/ml ethyl acetate and dichloromethane have greater effect than water and n-hexane. For 6.25mg/ml dichloromethane, n-hexane and ethyl acetate show better effect than water. The maximum anti parasitic activity within the first 30 min was exerted by n-hexane extracts and after 1hr exposure dichloromethane and n-hexane exerts comparable efficacy. 100% mortality in all fractionates and concentrations attained after 6 hr exposure. Both methanol and ethanol extracts with different solvent fractionates show almost similar effect.

Table 1. Mean mortality percentages for the assay of malopaagus Ovinus, using different fractionate concentrations of crude for methanol extracts of C. aurea

Fraction	Concentration	Mortality			P-value
		30min	1hr	6 hr	
Ethyl acetate	100mg/ml	66.67±8.819	80±5.774	100±0.00	0
	50mg/ml	50.00±5.77 4	70±5.774	100±0.00	
	25mg/ml	40±5.77 4	50.00±5.77 4	100±0.00	
	12.5mg/ml	30±5.77 4	50.00±5.77 4	100±0.00	
	6.25mg/ml	26.67±6.667	36.67±12.019	100±0.00	
Dichloromethane	100mg/ml	60±5.774	80±5.774	100±0.00	0
	50mg/ml	50±5.77 4	76.67±3.333	100±0.00	
	25mg/ml	46.67±3.333	66.67±3.333	100±0.00	
	12.5mg/ml	36.67±3.333	50.00±5.77 4	100±0.00	
	6.25mg/ml	30.00±5.77 4	46.67±3.333	100±0.00	
n-hexane	100mg/ml	63.33±3.333	73.33±3.333	100±0.00	0
	50mg/ml	56.67±3.333	70.00±5.77 4	100±0.00	
	25mg/ml	46.67±3.333	53.33±3.333	100±0.00	
	12.5mg/ml	40.00±0.00	40.00±5.77 4	100±0.00	
	6.25mg/ml	30.00±5.774	36.67±5.774	100±0.00	
Water	100mg/ml	53.33±3.333	53.33±3.333	100±0.00	0
	50mg/ml	43.33±3.333	50.00±5.774	100±0.00	
	25mg/ml	40.00±5.774	46.67±3.333	100±0.00	
	12.5mg/ml	36.67±3.333	46.67±3.333	100±0.00	
	6.25mg/ml	30.00±10.00	35.00±5.00	100±0.00	
Positive control	0.01% ivermectin	53.33±3.333	53.33±3.333	100±0.00	
Negative control	2%tween80	0	0	0	

Table 1. Continues

Fraction	Concentration	Mortality			P-value
		30min	1hr	6 hr	
Ethyl acetate	100mg/ml	66.67±3.333	76.67±3.333	100±0.00	0
	50mg/ml	60.00±5.774	66.67±3.333	100±0.00	
	25mg/ml	40.00±0.00	46.67±3.333	100±0.00	
	12.5mg/ml	36.67 ±3.333	36.67±8.819	100±0.00	
	6.25mg/ml	53.33±3.333	53.33 ±3.333	100±0.00	
Dichloromethane	100mg/ml	70.00±5.774	83.33±3.333	100±0.00	0
	50mg/ml	63.33±3.333	63.33±3.333	100±0.00	
	25mg/ml	53.33±3.333	60.00±5.774	100±0.00	
	12.5mg/ml	43.33±3.333	46.67±3.333	100±0.00	
	6.25mg/ml	36.67±3.333	40.00± 5.774	100±0.00	
n-hexane	100mg/ml	67.67±6.667	83.33±3.333	100±0.00	0
	50mg/ml	60.00±5.774	73.33 ±3.333	100±0.00	
	25mg/ml	56.67±5.774	56.67 ±3.333	100±0.00	
	12.5mg/ml	46.67±3.333	5.00+ 5.774	100±0.00	
	6.25mg/ml	30.00±5.774	40.00±5.774	100±0.00	
Water	100mg/ml	63.33±3.333	66.67±3.333	100±0.00	0
	50mg/ml	50.00±10.00	66.67±3.333	100±0.00	
	25mg/ml	36.67±3.333	43.33±3.333	100±0.00	
	12.5mg/ml	36.67±3.333	43.33±3.333	100±0.00	
	6.25mg/ml	30.00±0.00	43.33±3.333	100±0.00	
Positive control	0.01% ivermectin	53.33±3.333	53.33±3.333	100±0.00	
Negative control	2%tween80	0	0	0	

