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Full Length Research Paper

Acute toxicity and histopathological assessment of methanol extract of *Cleome viscosa* (Linn.) whole plant.

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Cleome viscosa **Linn (Cleomaceae) is a medicinal plant used widely in Nigeria for the management of various ailments. This research appraised the toxic potential of the plant with a view to validating or contesting its safety. Acute oral toxicity of the methanolic whole plant extract of *Cleome viscosa* was evaluated in mice using modified Lorke's method. Signs accompanying toxicity and possible death of animals were investigated for a period of two weeks to determine the median lethal dose (LD_{50}) of the extract. After two weeks observation period, all the animals in the respective dose groups 10, 100, 1000, 1600, 2900 and 5000 mg/kg were euthanized by cervical dislocation. The weight gained, absolute organ weight, and mean organ-body weight ratios (OBR) were determined and compared with values from those of the control group. The oral median lethal dose of the extract was found to be greater than 5000 mg/kg. There was a significant difference in weight gained on day 7 (P=0.052) among dose groups up to 1000 mg/Kg body weight. There was however, no significant difference in the relative organ weights between treated and control animals except for the Liver (p=0.048). Histopathological analysis showed mild congestion of the pulmonary vessels at dose 1600 mg/kg and above, mild diffuse vacuolar degeneration of hepatocytes across all tested dose as well as mild renal cortical congestion especially at high dose. The oral median lethal dose results indicate that the methanol extract of *Cleome viscosa* whole plant is non- toxic by oral administration at the tested doses.**

Key words: *Cleome viscosa*, methanol extract, acute toxicity, histopathology

INTRODUCTION

For centuries and in most of the cultures throughout the world, herbal prescriptions and natural remedies are commonly employed for relief or treatment of diseases (Maqsood et al., 2010). Also in modern world, herbal medicines are becoming popular as people resort to natural therapies. Novel clinically active drugs are been isolated from higher plants. Regrettably, there are limited scientific evidence as to the efficacy and safety to back up the continued therapeutic application of these medications. The justification for their use has rested largely on long term clinical knowledge (Zhu, 2002). Now, with the upsurge in the use of herbal medicines, a comprehensive scientific exploration of these plants will go a long way in substantiating their folkloric usage as

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well as their prophylactic properties (Sofowora, 1993). One foremost and prevailing benchmark in the selection of herbal medicines for use in health services is safety. Plants extracts should not only be efficacious but safe for consumption.

Cleome viscosa Linn. (Cleomaceae) is a weed distributed throughout the tropical regions of the world and plains of India. The plant is an annual, sticky herb with a strong penetrating odour, yellow flower and long slender pods containing seeds. In Ayurvedic system of medicine, the plant is used for the treatment of fever, inflammations, liver diseases, bronchitis and diarrhea (Chatterjee et al., 1991). The rural people use the fresh juice of the crushed seed for the treatment of infantile convulsions and mental disorder. The juice of the plant diluted with water is given internally in small quantities in fever and the leaves are useful in healing wounds and ulcer (Nadkarni, 1982; Kirtikar et al., 1984).

The smoke from its leaves is used by the locals to repel mosquitoes at night. Its extract exhibited larvicidal activity against the second and forth instar larvae of *Anopheles stephensi*, a vector of malaria in India (Saxena et al., 2000). *C. viscosa* is highly effective in a wide spectrum of diseases and reported to possess antidiarrhoeal (Devi et al., 2002), analgesic (Parimaladevi et al., 2003), antipyretic activity (Devi et al., 2003), psycho-pharmacological, anti-microbial properties including *in vitro Helicobacter pylori* and wound healing activity (Parimala et al., 2004a; Mahady et al., 2006; Panduraju et al., 2011), also against *Escherichia coli* , *Proteus vulgaris* and *Pseudomonas aeruginosa* (Sudhakar et al., 2006). In view of the reported effects of *C. viscosa*, the toxic potential of this plant was studied to generate information on its toxicity profile.

MATERIALS AND METHODS

Plant materials

The plant, *C. viscosa* Linn. was collected from Jeje area of Ibadan, Oyo State and authenticated at the Forestry Research Institute of Nigeria where voucher specimen was deposited under the reference number FHI 109669. The whole plant was dried at room temperature and powdered. About 2 kg of the powdered sample was soaked with 100% methanol for 48 h. The extract was concentrated using rotary evaporator and percentage yield was 5.12%. The dry extract was stored in a refrigerator at 4°C for further use.

Animals

The animals (ICR mice), both male and female, 6 to 7 weeks old (15 to 27g) used for these experiments were obtained from the Animal House, Department of Zoology, University of Ibadan. The mice were housed under standard conditions, fed with standard animal feed and given water *ad libitum* throughout the study period. They were allowed to acclimatize for seven days before the test was commenced. All experimental protocols were in compliance with University of Ibadan Ethics Committee Guidelines as well as internationally accepted principles for laboratory animal use, and care as found in the US guidelines (NIH publication Number 85-23, revised in 1985).

Phytochemical screening

Preliminary phytochemical screening was carried out according to Harborne, 1998.

Acute toxicity study

Acute toxicity study was carried out according to modified Lorke's method (Lorke, 1983). The study was conducted in two phases using a total of sixteen animals. The mice were fasted overnight prior administration of plant extract. In the first phase, twelve animals were divided into 4 groups of 3 mice each. Groups 1, 2 and 3 animals were given single dose of 10, 100 and 1000 mg/kg of the extract orally, respectively, to establish the possible range of doses producing any toxic effect. Group 4, the control group received a mixture of distilled water and dimethyl sulfoxide (DMSO). In the second phase, the first three animals received 1600, 2900 and 5000 mg/kg separately, while the forth (the control) received a mixture of distilled water and DMSO. All animals were observed frequently on the day of treatment and surviving animals were monitored daily for 2 weeks for signs of acute toxicity. Recovery and weight gain were seen as indications of having survived the acute toxicity. The weights of these organs were also taken and the mean organ-body weight ratios calculated and compared with those of the control group. Body weights of the mice were recorded on study days 0 (initiation), 7 and 14 (termination). At the end of 14 days, all surviving mice were euthanized by cervical dislocation. Five organs, heart, lungs, liver, kidney and spleen were isolated and subjected to complete gross necropsy and histopathological study. Histopathological assessment and photomicrography of prepared slides were done using an Olympus light Microscope with attached Kodak digital camera. % Relative organ weight= Absolute organ weight (g)/Body weight of mice on sacrifice day x100. Figure 1 to 5.

Statistical analysis

The statistical analyses were carried out using Statistical Package for Social Sciences (SPSS-17 computer package) and ANOVA (one-way) followed by Duncan's Multiple Comparism Test. All data were expressed as mean ± SD of triplicate parallel measurements. Differences between means at 5% level ($p \leq 0.05$) were considered significant.

RESULTS AND DISCUSSION

Despite the widespread use of medicinal plants, few scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies. To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a 'safe' dose in humans. The highest overall concordance of toxicity in animals with humans is with hematological, gastrointestinal, and cardiovascular adverse effects (Olson et al., 2000), while certain adverse effects in humans, especially

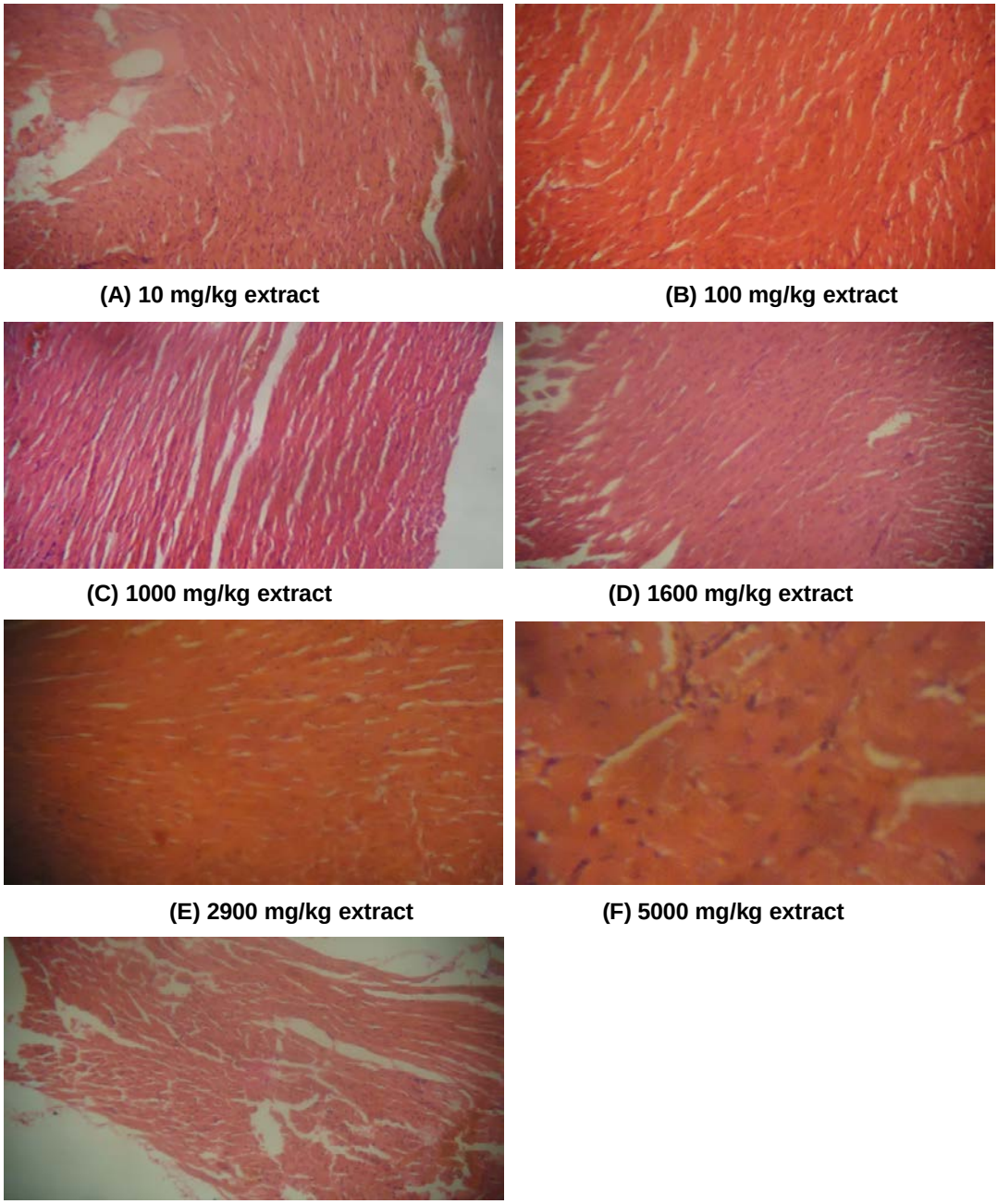

Figure 1. Histopathological assessment and photomicrography of the of the **heart** × 100 in mice treated with 10 mg/kg, 100mg/kg, 1000mg/kg, 1600mg/kg, 2900mg/kg, 5000mg/kg of methanol extract of *C. viscosa* whole plant and control group.

hypersensitivity and idiosyncratic reactions, are poorly correlated with toxicity observed in animals. Furthermore, it is quite difficult to ascertain certain adverse effects in animals such as headache, abdominal pain, dizziness and visual disturbances. In addition, interspecies differences in the pharmacokinetic parameters make it difficult to translate some adverse effects from animals to humans (Olson et al., 2000). The antipyretic, analgesic, and anti- inflammatory (Parimala et al., 2003a, b) as well as antimicrobial (Sudhakar et al., 2006), psychopharmacological effects (Parimala et al., 2004b) and immunomodulatory effects (Tiwari et al., 2004) of *C. viscosa* has been reported.

The biological/pharmacological activity, as well as

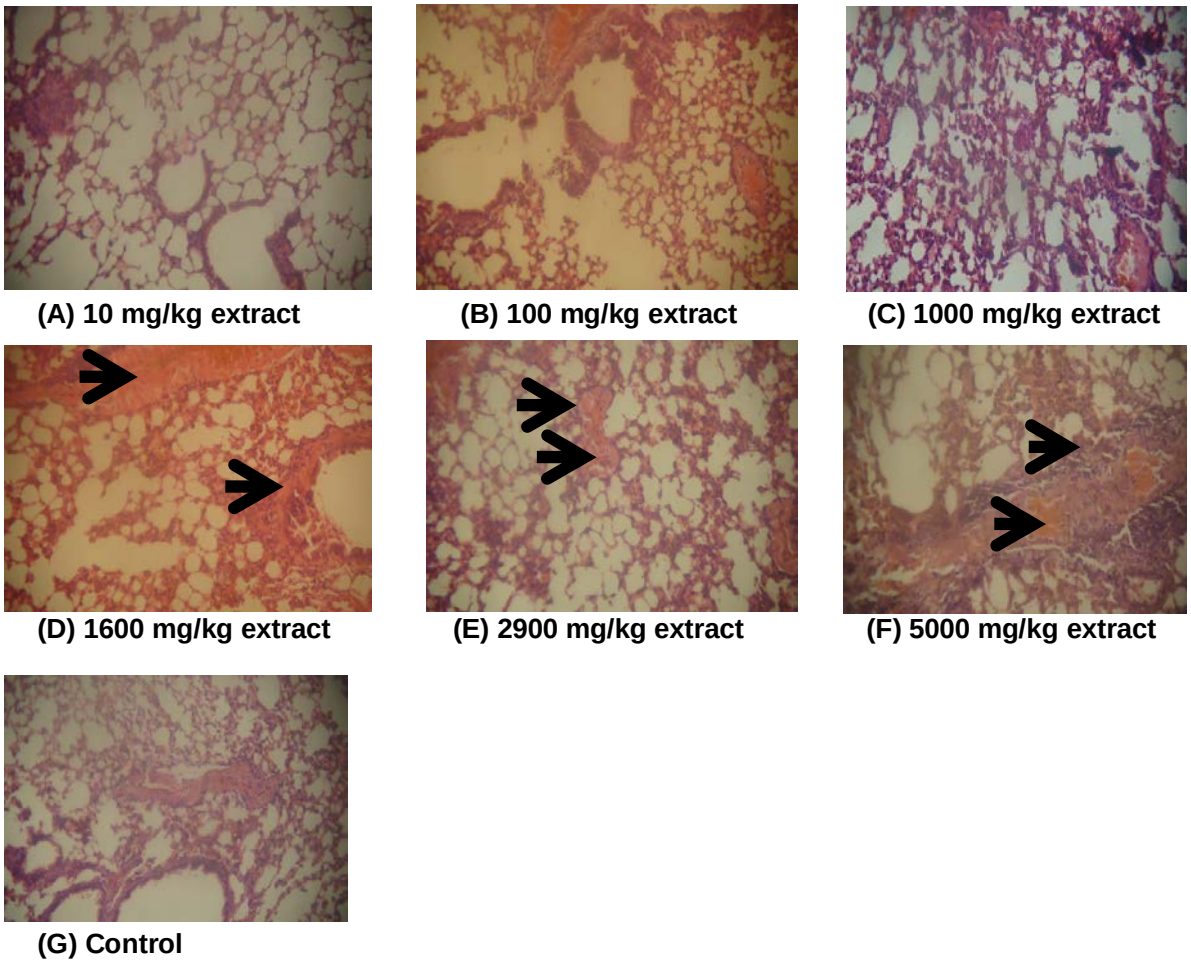

Figure 2. Histopathological assessment and photomicrography of the of the **lungs** × 100 in mice treated with 10 mg/kg, 100mg/kg, 1000mg/kg, 1600mg/kg, 2900mg/kg, 5000mg/kg of methanol extract of *C. viscosa* whole plant and control group.

toxicity potential of a plant is directly related to the type, nature and quantity of secondary metabolites present in it. Thus, screening for the presence of possible phytochemicals in a plant is imperative. The results of preliminary phytochemical screening are given in Table 1. It shows the presence of flavonoids, phenolic compounds, alkaloids, phytosterol, fatty acid and saponins. Anthraquinone, tannin and coumarins were absent in the methanolic extract of *C. viscosa* L.