Table 2. Mean mortality percentages for the assay of sheep ked parasite, using different fractionate concentrations of crude for ethanol extracts of *C. aurea*

Fraction	Concentration	Mortality			P-value
		30min	1hr	6 hr	
Ethyl acetate	100mg/ml	66.67±3.333	76.67±3.333	100±0.00	0
	50mg/ml	60.00±5.774	66.67±3.333	100±0.00	
	25mg/ml	40.00±0.00	46.67±3.333	100±0.00	
	12.5mg/ml	36.67 ±3.333	36.67±8.819	100±0.00	
	6.25mg/ml	53.33±3.333	53.33 ±3.333	100±0.00	
Dichloromethane	100mg/ml	70.00±5.774	83.33±3.333	100±0.00	0
	50mg/ml	63.33±3.333	63.33±3.333	100±0.00	
	25mg/ml	53.33±3.333	60.00±5.774	100±0.00	
	12.5mg/ml	43.33±3.333	46.67±3.333	100±0.00	
	6.25mg/ml	36.67±3.333	40.00± 5.774	100±0.00	
n-hexane	100mg/ml	67.67±6.667	83.33±3.333	100±0.00	0
	50mg/ml	60.00±5.774	73.33 ±3.333	100±0.00	
	25mg/ml	56.67±5.774	56.67 ±3.333	100±0.00	
	12.5mg/ml	46.67±3.333	5.00+ 5.774	100±0.00	
	6.25mg/ml	30.00±5.774	40.00±5.774	100±0.00	
Water	100mg/ml	63.33±3.333	66.67±3.333	100±0.00	0
	50mg/ml	50.00±10.00	66.67±3.333	100±0.00	
	25mg/ml	36.67±3.333	43.33±3.333	100±0.00	
	12.5mg/ml	36.67±3.333	43.33±3.333	100±0.00	
	6.25mg/ml	30.00±0.00	43.33±3.333	100±0.00	
Positive control	0.01% ivermectin	53.33±3.333	53.33±3.333	100±0.00	
Negative control	2%tween80	0	0	0	

Table 3. Phytochemical constitutes and Mortality rate within the certain concentration and given time value

Fraction	Concentration	Mortality			P-value
		30min	1hr	6 hr	
Ethyl acetate	100mg/ml	66.67±3.333	76.67±3.333	100±0.00	0
	50mg/ml	60.00±5.774	66.67±3.333	100±0.00	
	25mg/ml	40.00±0.00	46.67±3.333	100±0.00	
	12.5mg/ml	36.67 ±3.333	36.67±8.819	100±0.00	
	6.25mg/ml	53.33±3.333	53.33 ±3.333	100±0.00	
Dichloromethane	100mg/ml	70.00±5.774	83.33±3.333	100±0.00	0
	50mg/ml	63.33±3.333	63.33±3.333	100±0.00	
	25mg/ml	53.33±3.333	60.00±5.774	100±0.00	
	12.5mg/ml	43.33±3.333	46.67±3.333	100±0.00	
	6.25mg/ml	36.67±3.333	40.00± 5.774	100±0.00	
n-hexane	100mg/ml	67.67±6.667	83.33±3.333	100±0.00	0
	50mg/ml	60.00±5.774	73.33 ±3.333	100±0.00	
	25mg/ml	56.67±5.774	56.67 ±3.333	100±0.00	
	12.5mg/ml	46.67±3.333	5.00+ 5.774	100±0.00	
	6.25mg/ml	30.00±5.774	40.00±5.774	100±0.00	
Water	100mg/ml	63.33±3.333	66.67±3.333	100±0.00	0
	50mg/ml	50.00±10.00	66.67±3.333	100±0.00	
	25mg/ml	36.67±3.333	43.33±3.333	100±0.00	
	12.5mg/ml	36.67±3.333	43.33±3.333	100±0.00	
	6.25mg/ml	30.00±0.00	43.33±3.333	100±0.00	
Positive control	0.01% ivermectin	53.33±3.333	53.33±3.333	100±0.00	
Negative control	2%tween80	0	0	0	

Table 4. Phytochemical analysis of the leaves of *C. aurea*

Chemical constitute	Type of plant
	C. aurea
Tannins	+
Saponins	+
Flavonoid	+
Phenol	+
Alkaloids	+

Thin layer chromatography

During visualization under ultra-violate radiation light at 254 nm there were different color band movement on the plat and the invisible bands were stained by iodine and examined under UV. The different color bands move over the plat have different color weight.

DISCUSION

The findings of this research have confirmed that different fractionates of both methanolic and ethanolic extracts have almost similar anti-parasitic activity against M. *ovinus*. Even though insecticidal effect of the extracts depends on exposure time and concentration, 100% mortality was recorded after six hours of exposure. The mortality of the parasite may be due to the complex mixture of secondary metabolites like tannin, saponin, alkaloid, phenolic compounds and flavonoids (Koul et al., 2008). Plants which have chemical compositions similar to this plant may have anti parasitic activity. The difference between different fractionates of both methanol and ethanol extracts may be due to different chemical composition of the active ingredients (Megalla et al., 1980). The secondary metabolites might be toxic to the parasite in combination or individually (George et al., 2009). Above 70% mortality of the parasite was recorded at 100% concentration with 30 minutes exposure. Except 6.25mg/ml, all fractionates of

methanol and ethanol with all concentrations has almost similar effect with positive control ivermectin. Extracts of C. *aurea* have been used to treat scabies (Jansen, 1981), for tick control (Regassa, 2000). Additionally, there are reports regarding the antibacterial and antioxidant activity of C. *aur*ea (Adedapo et al., 2008), it is also used to treat bacterial dermatitis (Tadeg et al., 2005). These reports indicate the broad spectrum activity and the common traditional and medicinal value of the plant. These activities against different micro-organisms indicate that the plant contains different chemicals that can act on different parts of the microbes. These broad spectrum activities may be due to the potential effects of secondary metabolites. Crude extracts having similar secondary metabolites might have a potential to treat similar diseases. These might be the potency of the chemicals in the plant and comparable anti parasitic effect of the different fractionates have things in common. The phytochemical composition of the acetone extracts of C.*aurea* showed hypotensive effect of intravenous injection to normotensive rats (Riedel, 2010). This might be the cause that different concentrations had similar effect that can relax the vessels of M.*ovinus* and induce to take more active ingredient within short period of time after being induced into the system. N-hexane and acetone extracts have little tick repellency effect to tick (Zorloni, 2010). These might be because the crude does not have aromatic effect. Different chromatographic images indicate that the plant has different chemicals with different polarities. Chemical constitutes having high polarity can retain the silica plat and do not show advanced movement from line. Whereas, molecules which are non-polar move with the volatile solvents stored in the tanker. Color bands which are not visualized by UV light were examined after staining with iodine.

CONCLUSION AND RECOMMENDATION

This study showed that *C. aurea* chemical compounds extracted by methanol and ethanol have different chemicals in it which have anti-parasitic effect. The insecticidal effect of C. *aurea* has comparable affect with the synthetic drug, ivermectin. Different plants have their own potent chemical substances to do its function. The result of this study provides scientific evidence to the traditional uses of C. aurea, for the management of M. ovinus of sheep. Different phytochemical screening results obtained from Methanol and ethanol extracts of C. aurea shows low polarity difference between solvents; since its metabolites have the same characterstics. The crude has different chemicals with different retention factor to the silica plat. Based on the above conclusion the following recommendations are forwarded

- ✓ The different constitutes of the plant after phytochemical screening and its host toxicity should be further investigated.
- ✓ Plants having alkaloid, falvonoid, tannin, saponnin and phenolic compounds should be studied
- ✓ Solvents with high different in polarity have to be used during extraction to obtain different active ingredients.
- ✓ Plants having medicinal value should be conserved and registered
- ✓ There should be linkage between traditional practitioner and researcher's.

DECLARATIONS

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MB2 conceived the study, coordinated the overall activity, and carried out the statistical analysis, drafted the manuscript. AK participated in drafting and reviewing the manuscript. MY conceived the study, coordinated the overall activity, and reviewed the manuscript. MB1 participated in drafting and reviewing the manuscript. NA participated in the design of the study, and reviewed the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

Data will be made available up on request of the primary author

Acknowledgment

The authors' heartfelt thanks to University of Gondar, College Veterinary Medicine and Animals Sciences, and Research and Community Service V/ President Office for finance and resource supporting

REFERENCE

Abadi Y (2000). Current problems of the leather industry," in the Opportunities and Challenges of Enhancing Goat

Production in East Africa, Proceedings of a Conference Held at Debub University, Hawassa, Ethiopia from November 10 to 12, 2000, pp. 139-143.