Flavonoids and other phenolics are ubiquitous in nature and can occur either in the free state or as glycosides. They constitute one of the most characteristic classes of compounds in higher plants and many are easily recognized as flower pigments in most flowering plants. However, their occurrence is not restricted to flowers but include all parts of the plant. They are widespread and have relatively low toxicity compared to other active plant compounds. Flavonoids have potential to be biological "response modifiers", such as anti-allergic, anti-inflammatory, anti-microbial and anti-cancer.

Phytosterols also have been implicated in lowering cholesterol (Pollak, 1953; Tilvis and Miettinen, 1986) and inhibiting lungs, breast, ovarian and stomach cancer (Woyengo et al., 2009). They also have long history of safety (Jones 2007). Medicinal use of alkaloid-containing plants has a long history (Hesse, 2002). The percentage of alkaloids in plants is usually small, and is not homogeneous over the plant tissues. Depending on the plants, the maximum concentration could be observed in the leaves fruits, seeds, root or bark (Grinkevich, 1983). Furthermore, different tissues of the same plants may contain different alkaloids (Orekhov, 1955). Consuming some secondary metabolites can have severe consequences. Alkaloids can block ion channels (Hamill and McBride, 1996), inhibit enzymes (Pastuszak et al., 1990), or interfere with neurotransmission producing hallucinations (Gaudreau and Gagnon, 2005), convulsion, vomiting and even death (Audi, 2005), diterpene gossypol blocks phosphorylation and is very toxic, spinasterol from spinach interferes animal hormone

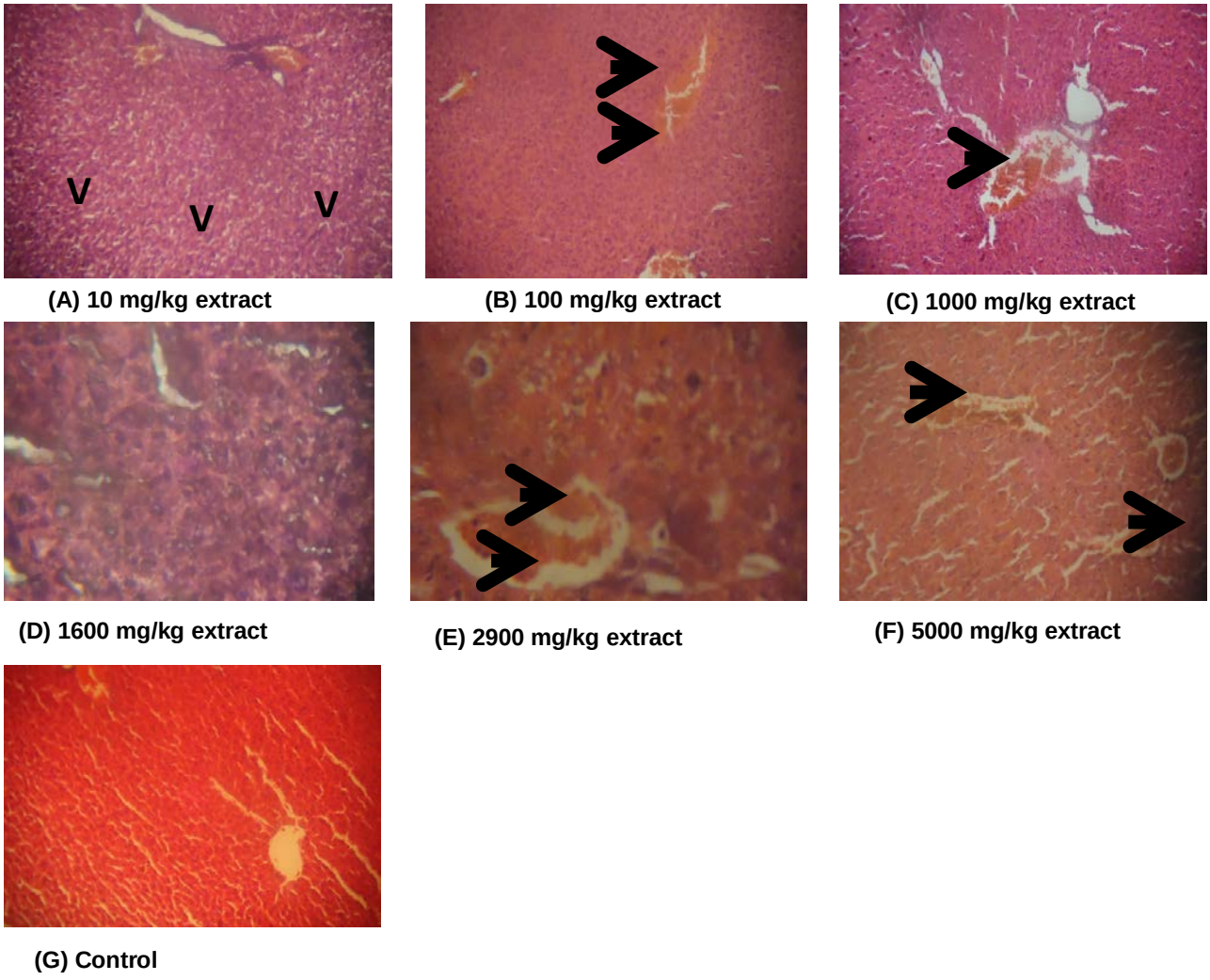

Figure 3. Histopathological assessment and photomicrography of the of the **liver** × 100 in mice treated with 10 mg/kg, 100mg/kg, 1000mg/kg, 1600mg/kg, 2900mg/kg, 5000mg/kg of methanol extract of *C. viscosa* whole plant and control group. VD= vacuolar.

actions, gallotannins also binds to protein and block digestion (Hartmann, 2007). Plants containing cyanogenic glycosides can liberate cyanide which blocks cytochrome C-oxidase thus, becoming potentially poisonous (Venturi, 2011). Some phenolics can be carcinogenic while tannic acid has been shown to cause damage to intestinal walls (Glenn, 2005). Saponins are known to have deleterious haemolyzing effect on circulating erythrocytes (Sofowora, 1993).

The acute lethal study of *C. viscosa* on mice (Table 5) showed that no animal died within 24 h after oral administration of the extract, and the LD_{50} was greater than 5000 mg/kg. The major signs of toxicity noticed within 24 h include ataxia, lethargy and asthenia. These signs were not seen in 10 mg/kg dose group but progressed and became increasingly pronounced as the dose increased towards 5000 mg/kg b.w. The LD50, being greater than 5000 mg/kg b.w., is thought to be safe as suggested by Lorke (Tijani et al., 1986; Deora et. al., 2010). Again, the absence of death among mice in all the dose groups throughout the two weeks of the experiment seems to support this claim. The LD50 value of more than 5,000 mg/kg, showed that the extract is practically safe.

Also in the toxicity studies, mice in all experimental group gained weight over the course of this study (Table 2 to 5). There was a significant difference in body weight gained on day 7 ($p>0.052$) among dose groups up to 1000 mg/Kg body weight. Mice in all experimental group gained weight over the course of this study especially those mice that took higher doses (Table 3). There was however no statistically significant differences ($p>0.05$) noted in absolute organ weights between the C. viscosa extract treated and control groups. Also, there was no statistically significant difference in relative organ weights between treated and control animals except for the liver

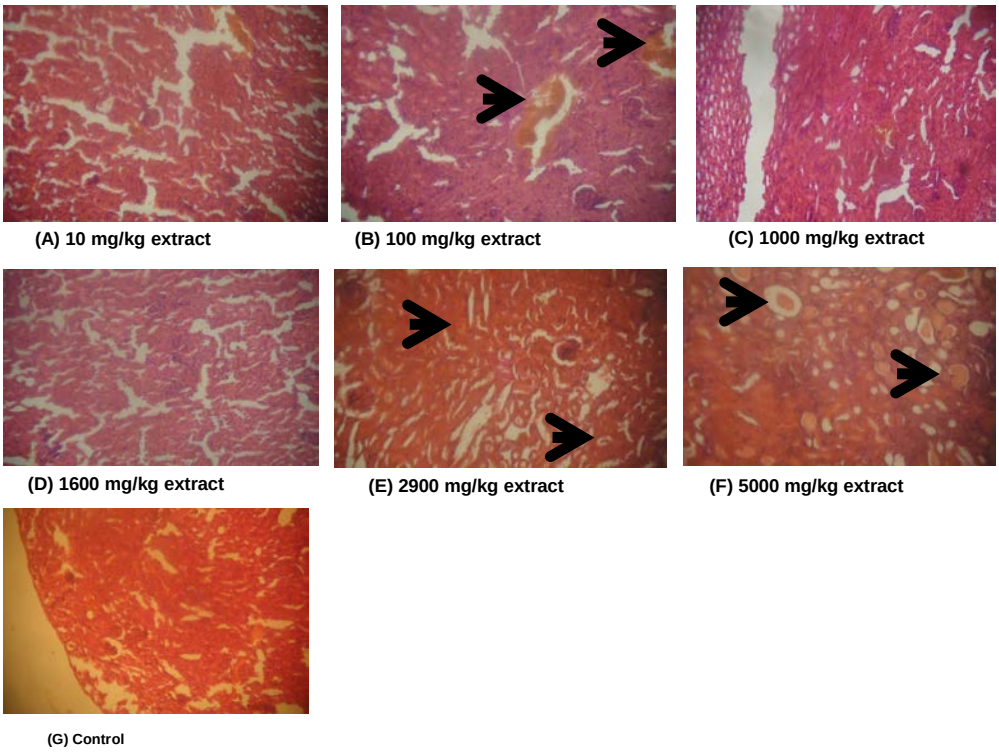

Figure 4. Histopathological assessment and photomicrography of the of the **kidney** × 100 in mice treated with 10 mg/kg, 100mg/kg, 1000mg/kg, 1600mg/kg, 2900mg/kg, 5000mg/kg of methanol extract of *C. viscosa* whole plant and (G) control group.

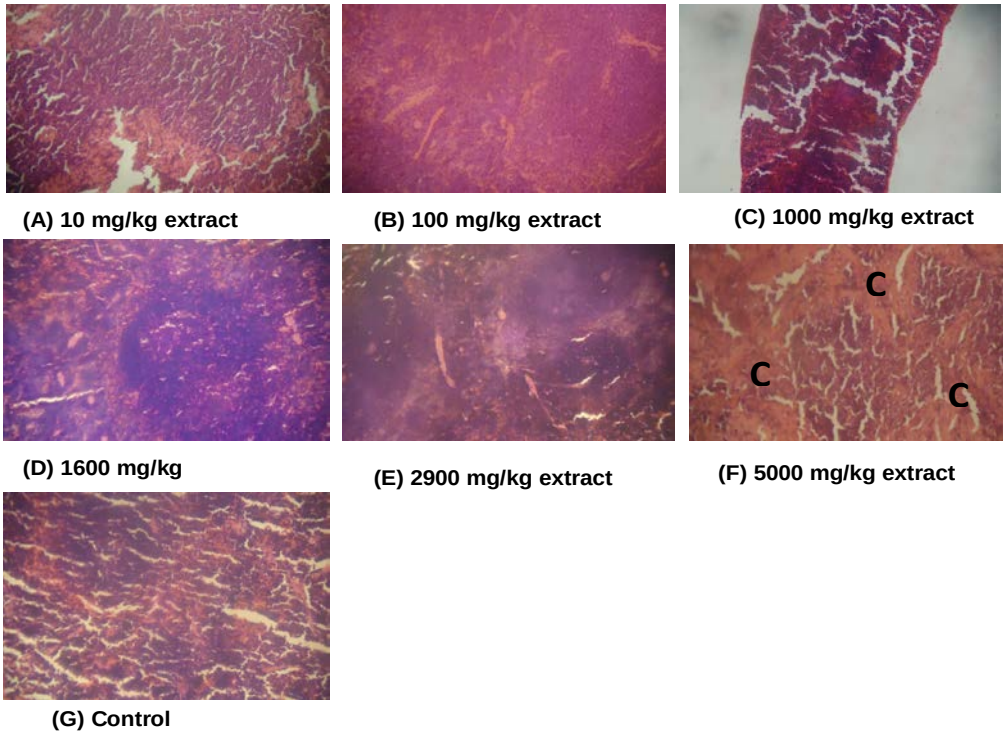

Figure 5. Histopathological assessment and photomicrography of the of the **spleen** × 100 in mice treated with 10 mg/kg, 100mg/kg, 1000mg/kg, 1600mg/kg, 2900mg/kg, 5000mg/kg of methanol extract of *C. viscosa* whole plant and control group. C= congestion.