Abate G, Demissew S and Etse D (1989). In Ethiopian Traditional Medicine. Addis Ababa University Press, Addis Ababa. Journal of ethnopharmacology, 85(1): p. 43-52.

Adedapo A, Jimoh O, Koduru S, Afolayan J, Masika J (2008). Antibacterial ant antioxidant properties of the methanol extracts of the leaves and stems of Calpurnia aurea. BMC Complement, Alternative Medicine, 1 (8): 53.

Alawa I, Adamu M, Gefu O, Ajanusi J, Abdu A, Chiezey P, Alawa N and Bowman D (2003). In vitro screening of two Nigerian medicinal plants (Vernonia amygdalina and Annona senegalensis) for anthelmintic activity. Veterinary Parasitology, 113: 73-81.

Ayoola A, Coker B, Adesegun A, Adepoju A, Obaweva K, Ezennia C and Atangbayila O, (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Tropical Journal of Pharmacy Research, 7: 1019-1024.

Bay D and Harris R (1988). Introduction to Veterinary Entomology (A Guide to Livestock Insects), Stonefly Publishing, Texas, pp. 111.

Bekele T (2002). Studies on seasonal dynamics of ticks of Ogaden cattle and individual variation in resistance to ticks in Eastern Ethiopia. Journal of Veterinary Medicine, 49: 285-288.

Berhanu W, Negussie H, Alemu S and Mazengia H (2011). Assessment on major factors that cause skin rejection at Modjo export tannery, Ethiopia, Tropical Animal Health Production, 43: 989-993.

Berriatua E, French P, Wall R, Smith E and Morgan L (1999). Within-flock transmission and degree of lesion development in naive sheep housed with single infected sheep. Veterinary Parasitology, 83: 277-289.

Blum A and Bekele A (2002). Storing grains as a survival strategy of small farmers in Ethiopia. Journal of International Agriculture, Ext. Ed. 9, 77–83.Cape Town

Coates k (1983). Trees of south Africa 2nd ed nstruik publisher cap town. Journal of Tropical Ecology, 19(4): p. 375-386.

Central Statistical Agency (2013). Federal Dominance of Democratic Republic of Ethiopia central statistical Agency Agricultural Sample Survey. Report on Livestock and livestock characteristics (private peasant holding). Addis Ababa, Ethiopia. pp 13-15.

Currie B, Harumal P, Mc Kinnon M and Walton F (2004). First documentation of in vivo and in vitro ivermectin resistance in Sarcoptes scabies. Clinical Infectious Disease, 39: 8–12.

Doughari H, Human S, Bennade S and Ndakidemi A (2009). Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. Journal of Medicinal Plants Research, 3: 839848.

Farhan H, Rammal H, Hijazi A, Hamad H and Badran B (2012). Phytochemical screening and extraction of polyphenol from stems and leaves of a Lebanese Euphorbia macrolada schyzoceras Boiss. Annual Biological Research, 3: 149-156.

Fuerte S, Nicoladis S and Berridge C (2000). Conditioned taste aversion in rats for a threonine-deficient diet: demonstration by the taste reactivity test. Physiological Behavior, 2000: 68:423-429.

Gemeda N, Mokonnen W, Lemma H, Tadele A, Urga K, Addis G, Debella A, Getachew M, Teka F, Yirsaw K, Mudie K and Gebre S (2014). Insecticidal Activity of some traditionally used Ethiopian Medicinal Plants against Sheep Ked Melophagus ovinus Journal of Parasitology Research, DOI: 10.1155/2014/978537

Germishuizen G and Meyer Z (2003). Plants of Southern Africa: An Annotated Checklist. Strelitzia 14. National Botanical Institute Pretorite. Journal of Tropical Ecology, 19(4): p. 375-386.

George D, Masic E, Sparagan O and Guy H (2009). Variation in chemical composition and acaricidal activity against Dermanyssus gallinae of four eucalyptus essential oils,"Experimental and Applied Acarology, vol. 48, no. 1-2 pp. 43-50: 2009.

Heukelbach J, Speare R and Canyon D (2006). Natural products and their application to control head lice: an evidence-based review. In: Brahmachari G(ed) chemistry natural products: recent trend and developments. Kerala, India, Pp: 1-26.

Hutching A (1996). Zulu medicinal plants, An inventory, university of natal press Pietermaritzburg

Jansen M (1981). Spices, Condiments and Medicinal Plants in Ethiopia, Their Taxonomy and Agricultural Significance. Centre for Agricultural Publishing and Documentation, Wageningen.

John C (2002). Livestock Insects-Sheep and Goats. High Plains IPM Guide, a cooperative effort of the University of Wyoming, University of Nebraska, Colorado State University and Montana State University.

Jones C, Hunt D and King W (1997). Veterinary pathology, 6th eds. Lippincott Williams and Wilkins, Baltimore, Maryland, USA, pp: 817-872.

Kamal K, and Anil K (2004). Ethno-veterinary Practices among the Konda Reddi of East Godavari District of Andhra Pradesh. Journal of Studies of Tribes and Tribal, Ind., 2:37-4.

Kassa B (1998). Control of sheep and goat skin diseases. By Ian, B.C. and Kassa B. (Eds.) In: Proceedings of Control of Sheep and Goat Skin Diseases for Improved Quality of Hides and Skins, FAO, Addis

Kassa B (2005). Pre-slaughter defects of hides/skin and intervention options in east Africa: Harnessing the leather industry to benefit the poor. Regional Workshop Proceedings, April 18-20, Addis Ababa, Ethiopia pp 71-84.

Khater F, Ramadan Y and Mageid D (2013). In vitro control of the camel nasal botfly, Cephalopina titillator, with doramectin, lavender, camphor, and onion oils. Parasitology Research, 112:2503-10.

Koul S, Walia G and Dhaliwal S (2008). Essential oils as green pesticides: potential and constraints," Biopesticides International vol. 4, no. 1, pp. 63-84: 2008

Krishnaveni S and Venkatalakshmi P (2014). Antimicrobial, larvicidal and acaricidal activities of the ethanolic extract of andrographis paniculata and carica papaya leaves. World Journal of Pharmacology Research, l3:660-669.

Kumsa B, Tamirat H, Tadesse G, Aklilu N and Cassani R (2012). Prevalence and species composition of ixodid ticks infesting horses in three agroecologies in central Oromia, Ethiopia, Tropical Animal Health Production, 44: 119-124

Lee H (2004). Acaricidal activity of constituents identified in Foeniculum vulgare fruit oil against Dermatophagoides spp. (Acari: Pyroglyphidae)," Journal of Agricultural and Food Chemistry, vol. 52, no. 10, pp. 2887–2889, 2004.

Legg E, Kumar R, Watson W and Lloyed E (1991). Seasonal movement and spatial distribution of the sheep ked (Dipetra: Hippoboscidae) on Wyoming lambs. Journal of Economics and Entomology, 84: 1532-1539.

Levot W (2000). Resistance and the control of lice on humans and production animals. International Journal of Parasitology, 30: 291-297.

Liu H (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of Action. Journal of Nutrition, 134:3479-3485.

McCorkle M (1986). An introduction to ethno veterinary research and development, Journal of Ethno biology, 6:129-49.

Megalla M, El-Keltawi S and Ross A (1980). "A study of antimicrobial action of some essential oil constituents," Herba Polonical, vol. 3, pp. 181-1186: 1980.

Megersa M, Asfaw Z, Kelbessa E, Beyene A and Woldeab B (2013). An ethno botanical study of medicinal plants in Wayu Tuka District, East Welega Zone of Oromia Regional State, West Ethiopia. Journal of Ethno biology and Ethno medicine, 9: 68.

Mersha C, Tamiru N and Asegedech S (2010). Ectoparasites are the major causes of various types of skin lesions in small ruminants in Ethiopia, Tropical Animal Health and Production, 42: 1103-1109.

Mwale M., Bhebhe E, Chimonyo M and Halimani E (2005). Use of herbal plants in poultry health management in the Mushagashe small-scale commercial farming area in Zimbabwe. International Journal of Applied Research and Veterinary Medicine, 3: 163-170.

National Bank of Ethiopia (2000). Annual Report. Addis Ababa, Ethiopia.

Plant W and Lewis J (2011). "Treatment and control of ectoparasites in sheep," Veterinary Clinics of North America Food Animal Practice, 27:203-212.

Pooley E (1993). The complete field guide to trees of natal, Zululand and Transkei. Natal flora publicayion trus, natal hebarium, durban

Provenza D, Villalba J, Cheney D and Werner J (1998). Selforganisation of foraging behaviour: from simplicity to complexity without goals. Nutrition Research Review, 1998 11: 199-222

Radostits M, Blood C, and Gay C (1994). Veterinary Medicine: A textbook of the disease of cattle, sheep, pigs, goats and horses, eighth ed. Bailliere Tindall, UK.