Table 1. Preliminary phytochemical screening of *Cleome viscosa* methanolic extract.

Chemical tests	*C. viscose* methanolic extract
Detection of alkaloids	
Dragondroffs test	+
Hager's test	+
Detection of phenols	
Ferric chloride test	+
Detection of flavonoids	
Alkaline reagent test	+
Detection of anthroquinones	
Free anthroquinones test	-
Modified borntrager's test	-
Detection of phytosterols	
Salkowski's test	+
Detection of fatty acids	+
Detection of tannins	
Ferric chloride test	-
Detection of saponins	
Froth test	+
Coumarins	**-**

Keys: (+) = Present and (-) = Absent.

Table 2. Effect of oral administration of methanol extract of *C. viscosa* on the body weights of mice.

Experiment	Dose (mg/kg b.w.)	Initials (g) 0 days	Weight gain (g) After 7 days	Weight gain (g) 14 days
Phase 1	10	17.6667^{a}±2.0816	18.0000^{a}±1.0000	18.6667^{a}±0.5773
	100	20.0000^{a}±1.0000	22.0000^{ab}±1.0000	22.0000^{a}±0.0000
	1000	20.6667^{a}±1.5275	23.6667^{b}±1.1547	22.6667^{a}±1.1547
Control	0	18.6667^{a}±3.2145	19.3333^{ab}±4.0414	19.6667^{a}±4.5092
	1600	22.0000	23.0000	28.0000
Phase 2	2900	27.0000	25.0000	30.0000
	5000	20.0000	22.0000	28.0000

Test of significance was done in rows. Values are presented as mean ± standard deviation (n=3) in the same row with different superscripts differ significantly ($p < 0.05$) compared to the control group by one-way ANOVA followed by Duncan's Multiple Comparison Test. Weight values in phase-2 (were n<3) were not compared due to absence of measure of variability.

except for the Liver (p=0.048). Liver weight relative to body weights increased in a dose dependent manner in all group with the test extract (Table 4) with the highest liver weights at dose 2900 mg/kg body weight. However, the magnitudes of the alterations were small and were not considered treatment-related. Mild diffuse vacuolar degeneration of hepatocytes and moderate portal congestion of the liver appears to be the major gross pathology accompanying treatment of mice with methanolic

Table 3. Effect of oral administration of methanol extract of *C. viscosa* on absolute organ weight.

Organ	10 mg/kg	100 mg/kg	1000 mg/kg	1600 mg/kg	2900 mg/kg	5000 mg/kg	Control
Heart	0.1200^{a}±0.0435	0.1200^{a}±0.0100	0.1333^{a}±0.0288	0.12	0.19	0.23	0.1000^{a}±0.0360
Lungs	0.1767^{a}±0.0152	0.1533^{a}±0.0305	0.1667^{a}±0.0251	0.16	0.21	0.22	0.1500^{a}±0.0519
Liver	0.8833^{a}±0.0230	1.0300^{a}±0.0173	0.8933^{a}±0.2396	1.63	1.77	1.41	1.0633^{a}±0.3412
Kidney	0.1400^{ab}±0.0200	0.1667^{b}±0.0057	0.1667^{b}±0.0057	0.16	0.27	0.21	0.1300^{a}±0.0264
Spleen	0.0833^{a}±0.0115	0.1233^{a}±0.0321	0.0933^{a}±0.0152	0.27	0.16	0.09	0.1233^{a}±0.0611

Test of significance was done in rows. Values are presented as mean ± standard deviation(n=3) in the same row with different superscripts differ significantly ($p < 0.05$) compared to the control group by one-way ANOVA followed by Duncan's Multiple Comparism Test. Dose groups with single mice per group (n<3) were not compared due to absence of measure of variability.

Table 4. Effect of oral administration of methanol extract of *C. viscosa* on organ-body weight.

Organ	10 mg/kg	100 mg/kg	1000 mg/kg	1600 mg/kg	2900 mg/kg	5000 mg/kg	Control
Heart	0.6481^{a}±0.2579	0.5454^{a}±0.0454	0.5939^{a}±0.1531	0.4285	0.6333	0.8214	0.4999^{a}±0.0726
Lungs	0.9483^{a}±0.1067	0.6969^{a}±0.1388	0.7360^{a}±0.1161	0.5714	0.7000	0.7857	0.7833^{a}±0.2753
Liver	4.7338^{ab}±0.1359	4.6817^{ab}±0.0786	3.9128^{a}±0.8335	5.8214	5.9000	5.0357	5.3333^{b}±0.5166
Kidney	0.7523^{a}±0.1293	0.7575^{a}±0.0262	0.7360^{a}±0.0331	0.5714	0.9166	0.7500	0.6708^{a}±0.0273
Spleen	0.4453^{a}±0.0489	0.5605^{a}±0.1460	0.4141^{a}±0.0834	0.9642	0.5333	0.3214	0.6027^{a}±0.1687

Test of significance was done in rows. Values are presented as mean ± standard deviation(n=3) in the same row with different superscripts differ significantly ($p < 0.05$) compared to the control group by one-way ANOVA followed by Duncan's Multiple Comparism Test. Dose groups with single mice per group (n<3) were not compared due to absence of measure of variability.

Table 5. Acute lethal effect of methanol extract of *Cleome viscosa* administered orally mice.

Experiment	Dose (mg/kg b.w.)	Mortality of mice after 24hrs of administration	Mortality at 14 days observation
Phase 1*	10	0/3	0/3
	100	0/3	0/3
	1000	0/3	0/3
Control	0	0/3	0/3
Phase 2	1600	0/1	0/1
	2900	0/1	0/1
	5000	0/1	0/1
Control	0	0/1	0/1

(*Experiment was conducted in two phases; each dose group of phase-1 made up of 3 mice while those in phase 2 have 1 mice per group).

Table 6. Post mortem result for acute toxicity of methanol extract of *C. viscosa* administered orally to mice.

Organ	10 mg/kg	100 mg/kg	1000 mg/kg	1600 mg/kg	2900 mg/kg	5000 mg/kg	Control
Heart	None	None	None	None	None	None	None
Lungs	None	None	None	Mild congestion of pulmonary vessels	Mild congestion of pulmonary vessels	Mild congestion of pulmonary vessels	None
Liver	Mild diffuse vacuolar degeneration of hepatocytes	Moderate portal and central venous congestion	Mild portal congestion	Moderate central venous congestion	Mild diffuse vacuolar degeneration of hepatocytes	Moderate portal and central venous congestion	None
Kidney	None	Renal cortical congestion	None	None	Few tubules have protein casts in their lumen	Renal tubules have copious amount of proteinaceous material in the lumen	None
Spleen	None	None	None	None	None	Congestion	None

with methanolic extract of *C. viscosa* (Table 6). Again, liver congestion could be attributed in part to its role in biotransformation of xenobiotics or to a slight clog of liver which is a function of lipid metabolization at that dose apart from vascular changes which could be attributed to the treatment.

The findings of this study indicate that the methanolic whole plant extract of *C. viscosa* may be considered safe for consumption since no animal died within 24 h after oral administration of the extract and the LD_{50} was greater than 5000 mg/kg.

CONCLUSION

The methanol extract of *Cleome viscosa* whole plant appears non- toxic by oral administration at the tested doses as indicated by the high oral median lethal dose.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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In vitro antimicrobial activity of "Antibact", an herbal medicinal product against standard and clinical bacterial isolates

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***In vitro* antimicrobial activities of ethanol and aqueous "Antibact", herbal products consisting of a combination of the leaves and branches of four different plants were evaluated against twenty one pathogenic bacteria. Saponins, reducing sugars, phenolics, polyuronides, and triterpenes were the major phyto-constituents of both the aqueous and ethanol "Antibact". The LD_{50} analysis revealed the products were safe (LD_{50}>5000 mg/kg bodyweight) for *in vivo* use. All the isolates (100%) were resistant to at least five of the 12 antibiotics used in the study. In total, the aqueous "Antibact" inhibited the growth of 5 out of the 21 (23.81%) microbes used with an average zone of inhibition of 9.73 ± 0.35 mm. Thirteen (61.90%) out of the 21 microbes used were susceptible to the ethanol "Antibact", registering an average inhibition zone of 10.80 ± 0.18 mm. In the case of the minimum inhibitory concentration (MIC), the aqueous "Antibact" exhibited MIC range of 4.00 to 32.00 mg/ml and 0.50 to 8.00 mg/ml, while the ethanol "Antibact" recorded MIC range of 2.00 to 8.00 and 1.00 to 2.00 mg/ml for the wild and standard strains, respectively. The average minimum bactericidal concentration (MBC) for the aqueous "Antibact" was 32.00 mg/ml while the ethanol "Antibact" had MBC range of 4.00 to 16.00 mg/ml and 4.00 to 8.00 mg/ml for the wild and standard strains, respectively. In conclusion, both "Antibact" were safe for human use and effective against some pathogenic bacteria *in vitro*. However, ethanol "Antibact" showed better antimicrobial activity.**

Key words: Antibact, antimicrobial activity, clinical isolate, drug-resistant.

INTRODUCTION

Medicinal plant may be defined as any plant whose some or all of its parts contain active compounds which can be used in the treatment or management of a disease. In the last decade, there have been global upsurge in the use of

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traditional medicine (TM), and complementary and alternative medicines (CAM) in both developed and developing countries. Currently, TM and CAM play increasingly important role in health care and health sector reform globally. Hence, the safety and efficacy, as well as the quality control of TM and CAM have become important concerns for both health authorities and the public (WHO, 2005).

In Africa, up to 80% of the populations in rural areas depend on traditional medicine to meet their primary health care needs, while in India the corresponding figure is 65% (WHO, 2002). These figures are expected to go up in recent years. Contrary to the presumption that the 21st century Ghanaian care less and knows less about herbal medicine and its role in the general wellbeing of Ghanaians, studies conducted by corporate bodies and individuals proved otherwise (Addo, 2007; Darko, 2009). It is also a known fact that, TM is the first choice of healthcare treatment for more than 80% of Africans suffering from high fever and other common ailments (Matur et al., 2009).

Resistance to antibiotics is a serious worldwide problem which is increasing and has implications for morbidity, mortality, and health-care both in hospitals and in the community (Franco et al., 2009). For decades, antimicrobial drugs have proven useful for the treatment of infectious diseases but lately most bacteria are inherently resistant to newly developed antimicrobial agents (Newman et al., 2006). The emergence of the acquired resistance to antimicrobial drugs has been observed in almost all pathogenic bacteria (Newman et al., 2006) and the emergence of multiple drug resistant bacteria (MDR) has become a major cause of failure of the treatment of infectious diseases (Gibbons, 2005; Mathias et al., 2000). As a result, society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance to many, and in some cases all effective first-line antibiotics (Mills-Robertson et al., 2009). Hence, there is need to look for alternative strategies for the management of resistant bacteria and one of the possible strategies towards this objective involves rational localization of bioactive phytochemicals which have antibacterial activity and this may be one of the important approaches for the containment of antibiotic resistance (Gottlieb et al., 2002).

This study therefore investigated the antimicrobial efficacy and safety of “Antibact”, herbal medicine products comprising of four plants namely: *Phyllanthus fraternus* G.L. Webster (Family Euphorbiaceae), *Hoslundia opposita* Vahl. (Family Lamiaceae), *Psidium guajava* L. (Family Myrtaceae) and *Cymbopongon citrates* (CD) Stapf (commonly called lemon grass). Studies have shown that these plants have varied degrees of antimicrobial activities and antioxidant properties (Mehta et al., 2014; Koffuor and Amoateng, 2011), but the combined effects of the plants have not been studies hence the need for the present study.

MATERIALS AND METHODS

Study site and plant

The study was carried out at the Microbiology Department of the Centre for Plant Medicine Research (CPMR) in Mampong-Akuapem. It is the main institute in Ghana where herbal products are certified for use before it is registered by the Food and Drugs Board of Ghana. All the medicinal plants including *Phyllanthus fraternus* G.L. Webster (Family Euphorbiaceae), *Hoslundia opposita* Vahl. (Family Lamiaceae), *Psidium guajava* L. (Family Myrtaceae) and *Cymbopongon citrates* (DC.) Stapf (commonly called lemon grass) used for the formulation of “Antibact” were identified and collected by a taxonomist at the Plant Development Department (PDD) of the CPMR. Voucher specimens of the plants were kept at the herbarium of the CPMR.

Pathogenic bacteria used

The 21 bacteria agents used in the study included standard strains of *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 33495), *Pseudomonas aeroginosa* (ATCC 27923), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus saprophyticus* (ATCC 15305), *Proteus mirabilis* (ATCC 49565), *Salmonella typhi* (ATCC 19430), and *Salmonella typhimurium* (ATCC 14028). In addition, identified clinical isolates consisting of two strains each of *E. coli*, *K. pneumoniae*, *P. aeroginosa*, *S. aureus*, *S. saprophyticus*, *P. mirabilis*, *S. typhi*, and *S. typhimurium* were obtained from the Department of Microbiology, Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana. These test strains were maintained on nutrient agar (NA) slopes at 4°C and were sub-cultured for use when needed.

Preparation of ethanol extract of “Antibact”

Five hundred grams (500 g) of equal proportions of each of the pulverized plant materials was cold macerated with 70% ethanol for two days (48 h). The ethanol extracts were concentrated using Heidolph rotary evaporator (LABOROTIA 4000, Germany) at a temperature of 65°C. Twenty five millilitres (25 ml) portions of the concentrated ethanol extracts were poured into 250 ml flasks and lyophilized using a Heto Power Dry LL3000 freeze-dryer (Jouan Nordic, R507/200 g, Germany) for 24 h. The freeze-dried powders obtained were also stored in air-tight containers and stored in a refrigerator at 4°C until needed.

Preparation of aqueous extract of Antibact

Aqueous fractions (decoctions) of the plant materials were prepared by boiling 1000 g of equal proportions of the dried plant materials in 2000 ml of clean water for about 45 min. The resultant extracts were concentrated using reduced temperature for another 60 min. The extracts were allowed to cool and subsequently lyophilized as described in the ethanol extracts. The freeze-dried powders obtained were also stored in air-tight containers and stored in a refrigerator at 4°C until needed.

Phytochemical analysis of aqueous and ethanol extracts of “Antibact” used

The phytochemical constituents of the aqueous and ethanol “Antibact” were determined by the protocols described by Krishnaiah et al. (2009). The phytochemical parameters assayed for, included alkaloid, flavonoids, polyuronides, reducing sugars,

cyanogenic glycoside, saponins, triterpenes, anthracenosides, phytosterols and phenols.

Safety of aqueous and ethanol "Antibact" obtained (acute toxicity (LD_{50}) test)

The LD_{50} study of the aqueous and ethanol extracts of "Antibact" was carried out using Sprague-Dawley female rats weighing between 250 and 300 g. The herbal extract was filtered and freeze dried to get the lyophilized extract. Dose levels of 5000, 2500, and 1250 mg/kg of the freeze dried extract were administered orally to the rats per kilogram body weight. The animals were observed for the first 24 h and then a period of 48 h for signs of toxicity such as: effect on eyes (eye colour, tears in eyes, bulging), effect on movement (sluggish movement or immobile), effect on breathing (quick or slow), arrangement of fur (pilo-errection), and twitching gait. General observations other than the aforementioned normal behavior were also observed and recorded. The LD_{50} value was expressed as the weight of extract administered per kilogram body weight of the experimental rats and the values obtained were compared to the Hodge and Sterner toxicity scale (CCOHS, 2005).

Antibiotics sensitivity test

The *in vitro* antibiotic sensitivity test was performed using Kirby-Bauer disc diffusion method as described by National Committee for Clinical Laboratory Standard (NCCLS, 1998). The antibiotics chosen were based on the Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI, 2007) as well as current treatment regimens for microbial infections in Ghana (MOH, 2010). Briefly, 2 to 6 h cultures of the microbes in peptone water that had achieved 0.5 McFarland standard turbidity were seeded over Muller-Hinton agar by the swabbing technique. Selected antibiotic disc were carefully placed on the surface of the agar and incubated at 37°C for 16 to 18 h. The zones of inhibition of the various antibiotics were measured with a meter rule by taking the diameter of the zones. The measured zones were compared to standard antimicrobial sensitivity chart and recorded as sensitive or resistant to the respective antibiotics. The antibiotics that were tested included: Amikacin (30 µg/disc), Ampicillin (10 µg/disc), Penicillin (10 µg/disc), Cloxacillin (5 µg/disc), Erythromycin (15 µg/disc), Tetracycline (30 µg/disc), Gentamicin (10 µg/disc), Cotrimoxazole (25 µg/disc), Chloramphenicol (30 µg/disc), and some of the newer generation antibiotics including Cefixime (30 µg/disc), Cefuroxime (30 µg/disc), and Cefotaxime (30 µg/disc).

Antibacterial activity of "Antibact"

Antibacterial activity of the aqueous and ethanol "Antibact" was determined by the agar-well diffusion method as described by CLSI (2007). Sixteen hours old overnight broth cultures were sub-cultured for another 2 h and their turbidity adjusted to 0.5 McFarland standards. Muller-Hinton agar plates were seeded with the 2 h old culture using the swabbing technique. A sterilized cock borer with 4 mm internal diameter was used to punch holes in the medium and about 100 µl of 32% w/v (using sterile distilled water and DMSO as diluents for aqueous and ethanol extracts respectively) of "Antibact" dispensed into the respective labelled holes. Disc of standard drugs 30 µg/disc chloramphenicol was used as positive controls, while 20% v/v DMSO and sterile distilled water were used as negative controls. Triplicates of each plate were made and the procedure repeated for the other microbes. The plates were kept in the refrigerator for about 4 h for complete diffusion of the extract and subsequently incubated at 37°C for 48h. After the incubation period, the diameter of each zone of inhibition was measured in millimeters (mm) with a standard ruler. The minimum inhibitory concentration (MIC) of the "Antibact" was determined for each organism as described previously (Eloff, 1998). The minimum bactericidal concentration (MBC) values were deduced from those wells with lowest concentrations at which no growth took place after sub-culturing for 24 h of incubation as described by Nester et al. (2004).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 16 version software was used to analyze the frequencies and averages for the resistance patterns of the test organisms and the antimicrobial activity of "Antibact".

RESULTS

Phytochemical constituents

The aqueous and ethanol "Antibact" were subjected to phytochemical screening and the results summarized as shown in Table 1. The study revealed the presence of saponins, reducing sugars, phenolics, polyuronides, and triterpenes as the major phyto-constituents of both aqueous and ethanol "Antibact". Alkaloids and flavonoids were however present only in the ethanol "Antibact" whilst phytosterols were only present in the aqueous "Antibact".

Toxicity test (LD_{50})

As shown in Table 2, the LD_{50} values obtained in this study for both aqueous and ethanol "Antibact" were greater than 5000 mg/kg. This suggests that both herbal medicinal products are practically non-toxic according to Hodge and Sterner scale.

Antibiotic sensitivity test

Twenty one strains of pathogenic bacteria were examined for their susceptibility to standard antibiotics. As shown in Figure 1, all the isolates (100%) were found to be resistant to five or more of all the antibiotics used, namely, Ampicillin (AMP), Chloramphenicol (CHL), Tetracycline (TET), Gentamicin (GEN), Amikacin (AMK), Cotrimoxazole (COT), Erythromycin (ERY), Penicillin (PEN), Cefixime (CXM), Cefotaxime (CTX), Cefuroxime CRX) and Cloxacillin (CXC). All the test microbes were found to be resistant to ampicillin, penicillin, cloxacillin and tetracycline but were variedly susceptible or resistant to the rest of the antibiotics used. Thus, all the microbes used for this study were multiple resistant, that is, resistance to 3 or more antibiotics (Figure 1).

Susceptibility of the microbes to aqueous and ethanol "Antibact"

The aqueous "Antibact" inhibited the growth of 3 out of 7

Table 1. Phytochemical components of “Antibact”.

Phytochemical parameter	Aqueous extract of antibact	Ethanol extract of antibact
Saponins	+	+
Reducing sugar	+	+
Cyanogenic glycosides	-	-
Phenolics	+	+
Polyuronides	+	+
Alkaloids	-	+
Anthracenosides	-	-
Flavonoids	-	+
Phytosterols	+	-
Triterpenes	+	+

+ = Present, - = Absent.

Table 2. Acute toxicity test (LD_{50}) of aqueous and ethanol “antibact”.

Aqueous extract						
Species and strain	No. of animals Sex/group	Route of admin.	Formulation and dosage	Time of deaths and period of observation	Approx. lethal dose (LD_{50})	Signs of toxicity
Sprague-Dawley rats	12 females; 3 groups (N=4)	Oral	Freeze-dried aqueous extract 5000, 2500 and 1250 mg/kg	No death occurred during the period of observation; 48 h of observation.	>5000 mg/kg body weight	Nil
Ethanol extract						
Species and strain	No. of animals Sex/group	Route of admin.	Formulation and Dosage	Time of deaths and period of observation	Approx. lethal dose (LD_{50})	Signs of toxicity
Sprague-Dawley rats	12 females; 3 groups (N=4)	Oral	Freeze-dried aqueous extract 5000, 2500 and 1250 mg/kg	No death occurred during the period of observation; 48 h of observation.	>5000 mg/kg body weight	Nil

(42.86%) standard strains with zones of inhibition ranging from 9.00 ± 0.00 to 9.67 ± 0.58 mm, while 2 (14.29%) wild strains out of a total of 14 were inhibited with zones of inhibition ranging from 10.00 ± 0.00 to 10.33 ± 0.58 mm. Four (66.67%) of the 6 Gram positive bacteria used in the study were susceptible to the aqueous “Antibact”. However, only 1 (6.67%) out of the 15 Gram-negative bacteria was inhibited in growth by the aqueous “Antibact”. In total, the aqueous “Antibact” inhibited the growth of 5 out of 21 (23.81%) microbes used with an average zone of inhibition of 9.73 ± 0.35 mm (Figure 2). In the case of the ethanol “Antibact”, the growth of 4 out of 7 (57.14%) standard strains were inhibited with zones of inhibition ranging from 9.00 ± 0.00 to 14.00 ± 0.00 mm. Nine (64.29%) out of 14 wild strains were however susceptible to the ethanol “Antibact” with zones of inhibition ranging from 9.00 ± 0.00 to 16.00 ± 1.73 mm. The ethanol “Antibact” inhibited all 6 (100%) Gram-positive bacteria used in the study. It however inhibited 9 (60.00%) of the 15 Gram-negative bacteria used. The ethanol “Antibact” in total inhibited the growth of 13 (61.90%) out of the 21 microbes used with an average inhibition zone of 10.80 ± 0.18 mm (Figure 2).

MICs and MBCs of aqueous and ethanol "Antibact "

Results of the MICs and MBCs of “Antibact” are as shown in Tables 3 and 4. The aqueous “Antibact” exhibited MICs ranging from 0.5 to 16.0 mg/ml for the standard strains whilst the wild strains showed MIC ranged of 4.0 and 32.0 mg/ml (Table 2). In the case of the ethanol “Antibact”, the MICs ranged between 1.0 and 2.0 mg/ml for the standard strains whilst that of the wild strains ranged from 2.0 to 8.0 mg/ml (Table 3). Results from the MBCs showed that the aqueous “Antibact” is bacteriostatic at concentrations < 32 mg/ml while the ethanol “Antibact” demonstrated better bactericidal activity

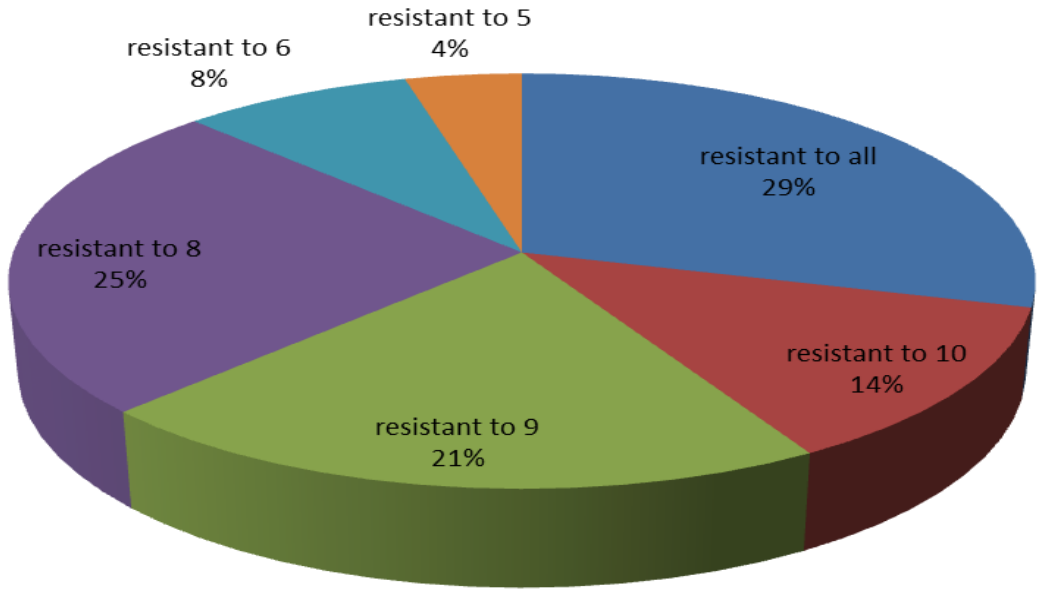

Figure 1. Percentage resistance of the microbes to the antibiotics used.