Robert O, Akol M and Okello-Onen J (2010). Ethnoveterinary botanicals used for tick control in the acholi subregion of Uganda. Journal of Animal Veterinary Advance, 9: 29512954.

Rogers W and Rozin P (1996). Novel food preferences in thiamine-deficient rats. Journal of Comparative Physiology, 1996 61:1-4

Sewell H and Brockesby W (1990). Hand Book on Animal Disease in the Tropics, 4th Edition, Bailliere Tindall, pp.2 - 28.

Sirikatitham A, Chuchom T and Itharat A (2007). Development of the Chromatographic Fingerprint Analysis of Dioscorealides and Dioscoreanone from Dioscorea membranacea Pierre, Journal Science and Technology, 29:101-108

Small W (2005). A review of Melophagus ovinus (L.), the sheep ked. Veterinary parasitology, 130:141–155.

Tadeg H, Mohammed E, Asres K and Gebre-Mariam T (2005). Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. Journal of Ethno pharmacology, 100: 168-175.

Tadesse H (2005). Pre-slaughter defects of hides/skin and intervention options in east africa: harnessing the leather industry to benefit the poor. Regional Workshop

Tefera S and Abebe W (2007a). A study on ectoparasites of sheep and goats in eastern part of Amhara region, Northeast Ethiopia. Ethiopian Small Ruminant Research, 69: 62-67.

Tefera S and Abebe W (2007b). Effect of Ectoparasites on quality of pickled skins and their impact on the tanning industries in Amhara regional state, Ethiopia, Small ruminant research, 69: 55-69.

Urquhart L, Armour M. Dunn D and Jennings W (1996). Veterinary Parasitology, 5th edition Longman Scientific and Technical, UK, pp: 141-205.

Van den Broek M, Huntley F, Halliwell W, Machell J, Taylor M, and Miller P (2003). Cutaneous hypersensitivity reactions to Psoroptes ovis in sheep previously infested with P. ovis the sheep scab mite. Veterinary Immunopathology, 91: 105-117.

Van Wyk E, Verdoorn H, Greinwald R, Bachmann P (1991). Taxonomic significance of major alkaloids in the genus Priestleya. Biochemistry Systemic Ecology, 19, 595–598.

Vermin J, de Kok J, Romers C, Radema H and Van Eijk L (1979). Calpurmenin and ist 13a-**(**20-pyrrolecarboxylic acid) ester. Acta Crystallogrer, 35: 1839-1842.

Vitazkova S, Long E and Glendinning J (2001). Mice suppress malaria infection by sampling a "bitter" chemotherapy agent. Animal Behavior, 2001; 61:887–894.

Wagner H and Bladt S (1996). Plant Drug Analysis: A thin Layer Chromatography Atlas, 2nd edition,Munchcn, Germany)

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

Wall R and Shearer D (2001). Veterinary external parasites: Biology, pathology and control. Third edition, Blackwell.

Wall R and Shearer D (1997). Veterinary entomology: arthropod ectoparasites of veterinary importance, 1st eds. Champan and Hall, United Kingdom pp 1-439.

WHO (2012). The top 10 causes of death. World Health Organization, Geneva. [Online] available at: http://www.who.int/mediacentre/factsheets/fs310/en/index2.html [accessed on October 18 2015].

Wrangham W (1977). Feeding behaviour of chimpanzees in Gombe National Park, Tanzania, In: Clutton-Brock TH, ed. Primate Ecology. New York: Academic Press; 1977:504–538.

Yineger H and Yewhalaw D (2007). Traditional medicinal plant knowledge and use by local healers in Seroku District, Jimma Zone, Southwestern Ethiopia. J. Ethnobiol. Ethnomed. 3 (1): 24.

Yineger H, Yewhalaw D and Teketay D (2008). Ethnomedicinal plant knowledge and practice of the Oromo ethnic group in southwestern Ethiopia. Journa l of Ethno biology Ethno medicine, 4 (1), 11.

Zewdu K (1995). Hides and skins in Ethiopia. Proceedings of the 2nd Annual Conference of the Ethiopian Society of Animal Production. Addis Ababa, Ethiopia pp 6-14.

Zorloni A (2007). Evaluation of plants used for the control of animal ectoparasitoses in southern Ethiopia (Oromiya and Somali regions). MSc Thesis. Phyto-medicine Programme, Univers