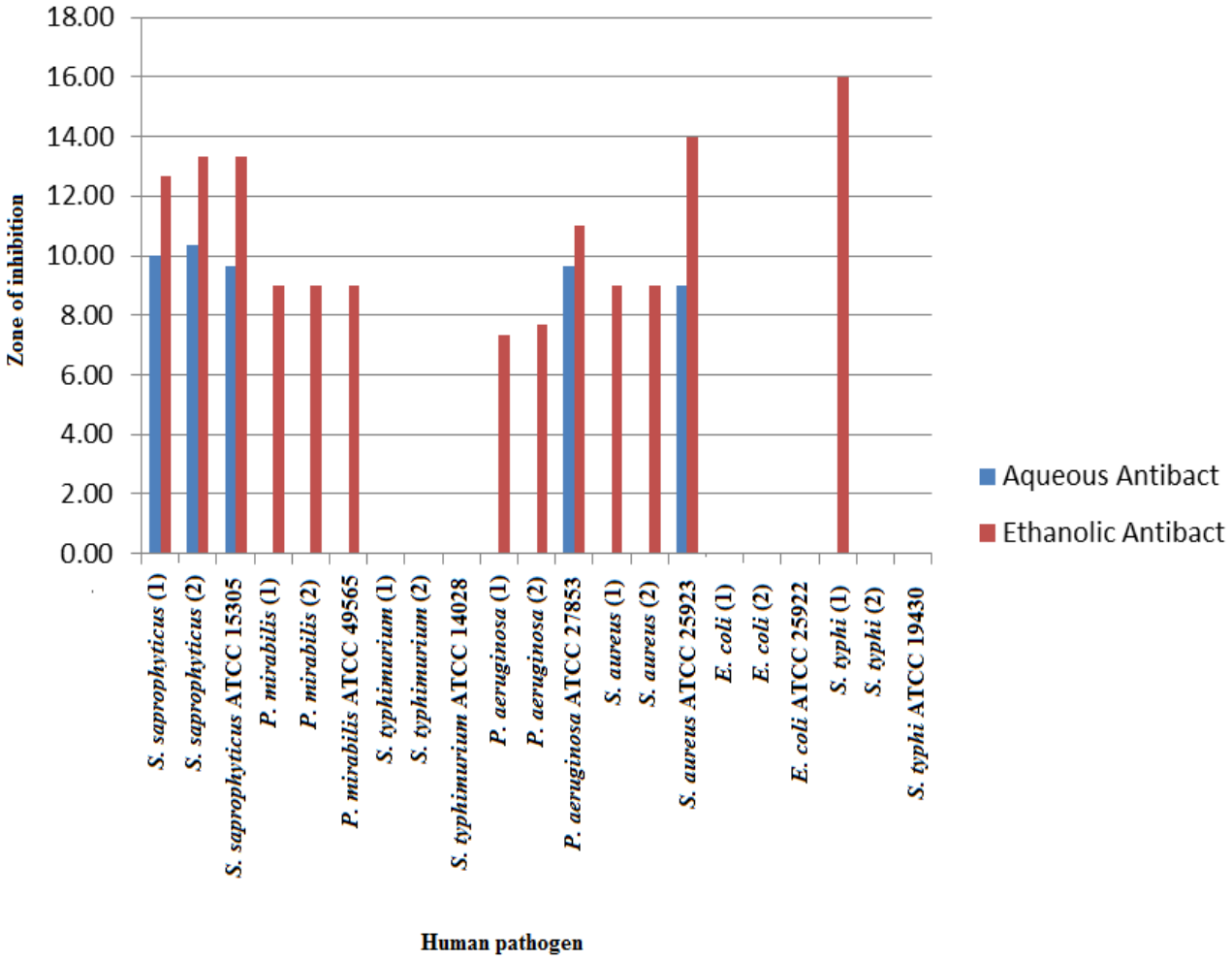

Figure 2. Susceptibility of microbes to the aqueous and ethanol “Antibact”

Table 3. MICs (mg/ml) of the aqueous and ethanol "Antibact".

Pathogenic bacteria	MICs (mg/ml)							
	Aqueous "Antibact"				Ethanolic "Antibact"			
	1	2	3	Average	1	2	3	Average
Wild strains								
S. saprophyticus (1)	16.0	16.0	16.0	16.00	2.0	2.0	2.0	2.00
S. saprophyticus (2)	16.0	16.0	16.0	16.00	2.0	2.0	2.0	2.00
P. mirabilis (1)	16.0	16.0	16.0	16.00	4.0	4.0	4.0	4.00
P. mirabilis (2)	16.0	16.0	16.0	16.00	2.0	2.0	2.0	2.00
S. typhimurium (1)	16.0	16.0	16.0	16.00	8.0	8.0	8.0	8.00
S. typhimurium (2)	16.0	16.0	16.0	16.00	4.0	4.0	4.0	4.00
P. aeruginosa (1)	4.0	4.0	4.0	4.00	8.0	8.0	8.0	8.00
P. aeruginosa (2)	32.0	32.0	32.0	32.00	4.0	4.0	4.0	4.00
S. aureus (1)	16.0	16.0	16.0	16.00	8.0	8.0	8.0	8.00
S. aureus (2)	16.0	16.0	16.0	16.00	2.0	2.0	2.0	2.00
E. coli (1)	4.0	4.0	4.0	4.00	4.0	4.0	4.0	4.00
E. coli (2)	32.0	32.0	32.0	32.00	4.0	4.0	4.0	4.00
S. typhi (1)	4.0	4.0	4.0	4.00	4.0	4.0	4.0	4.00
S. typhi (2)	32.0	32.0	32.0	32.00	8.0	8.0	8.0	8.00
Standard strains								
S. saprophyticus ATCC 15305	2.0	2.0	2.0	2.00	2.0	2.0	2.0	2.00
P. mirabilis ATCC 49565	4.0	4.0	4.0	4.00	2.0	2.0	2.0	2.00
S. typhimurium ATCC 14028	8.0	8.0	8.0	8.00	2.0	2.0	2.0	2.00
P. aeruginosa ATCC 27853	8.0	8.0	8.0	8.00	2.0	2.0	2.0	2.00
S. aureus ATCC 25923	16.0	16.0	16.0	16.00	1.0	1.0	1.0	1.00
E. coli ATCC 25922	0.5	0.5	0.5	0.50	2.0	2.0	2.0	2.00
S. typhi ATCC 19430	2.0	2.0	2.0	2.00	2.0	2.0	2.0	2.00

(Table 4).

DISCUSSION

Undoubtedly, infectious diseases are the leading cause of morbidity and mortality globally. The situation has been compounded with the continuous emergence of multi-drug resistant infectious agents, particularly pathogenic bacteria. This phenomenon has led to an increase in investigations into natural products, particularly plant products, as a source of new biomolecules for human disease management (Mohana et al., 2009). Considering the fact that different plants have various medicinal and antimicrobial properties, we put together four medical plants to form two products, aqueous "Antibact" and ethanol "Antibact", potential antimicrobial agents for systemic use. The antimicrobial activities of the products were investigated, and their phyto-constituents and LD_{50} levels established.

The antibiogram of the organisms used showed that none of the microbes examined in this study were susceptible to relatively affordable, commonly prescribed "first-line" antibiotics such as Ampicillin, Penicillin, Tetracycline, and Cloxacillin (Figure 1). In recent times, one of the challenges hampering the smooth treatment of infectious diseases is microbial resistance to anti-microbial agents. For example, beta-lactamase producing bacteria are mostly resistant to beta-lactam drugs such as Penicillins, Cephalosporins, Carbapenems, Monobactams and others (Del Carmen Rodrigue et al., 2004). Quinolones such as norfloxacin, ciprofloxacin, nalidixic acid and others which block bacteria DNA synthesis by inhibiting DNA gyrase (topoisomerase) are now mostly not effective because of mutagens which modify the bacterial DNA gyrase (Baceiro et al., 2013; Fournier et al., 2000). Resistance to Aminoglycosides antibiotics, Tetracycline, Chloramphenicol, Erythromycin, clindamycin and others have been reported (Greenwood et al., 2007).

The emergence of resistant bacterial strains to almost all the "first-line" antibiotics raises public health concerns especially in most developing countries where antibiotics are purchased as over-the-counter drug. Even though there are natural causes of this growing worry of microbial resistance to antimicrobials, the abuse of

Table 4. MBCs of the aqueous and ethanol "Antibact".

Pathogenic bacteria	MBCs (mg/ml)	
	Aqueous "Antibact"	Ethanol "Antibact"
Wild strains		
S. saprophyticus (1)	32.0	8.0
S. saprophyticus (2)	32.0	8.0
P. mirabilis (1)	32.0	4.0
P. mirabilis (2)	32.0	8.0
P. aeruginosa (1)	32.0	8.0
P. aeruginosa (2)	32.0	8.0
S. aureus (1)	32.0	8.0
S. aureus (2)	32.0	8.0
E. coli (1)	32.0	16.0
E. coli (2)	32.0	4.0
S. typhi (1)	32.0	16.0
S. typhi (2)	32.0	16.0
Standard strains		
S. saprophyticus ATCC 15305	32.0	8.0
P. mirabilis ATCC 49565	32.0	4.0
P. aeruginosa ATCC *27853*	32.0	4.0
S. aureus ATCC *25923*	320	8.0
E. coli ATCC *25922*	32.0	4.0
S. typhi ATCC 19430	32.0	4.0

antibiotics by both patients and clinicians, and the widespread use of antimicrobial agents in veterinary medicine are huge contributing factors. In addition to the hunt for new antimicrobials/antibiotics, there should be collective efforts aimed at educating the general public on the safe use of antimicrobial agents.

The results of the phytochemical analysis showed that the herbal medicinal products "Antibact" contain saponins, reducing sugars, phenolics, polyuronides and triterpenes as the major phyto-constituents. Alkaloids and flavonoids were present in only the ethanol "Antibact" while phytosterols were found in the aqueous "Antibact" (Table 1). Terpenoids have been reported to have anti-microbial properties (Scortichini and Pia, 1991). Studies have shown that triterpenes, terpenoids or isoprenoids have relatively high antifungal or antimicrobial properties which affect the non-mevalonate pathway. This pathway is critical for the synthesis of cell membrane components, prenylation of proteins and as a secondary source of carbon for fungi, protozoans, Gram-negative bacteria and other micro-organisms (Nayak et al., 2010). Reducing sugars have been reported to have antibacterial property (Dhale and Markandeya, 2011; Mabeku et al., 2007). Various oils from plants have shown varying degrees of antimicrobial activity (Akgul and Saglikoglu, 2005). The ethanol "Antibact" may contain some oils from the plants which contributed to the enhanced activity it exhibited. In general, the activity of these phytochemical-constituents may be responsible for the antimicrobial activities observed in the study.

Phyllanthus fraternus leaves are reportedly used to treat hepatitis, tuberculosis, viral infections, liver diseases, anemia, dysentry , cystitis, prostatitis, veneral diseases and urinary tract infections (Bapat and Mhapsekar, 2014; Singh et al., 2011). Koffuor and Amoateng (2011) also established in their study that the plant has antioxidant and anticoagulant properties hence confirming its potential in the management of conditions caused by oxidative stress. Study conducted in Kenya re-vealed the use of *Hoslundia opposita* in the treatment of colds, sore throat, gonorrhea, convulsion, stomach pains, and ringworms (Okach et al., 2013). Usman et al. (2010) indicated the plant contains essential oils, and this could be responsible for it broad us in treatment by traditional folks. Crude extract from *Psidium guajava* exhibited similar finding as in the present study (Ofodile et al., 2013; Biswas et al., 2013). Essential oil of *Cymbopongon citrates* has also been reported as potential source of bacteriostatic, fungistatic and microbicide agents against a wide range of infectious organisms (Soares et al., 2013; Vazirian et al., 2012; Lodhia et al., 2009). The antimicrobial activity of the plants confirms their use by

by traditional healers in the treatment and management of some diseases caused by infectious agents.

The LD_{50} test performed on the products revealed that both aqueous and ethanol "Antibact" are safe and non-toxic (Table 2). Our present investigation is the first study indicating the effectiveness of "Antibact", as significant antimicrobial agent against both Gram negative and Gram positive bacteria (Table 3). The ethanol "Antibact" was significantly effective as compared to the aqueous, inhibiting the growth of 13 out of 21 (62%) microbes used while the aqueous "Antibact" inhibited the growth of 5 out of 21 (23%) microbes used with respective average zones of inhibition of 6.64 ± 1.51 and 8.95 ± 1.42 mm. Several other studies have reported similar observations regarding various solvent systems used in the extraction processes (Bakht et al., 2014; Mills-Robertson et al., 2009). Probably the 70% ethanol has the potential of extracting active ingredients consisting of both polar and non-polar compounds from the product compared to the water which extracts mostly polar compounds. In general, the Gram positive organisms were found to be more susceptible to the "Antibact" than the Gram-negative bacteria used as indicated by previous studies (Biswas et al., 2013; Mills-Robertson et al., 2012).

The porous nature of the cell wall of Gram positive bacteria has been the reason for this observation, as about 90% of the cell wall of Gram-positive bacteria is made of peptidoglycan, which is not a regulatory structure compared to the cell membrane, and therefore, allows most compounds that fit to pass through it. Gram-negative bacteria, on the other hand, have cell wall made of approximately 20% peptidoglycan surrounding a periplasmic space that contains proteins which destroy potentially dangerous foreign matter (Drawz and Bonomo, 2010; Greenwood et al., 2007).

Regardless of the fact that the agar-well diffusion recorded some non-susceptibility by the microbes, the MIC test was performed on all the bacteria strains used in the study. Hundred percent inhibitory activities were seen in all the microbes, suggesting limitations of the agar-well diffusion techniques. It will therefore be appropriate to always use MIC test as the first step when screening medicinal plants for antimicrobial properties. The relatively low MIC values observed (Table 4), especially those exhibited by the ethanol "Antibact" (1.00 to 8.00 mg/ml) give an indication of the effectiveness of the products. Generally, lower MIC values were recorded among the standard strains as compared to the wild strains. Since the wild strains are clinical isolates, it is possible that their exposure to various antibiotics has led to the development of some levels of resistance. The study further revealed that the products have bactericidal properties. However, higher concentrations of aqueous "Antibact" were required to kill the bacteria as compared to that of the ethanol "Antibact". The observed bactericidal effect of "Antibact" products on the test bacterial isolates is justification for the need to explore the various medicinal plants in order to determine their antimicrobial efficacy and safety.

Conclusion

Conclusively, this study revealed that "Antibact", herbal medicinal products containing extracts from four plants have antimicrobial properties against selected pathogenic bacteria. However, the ethanol "Antibact" showed better activity than the aqueous "Antibact". The products are also safe for human use as their LD_{50} values are >5000 mg/kg body weight.

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Conflict of interests

The authors declare that they have no conflicting interests.

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Antimicrobial activity of *Thymus schimperi* Ronninger (Lamiaceae) against standard and clinical isolates of human pathogenic bacteria

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Thymus schimperi **Ronninger (Lamiaceae) locally known as Tosign, is a multipurpose endemic plant that has been used for various remedies as constituents of traditional medicine in Ethiopia. The objective of this study is to evaluate antibacterial activity of water, ethanol, methanol and chloroform extracts of *T. schimperi using* agar well diffusion and broth dilution methods against human pathogenic bacterial strains. Amongst the solvents used for this study, chloroform extract possess the highest potential of inhibiting the growth of all bacteria under study at concentrations of 50 mg/ml while ethanol and methanol extract fail to inhibit three gram negative bacteria, namely: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (clinical isolate) and *Shigella flexneri* (ATCC 12022) at the same concentration. Water extract did not show any zone of inhibition on all test bacteria as compared to other solvents. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were performed only for chloroform extracts that showed inhibition against all test organisms. This study revealed that, the highest inhibition with chloroform extract was exhibited against Methicillin-Resistant *S. aureus* (MRSA) with mean zones of inhibition of 22.6±2.5 mm whilst the minimum inhibition zone was observed for *E. coli* with mean zone of inhibition of 14.6±2.3 mm. The MIC value ranged from 6.25 to 12.5 mg/ml while the MBC value ranged from 6.25 mm to 25 mg/ml. This study clearly indicates that the crude chloroform extract of *T. schimperi* showed highest antibacterial activity against all studied bacterial strains as compared to the three solvents used in this study. Thus, further study and characterization of active compounds of chloroform extract of this plant is required.**

Key words: Antibacterial activity, MBC, MIC, *Thymus schimperi*, Tosign, zone of inhibition.

INTRODUCTION

Herbal drugs have got official recognition and gained a lot of acceptance worldwide due to their high therapeutic worth, fewer side effects, and economic value (Gupta et al., 2010; Kumar et al., 2010). Ethiopia has between 650

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and 1,000 medicinal plants, comprising about 10% of the entire flora of the country (Fulas, 2007). The current literature survey revealed that a large number of these plants have long been in use by local people; however, many of them are lacking modern scientific investigations. It has been widely claimed that about 80% of Ethiopian population rely on traditional medicine (Birhanu et al., 2015; Geleta et al., 2015). According to World Health Organization, majority of the population in developing countries like: Ethiopia (90%), Benin (80%), India (70%), Rwanda (70%), Uganda (60%), and Tanzania (60%) extensively use traditional medicines in health care (WHO, 2003).

The life-threatening infection of pathogenic microorganisms has increased worldwide and has become the cause of mortality in many of developing countries (Al-bari et al., 2006; WHO, 2015). The increasing prevalence of multi-drug resistant strains of bacteria and the appearance of strains with reduced susceptibility to antibiotics resulted in the formation of untreatable bacterial infections that open the door to search for new source of medicine (Rojas et al., 2006; Sosa et al., 2010; Biadglegne et al., 2014). Based on pre-existing indigenous knowledge, the numbers of modern drugs have been prepared from existing natural sources and many more had showed promising results.

Thymus species are well known for their medicinal importance because of their biological and pharmacological properties. The substances extracted from thyme especially the phenolic components *thymol* and *carvacrol* showed antibacterial activity against gram-positive and gram-negative bacteria due to their effects on the bacterial membrane (Asfaw et al., 2000). Because of its antibacterial activity, thyme is also useful as an antiseptic for the urinary tract, mouth and skin wounds. Tea and decoction prepared from thyme have successfully been used against gastro-intestinal complaints. Thyme oils are remedies to expel intestinal parasites, particularly hookworm (Mufti, 2011). *Thymus schimperi* (locally called Tosign) was found to have significant antioxidant activity and food preservative effect (Hailemariam and Emire, 2013). However, it is not well investigated on the modern scientific grounds. Keeping in view the common use of *T. schimperi* in traditional medicine, the present study was designed to evaluate its antibacterial activity against some human pathogenic bacteria to bridge information gap pertaining in the community.

MATERIALS AND METHODS

Plant material

Partially dried 2 kg leaves of *T. schimperi* R. (Lamiaceae) were purchased from Addis Ababa, Capital city of Ethiopia, and brought to Biotechnology laboratory of Gondar University. The plant material was properly screened from unwanted woody parts, giving 1.5 kg final weight. The scientifically authenticated leaves were then fully dried for 10 days at room temperature.

Preparation of plant extracts

The cleaned and dried leaves of Tosign were grinded using a grinder. The obtained powder was passed through a sieve (pore size: 30 µm diameter) and made ready for extraction. Four different solvents were used for extraction namely; chloroform, ethanol, methanol, and distilled autoclaved water. About 100 g of powder was taken and mixed with 300 ml of each solvent sequentially. The extraction was done using orbital shaker with continuous shaking for 8 h per day for 3 consecutive days. The extract was filtered using Whatman #1 filter paper. The debris was discarded and filtrate was evaporated under reduced pressure at 40°C. Finally the extract was dried; stock solution of 100 mg/ml was prepared in 50% Dimethyl sulfoxide (DMSO) (Anas et al., 2008), vortexed well, labeled and stored at 4°C in refrigerator until used. Chloroform crude extract was dissolved by the help of microwave for about one minute.

Preparation of test organisms

Both gram positive and gram negative bacterial strains: namely; *Escherichia coli* (ATCC 25922), methicillin resistant *Staphylococcus aureus* (MRSA), *Shigella flexneri* (ATCC 12022), *S. aureus* (ATCC 25923), *Klebsiella pneumoniae* (clinical isolate), *S. pneumoniae* (ATCC 63), and *S. pneumoniae* (clinical isolate) were used. The test microorganisms were grown on nutrient agar at 37°C for 24 h. The standard 0.5 McFarland known to form 1.5 x 10^8 CFU/ml was prepared by taking two to four colonies in normal saline solution following standard procedure (Andrews, 2006).

Antibacterial activity assay

The antibacterial activity of water, ethanol, methanol, and chloroform extracts of *T. schimperi* were determined using agar well diffusion method (Taye et al., 2011). The inoculums were prepared by taking overnight bacterial culture and adjusting to 0.5 McFarland standard in 0.9 % autoclaved NaCl (Normal saline). For sensitivity test, 38 g of Muller Hinton Agar was dissolved in 1000 ml distilled water and autoclaved at 121°C for 15 min. The media was then poured into steriled petri-dishes with uniform thickness and the agar was allowed to set at ambient temperature under laminar hood until solidification. The inoculums were spread evenly on the surface of solidified Muller Hinton agar with the help of sterilized cotton swab. On each plate, six equidistant wells were made with a 6 mm diameter sterilized cork borer. Then 100 µL of each plant extract adjusted to the same concentration (50 mg/ml) was aseptically added into a respective well. Chloramphenicol (30 µg/disc) and Vancomycin (30 µg/disc) were used as a positive control whilst 50% DMSO was used as a negative control. This was followed by allowing the agar plate to stay for 30 min under laminar hood and then incubated at 37°C for 24 h. The formation of clear inhibition zone of $\geq$ 7 mm diameters around the wells were taken as significant susceptibility measurement. The experiment was prepared in triplicate and mean value was used for further analysis.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined for extracts that showed inhibition zone of $\geq$ 7 mm diameter and for extract that inhibited the growth of all tested bacteria at concentration of 50 mg/ml. Among the extracts, only chloroform extract inhibited the growth of all tested microorganisms. The test was performed by using standard methods: agar well diffusion and microtiter plate (micro-tube dilution method). In former method, double serial dilution was employed from 50 mg/ml to obtain 1:2,

1:4, 1:8, 1:16, 1:32, and 1:64 in order to get 25, 12.5, 6.25, 3.125, 1.56 and 0.78 mg/ml concentration of extract respectively using 50% DMSO. Then 100 µL of diluted extract was added to prepared wells on Mueller Hinton agar and followed by identifying MIC concentration. In later method, the same principle was followed as above serial dilution except dilution was made in 1ml of nutrient broth. A 30 µL of standard suspension of test organisms were then added to each labeled concentration. Control was prepared with no inoculation of test organisms. The micro-tube was incubated at 37°C for 24 h and the presence of growth was evaluated by observing the bacterial turbidity of each tube before and after incubation and comparing the tube to the control.

Minimum bactericidal concentration (MBC)

For MBC, dilutions with no visually visible growth were taken and sub cultured on Mueller Hinton agar, and incubated for 24 h at 37°C. The concentration that resulted in no visible growth was then taken as MBC value.

Data analysis

Data was analyzed using statistical software package SPSS version 16.0 and presented as means ± standard deviation (SD). The one-way ANOVA was performed to examine the differences among test organisms and P value <0.05 was considered to be statistically significant.

RESULTS

The results of this study are presented in Table 1, and Figure 1. A 50 mg/ml concentration of all the four extracts prepared was tested for antimicrobial activity. Water extract did not show any antibacterial activity against all tested bacteria. Methanol and ethanol extract failed to inhibit the growth of: *E. coli, S. flexneri*, *and K. pneumonia* (clinical isolate). However, ethanol and methanol extract inhibited all studied gram positive bacteria; *MRSA, S. aureus, S. pneumoniae* (standard) and *S. pneumoniae* (clinical isolate). Methanol extract showed higher (P<0.05) inhibition zone against the tested bacteria as compared to ethanol extract (Table1). It is worth mentioning that chloroform extract showed 100% inhibition against all test bacteria with higher inhibition zone for most of test bacteria except for standard *S. aureus* (17 mm).

The maximum inhibition zone of ethanol extract (19 mm) was recorded for *MRSA* and standard *S. pneumoniae* while the minimum was obtained for standard *S. aureus* (17 mm). Likewise, the maximum inhibition zone for methanol extract was obtained for *MRSA* (19.3 mm) whilst the minimum was obtained for standard *S. aureus* (18.3 mm). For chloroform extract, the maximum inhibition zone was recorded for *MRSA* (22.7mm) while the minimum value was for standard *E. coli*. Hence, the currently evolving *MRSA* showed highest inhibition zone with chloroform extract as compared to other test bacteria (Table 1).

Standard antibiotic chloramphenical (30 µg/disc) and vancomycin (30 µg/disc) were used as positive control while 50% DMSO was used as negative control. As compared to standard antibiotics, chloroform extract showed high inhibition value than vancomycin (19 mm) for *MRSA* (22.7 mm) and standard *S. flexneri* (19.7 mm). Likewise, the chloroform extract resulted in higher zone of inhibition than chloramphenicol for Clinical isolate *K. pneumoniae*. Similar to water extract there was no inhibition with negative control and the data was not included in Table-1.

The MIC and MBC test was only conducted for chloroform extract because of the higher inhibition value recorded and its strong potential against all tested bacterial strains. The MIC value ranged from 6.25 mg/ml to 12.5 mg/ml. Five of test bacteria (*MRSA, S. flexneri, K. pneumoniae, S. pneumoniae* standard and clinical isolate) got high MIC value (12.5) with both micro dilution and agar well diffusion method, whilst only two of test organisms (*E. coli* and *S. aureus*) showed lowest MIC value (6.25 mg/ml). However, the maximum MBC value was 25 mg/ml, while the minimum value was 6.25mg/ml. The highest MBC value (25 mg/ml) was obtained for three test bacteria (*K. pneumoniae,* clinical *S, pneumoniae* and standard *S. pneumoniae*). The lowest MBC value (6.25 mg/ml) was recorded only for *E. coli*. The MBC and MIC value of *E. coli, MRSA,* and *S. flexneri* were found to be similar, whilst the remaining has different values for both tests (Figure 1).

DISCUSSION

Ethno-botanical screenings have been found to offer information on the importance of traditional medicines especially for those that do not have enough scientific evidence to prove their traditional use. This study is a continuation of earlier work justifying the utilization of Ethiopian folk medicine (Taye et al., 2011; Asressu, 2013). In the present study, attempts were made to validate the use of *T. schimperi* as antimicrobial agent and to substantiate the earlier findings (Hailemariam and Emire, 2013). To the best of our knowledge, there was no earlier work conducted on these bacterial strains. Though, local people have been using Tosign as cultural remedies, however, the information available is very minimal on this indigenous herb since it is availability is restricted to Ethiopia (Asressu, 2013).

The results of this study, clearly indicate that ethanol and methanol extracts could not inhibit the growth of all gram-negative bacteria (*E. coli*, *S. flexneri K. pneumoniae).* However, both extracts were effective against the remaining gram-positive bacteria. The observed difference in antibacterial activities between gram-negative and gram-positive were attributed due to the differences in composition and structure of bacterial outer membrane and cell wall which are among primary site of drug action in these organisms (Kenneth and George, 2004). Outer membrane of gram-negative

Table 1. Mean inhibition zone of four solvent extracts from *T. schimperi* at concentration of 50 mg/ml on different test bacteria.

Test organism	Inhibition zone (mm) Mean±S.D				Control	
	Water extract	Ethanol extract	Methanol extract	Chloroform extract	C30	V30
E. coli (ATCC 25922)	0.0	0.0	0.0	14.6±2.3	25	28
MRSA	0.0	19.0±3	19.3±1.5	22.7±2.5	26	20
S. flexneri (ATCC 2022)	0.0	0.0	0.0	19.7±0.5	27	19
S. aureus (ATCC 25923)	0.0	17.0±3.4	18.3±2.8	17.0±2.6	20	19
K. pneumonia (clinical isolate)	0.0	0.0	0.0	18.3±2.0	15	20
S. pneumoniae (ATCC 63)	0.0	19.0±1	19.7±1.1	20.7±3.0	24	21
S. pneumoniae (clinical isolate)	0.0	18.7±.05	19.0±1.0	19.3±1.1	21	20

Statistically significant: *P<0.05; **P<0.01; ***P<0.001 (One way ANOVA). C30 = Chloramphenicol, V30 = Vancomycin.

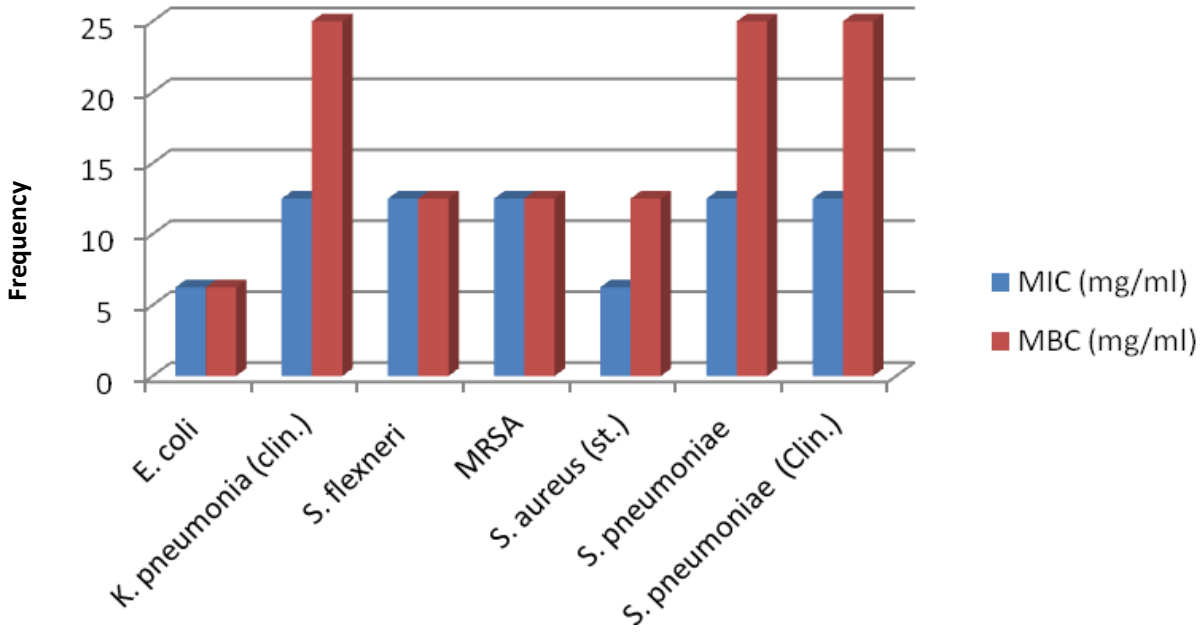

Figure 1. Mic and BMC values of chloroform extract against all tested bacteria.

bacteria is rich in lipopolysaccharide which can hinder penetration of different antibiotic molecules (Kenneth and George, 2004; Abdollah et al., 2010). However, in this study the chloroform extract of *T. schimperi* demonstrated total inhibition of all gram-negative as well as gram-positive tested bacteria. These results are in agreement with an earlier study where twenty different solvents were evaluated and chloroform was found to be the best solvent for the extraction of non-polar biological active compounds that were lethal to many bacteria (Harmala et al., 1992).

The aqueous extract of *T. schimperi* showed no antibacterial activities against all tested bacteria. This is not surprising since plant extracts from organic solvents have been found to give more consistent antimicrobial activity as compared to water extract (Parekh et al., 2005). The results gotten in this study are supported by previous studies where water was not found to be a suitable solvent for the extraction of antibacterial compounds from medicinal plants. Nonetheless, organic solvent, such as methanol and ethanol were suggested to be better than water as a solvent for antimicrobial agent extraction (Majhenic et al., 2007; El-Safey et al., 2011). Various reports suggested that water soluble flavonoids are not important as antimicrobial activity, though, water soluble phenolics were found to exhibit antioxidant potential (Nang et al., 2007). The aqueous extract of *Leonotis ocymifolia* was also found not to inhibit the growth of any bacterial species (Habtamu et al., 2010). The antimicrobial activity study on another species of genus thyme, *T. serpyllum* indicated that the aqueous extracts did not show any significant activity (Mufti, 2011).

The antimicrobial activities of many plants can be attributed due to the presence of high concentrations of *carvacrol*, which is known to occur at very high concentrations in many plant oils, including the members of the *Labiatae* family, such as *T. serpyllum* (Bounatirou et al., 2007). *T. schimperi* contained important antifungal substances such as *thymol, linalool,* and *carvacrol* (Lakew, 2011). The pharmacological actions of the plant extracts were suggested to be parallel to their *carvacrol* contents (Aydin et al., 2007). *Carvacrol* is considered to be biocidal, resulting in bacterial membrane perturbations. Furthermore, carvacrol might cross the cell membranes, penetrate the interior of the cell and interact with intracellular sites critical for antibacterial activities (Cristani et al., 2007).

The activity of the plant extract against both gram-positive and gram-negative bacteria might indicate the presence of broad-spectrum antibiotic compounds in that plant (Vaghasiya and Chanda, 2007). Chloroform extracts which resulted in higher inhibition zone were compared to vancomycin (22.7 and 19 mm on MRSA, 19.7 and 19 mm on *S. flexneri*). At the same time, it showed high inhibition zone (18.3 mm) than chloram-phenicol (15 mm) on *K. pneumonia*. Similar studies, conducted on other medicinal plants were shown that their antibacterial activity was comparable to positive controls and even some times higher than that (Ahmet et al., 2004). The fact that this plant extract was being active against both standard and clinical isolates; it is an indication that it can be a source of very potent antibiotic substances that can be used against multidrug resistant microorganisms.

Methicillin-Resistant *S. aureus* (MRSA) continued to be a major pathogen causing infections in hospitals and in the community, and are increasingly isolate in hospitals worldwide starting from its initial isolation in the UK in 1961(Udo et al., 2006). Interestingly, this study indicated the highest inhibition of MRSA with chloroform extract when compared to other bacterial strains studied. Hence, there was no doubt that highly powerful anti MRSA substances would be isolated in future from medicinal plants like *T. schimperi.*

Conclusion

Traditionally, *T. schimperi* (Tosign) has been used in various liquid and solid foods as flavor and medicinal uses. Our result indicated that it is a promising source of antimicrobial agents specially when extracted using chloroform. The broad antimicrobial activity of chloroform extract indicates the presence of highly active anti-microbial agents that can treat wide spectrum of human pathogens including the resistant ones. Thus, Tosign might represent an inexpensive source of natural antibacterial substances for use in treating various diseases, drug design as well as to prevent the growth of bacteria and extend the shelf life of the processed food.

In nutshell, from this present study, further information could be generated from several angles to validate the utility of this plant for medical application. The need to characterize and describe the antimicrobial activities, and investigate the suitability of these antimicrobial properties in practical applications is also important.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Systematic significance and pharmaceutical potentials of trichomes in accessions of *Sesamum indicum* L. Pedaliaceae

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Fresh leaves of twelve accessions of *Sesamum indicum* were collected after 12 weeks of planting and morphological-histology of trichomes were studied. These features may be used for the delimitation and determination of pharmaceutical potentials of the accessions. These accessions were found to exhibit high degree of heterogeneity in their trichome features. Nine types of trichomes were observed: unicellular, glandular peltate, capitate glandular, long unbranched uniseriate, short unbranched uniseriate, scale, multicellular, multiseriate capitate glandular and branched uniseriate trichomes. Lale-duk recorded the highest number (six types), four in accessions Ex-Sudan, Adaw-ting (Improved) and Ex-Gombe 1, three in accessions Ex-Gombe 3, Ex-Gombe 4, Ex-Gombe 5 and Ex-Gombe 6, two in kenana 4, Adaw-wula and Adaw-ting. The most frequent trichome type is short-unbranched uniseriate (76.52%), followed by long-unbranched uniseriate (72.73% and scale (65.11%). The least frequent was multiseriate capitate glandular (11.5%). The density of trichomes varied from accession to accession. Trichome density was the highest in Ex-Gombe 3 (63.2±0.49 mm^2) and the lowest was recorded by Ex-Gombe 4 (4.40±0.29 mm^2). The high variation in density coupled with the presence of glandular trichomes suggest that all the parts of these accessions probably contain or secrete chemicals that have many uses in the pesticide, pharmaceutical and flavour/fragrance industries and to conserve water. Furthermore, the trichome features varied from accession to accession; hence, are found to be good diagnostic and additional tool in identification as well as nomenclature of the accessions of *S. indicum*.

Key words: Trichomes, identification, pharmaceutical industries, sesame.

INTRODUCTION

Sesame (*Sesamum indicum* L. - Pedaliaceae) is native to Africa and India. The local names of the plants depend on the source areas of cultivations in the world, such as Yanmoti (Yoruba), ridi (Hausa) and beni (Tiv/Idoma and English) or gingelly (English) (Gill, 1992; Shittu, 2010) among others. It is widely naturalized in tropical regions of the world (Anilakumar et al., 2010). Sesame is an annual plant growing up to 100 cm tall. It has opposite

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leaves, broad lanceolate. Flowers are large, bell-shaped. They appear from the leaf axils on the lower stem, and gradually appear as the stem grows. Flowers vary in color. In some varieties they are yellow, in others purple or white. The plant is cultivated mainly for its seeds, small and flat ovals with mild, nutlike flavour.

Kanu (2011) reported that useful seed components of sesame include iron, magnesium, manganese, copper, calcium, vitamin B1, vitamin E, phytic acid, phytosterols and sesamin. Sesame seeds have been considered to be antioxidant, anticancer, demulcent, emollient and laxative properties. Due to its lignans content, sesame is very efficient in lowering cholesterol levels. One of its lignan components, sesamin, is proven to protect the liver from oxidative damage. As an excellent source of phytosterols, sesame seeds are efficient immune enhancer. It is also believed that they can help as prevention against certain forms of cancers (Farri, 2012). The result of paucity of knowledge and folkloric claim on the sesamum leaves effectiveness in treating infertility and infections was reported. In addition, sesame leaves extract consumption enhances the quality of the spermatozoa produced with improvement in the storage capacity of the epididymes for these spermatozoa in a dose related manner (Web 1, 2104).

Moreover, the methanolic leaf extracts showed antibacterial effect against *Staphylococcus aureus* at a higher concentration and was very effective against *Streptococcus pneumoniae* and *Candida albicans*; hence, possess both antibacterial and antifungal activity (R´ıos and Recio., 2005). This same natural antibacterial effect against common skin pathogens such as *Staphylococcus* and *Streptococcus* bacteria as well as common skin fungi including the athlete's foot fungus was reported in other similar study using the sesame oil (Sesame, 2000). Ogunsola and Fasola (2014) reported that the leaf ethanolic extracts of *S. indicum* had a very strong antimicrobial effect on *E. coli* and mildly effective against *Klebsiella pneumonia and Salmonella typhi* at 400 mg/ml.

Most plants have hairs on their aerial surfaces, called trichomes, superficially similar to the hairs on the human body (Peter and Shanower, 1998). These plant hairs, or trichomes, affect the plant in a number of ways. There are two major types of trichomes, glandular and non-glandular trichomes. Glandular trichomes contain or secrete chemicals that have many uses in the pesticide, pharmaceutical and flavour/fragrance industries. Daniel (2005) added that leaf cuticular study is becoming more important because taxonomists, drug industries, animal nutritionists, animal toxicologists and police department have found it useful in plant identification. Thus, there is today an increasing interest in understanding the morphology and chemistry of glandular trichome and its exudates and taking advantage of their potential uses. The ethno-botanical and medicinal uses of this commercially important, nutritionally rich oilseed need to be explored for better utilization.

Sesame is an age old important oilseed crop particularly from the oil and seed perspective. However, potential importance of its leaves is not much known. The aim of the study therefore is to evaluate the diagnostic features of *S. indicum* using leaf trichomes and to ascertain the pharmaceutical potentials of the accessions. The distinguishing trichome types of some accessions under study, in the long run, will be helpful as morphological-histological markers in systematics and pharmaceutical diagnostics.

MATERIALS AND METHODS

Collection

Fresh leaves of twelve sesame accessions after twelve weeks of planting were collected from Research Farm of Abubakar Tafawa Balewa University, Bauchi, Nigeria. The accessions consisted of Ex-Gombe 6, Kenana 4, Lale-duk, Ex-Gombe 5, Ex-Sudan, Adaw-wula, Adaw-ting (improved), Adaw-ting, Ex-Gombe 4, Ex-Gombe 1, Ex-Gombe 3, and Ex-Gombe 2.

Isolation of epidermal layers

Epidermal peels of the leaf surfaces of the accessions were made using the method of Metcalfe and Chalk (1988). The abaxial and adaxial surfaces of the leaves surfaces were carefully separated by using dissecting needle and forceps after being rinsed in tap water. Alternatively, the epidermal surfaces were sectioned with razor blade (free-hand section) and placed on microscope slides. The preparations were stained with 1% safranin and 50% glycerol or Formalin Acetic Acid (FAA) and observed under a light microscope (AbdulRahaman and Oladele, 2005).

Microscopic study

Using 35 fields of view at X40 objective as quadrats, the number trichomes was noted to determine the frequency of the different trichome types and was expressed as percentage occurrence of such types based on all occurrences (Obiremi and Oladele, 2001). Terminologies for naming followed those of Esua (1977) and AbdulRahaman and Oladele (2005). The trichome densities were determined as the number of trichome per square millimeter (Stace, 1965).

Statistical analysis

All data were processed using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Computer software used was IBM SPSS version 20.

RESULTS

The accessions studied exhibited heterogeneous types of trichomes except Ex-Gombe 2 which has homogenous types (Figures 1–12 and Table 1). Nine types of trichomes were observed: unicellular (Figures 1, 7 and 11), glandular peltate (Figure 2), capitate glandular (Figures 3 and 4), long unbranched uniseriate (Figure

Table 1. Trichomes types, densities, frequencies and basal cell shapes in accessions of *S. indicum*.

Accession	Trichome type	Major type of trichome	Trichome density (mm^2)	Total density (mm^2)	Frequency (%)	Basal cell shape
Adaw-ting	Short-unbranched uniseriate	Non-glandular	4.6±0.51	-	62.16	Unmodified
	Long unbranched uniseriate	Non-glandular	2.8±0.58	7.4±0.55	37.84	Unmodified
Adaw-ting (improved)	Short-unbranched uniseriate	Non-glandular	11.4±0.24	-	17.95	Radial
	Scale	Glandular	15.0±0.55	-	56.1	Unmodified
	Multiseriate- capitate glandular	Glandular	11.4±0.21	-	13.95	Radial
	Capitate glandular	Glandular	11.2±0.54	37.6±0.41	12.01	Unmodified
Adaw-wula	Short-unbranched uniseriate	Non-glandular	3.2±0.37	-	72.73	unmodified
	Capitate glandular	Glandular	1.2±0.20	4.4±0.29	27.27	unmodified
Ex-Gombe 1	Short-unbranched uniseriate	Non-glandular	18.4±0.51	-	29.02	Unmodified
	Capitate glandular	Glandular	11.4±0.24	-	17.98	Radial
	Scale	Glandular	23.2±0.49	-	36.59	Unmodified
	Long unbranched uniseriate	Non-glandular	10.4±0.33	63.4±0.38	16.4	Radial
Ex-Gombe 2	Short-unbranched uniseriate	Non-glandular	9.8±0.66	-	76.52	Radial
	Multicellular	Glandular	7.5±0.54	17.3±0.66	23.48	unmodified
Ex-Gombe 3	Short-unbranched uniseriate	Non-glandular	3.2±0.37	-	45.71	Radial
	Branched Uniseriate	Non-glandular	1.4±0.24	4.6±0.27	20	Radial
Ex-Gombe 4	Unicellular	Non-glandular	2.4±0.23	-	34.29	Radial
	Long unbranched uniseriate	Non-glandular	5.8±0.73	-	72.5	Unmodified
	Capitate glandular	Glandular	1.2±0.20	-	15	Radial
	Glandular peltate	Glandular	1.0±0.32	10.41±0.50	12.5	Unmodified
Ex-Gombe 5	Long unbranched uniseriate	Non-glandular	4.0±0.32	-	55.56	Radial
	Capitate glandular	Glandular	1.4±0.24	-	19.44	Unmodified
	Long-capitate glandular	Glandular	1.8±0.49	7.2±0.32	25.01	Radial
Ex-Gombe 6	Long unbranched uniseriate	Non-glandular	15.6±0.51	-	57.14	Radial
	Capitate glandular	Glandular	11.4±0.24	-	14.29	Unmodified
	Scale	Glandular	12.8±0.37	48.8±0.37	28.57	Unmodified
Ex-Sudan	Capitate glandular	Glandular	3.6±0.40	-	26.47	Unmodified
	Long-capitate glandular	Glandular	5.2±0.37	-	38.24	Radial
	Unicellular	Non-glandular	1.8±0.58	-	13.24	Radial
	Long unbranched uniseriate	Non-glandular	3.0±0.89	13.8±0.63	22.06	Radial
Kenana 4	Long unbranched uniseriate	Non-glandular	6.0±0.32	-	34.88	Radial
	Scale	Glandular	11.2±0.37	17.2±0.36	65.11	Radial
Lale-duk	Glandular peltate	Glandular	2.6±0.32	-	25.57	Unmodified
	Long unbranched multiseriate	Glandular	2.0±0.71	-	19.67	Unmodified
	Capitate glandular	Glandular	1.6±0.24	-	15.73	Unmodified
	Long-capitate glandular	Glandular	1.60±0.51	-	15.73	Unmodified
	Long unbranched uniseriate	Non-glandular	1.20±0.20	-	11.8	Radial
	Multiseriate- capitate glandular	Glandular	1.17±0.31	10.17±0.59	11.5	Unmodified

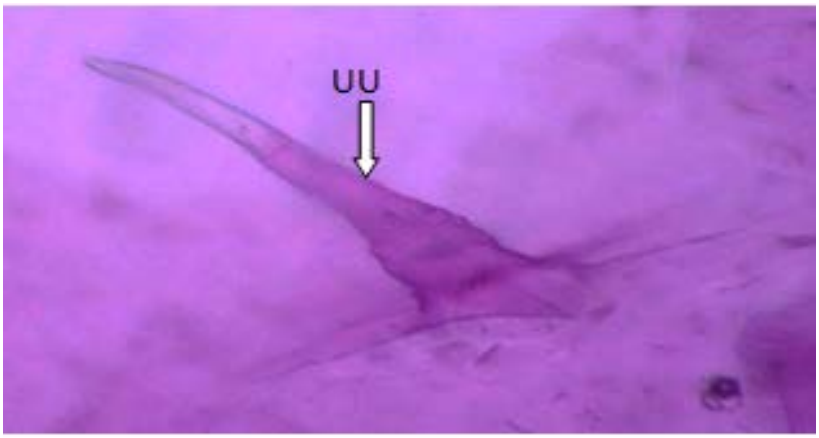

Figure 1: Unicellular uniseriate trichome in Ex-Gombe 4

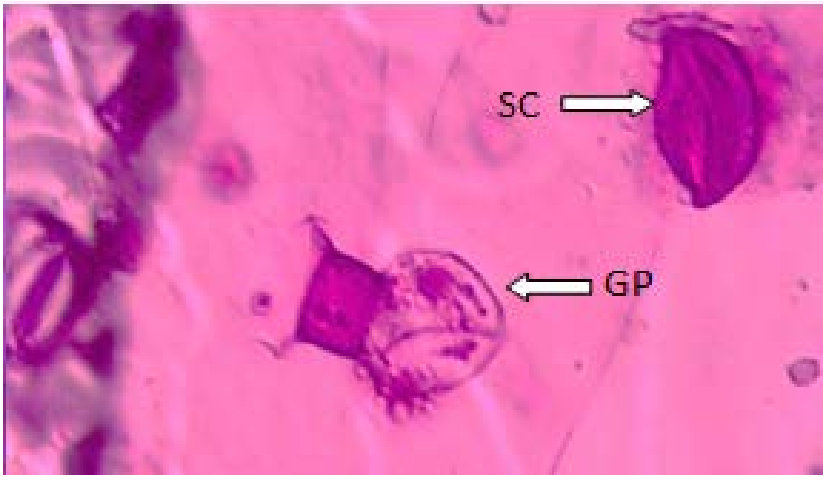

Figure 2. Glandular peltate and scale trichomes in Kenana 4

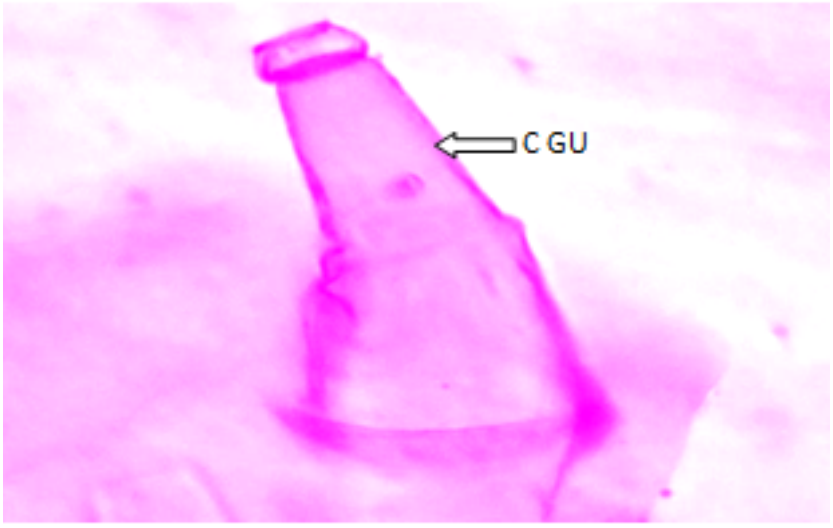

Figure 3. Capitate glandular uniseriate trichome in Ex-Gombe 5

Figure 4. capitate glandular uniseriate trichome in Adaw-ting

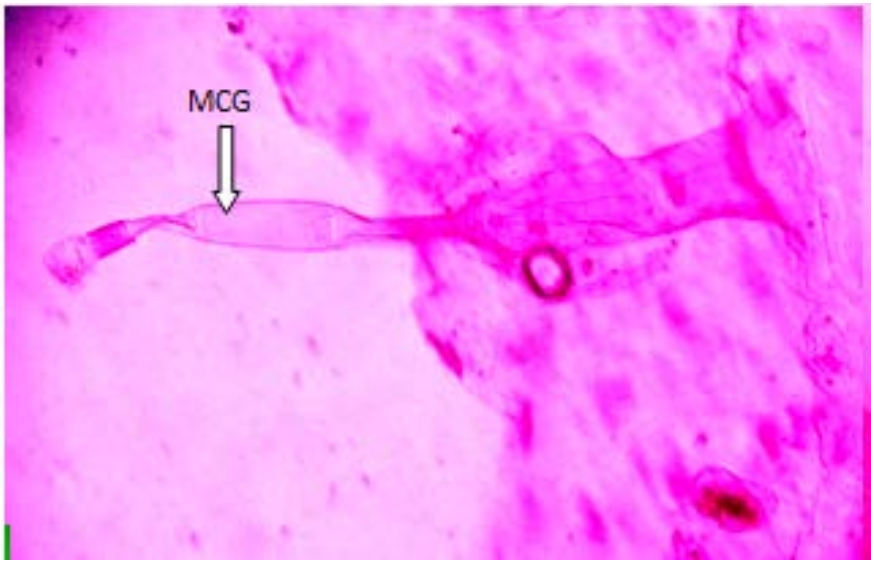

Figure 5. Multiseriate capitate glandular in Lale-duk

10), short unbranched uniseriate (Figures 10), scale (Figure 2), multicellular (Figure 9), multiseriate capitate glandular (Figure 5), branched uniseriate (Figure 11) and branched uniseriate (Figure 12). Alege et al. (2013) identified only unicellular and multicellular trichomes in *S. indicum*. Level of heterogeneity varies from accession to accession. It is the highest in accession Lale-duk with six types (Table 1), four in accessions Ex-Sudan, Adaw-ting (Improved) and Ex-Gombe 1, three in accessions Ex-Gombe 3, Ex-Gombe 4, Ex-Gombe 5 and Ex-Gombe 6, two in Kenana 4, Adaw-wula and Adaw-ting.

In all the trichome types, the most frequent are short unbranched uniseriate (76.52%) in Ex-Gombe 2, (72.73%) in Adaw-wula, they occur in six accessions and long unbranched uniseriate (72.5%) in Ex-Gombe 4, they occur in seven accessions. The lowest frequency was multiseriate capitate glandular (11.5%) observed in Lale-duk (Table 1). This information is meaningful from an ecological perspective because it can help us better understand what role these trichomes may have in the plant's ecology, and may lead to a better understanding of natural plant protection.

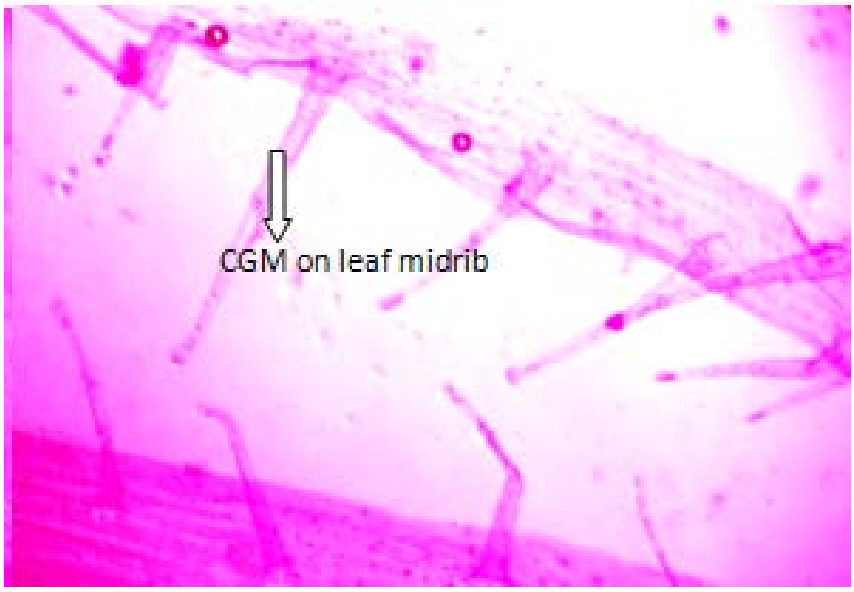

Figure 6. Capitate glandular multiseriate trichome in Ex-Gombe 6

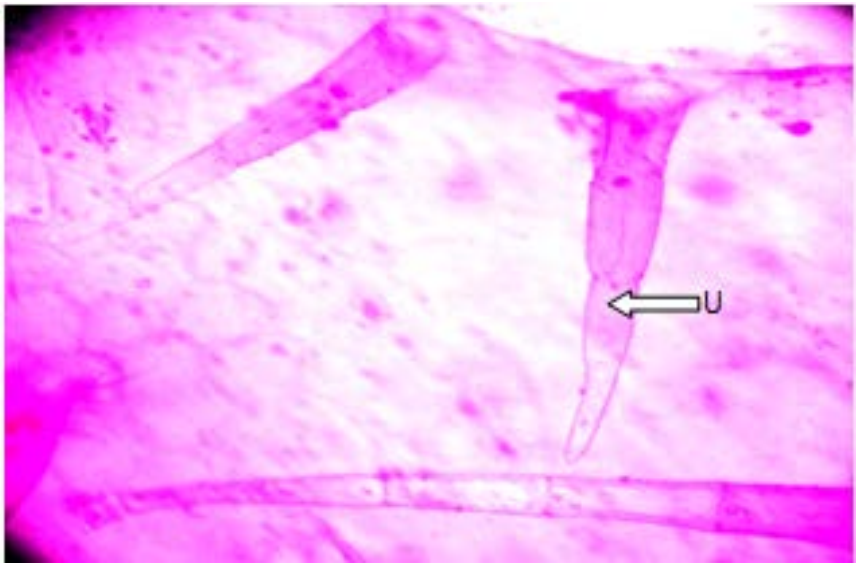

Figure 7. Unicellular trichome in Ex-Gombe 2

The density of trichomes varied from accession to accession. Trichome density (Table 1) is higher in Ex-Gombe 3 (63.4±0.38 mm^2) and the lowest was recorded by Ex-Gombe 4 (4.4±0.29 mm^2). The trichomes identified were also classified into two types based on their basal cell forms, namely, unmodified (Figure 12) and radial basal cells (Table 1). The former occurred in accessions Adaw-ting, Adaw-ting (improved), Adaw-wula, Lale-duk and Ex-Gombe 5 while the latter occur in Ex-Gombe 1, ex-Gombe 2, Ex-Gombe 3, Ex-Gombe 4, Ex-Gombe 6, Ex-Sudan and Kenana 4.

DISCUSSION

Trichomes are specialized hairs found on the surface of vascular plants and glandular trichomes in particular are responsible for a significant portion of a plant's secondary chemistry. Besides metabolically inactive or so-called non-glandular trichomes, biosynthetically active glandular trichomes also exist in *S. indicum* (Table 1). They sequester or store plant metabolites that are often characteristic for specific taxonomic groups e.g. monoterpenes in Lamiaceae, sesquiterpene lactones in

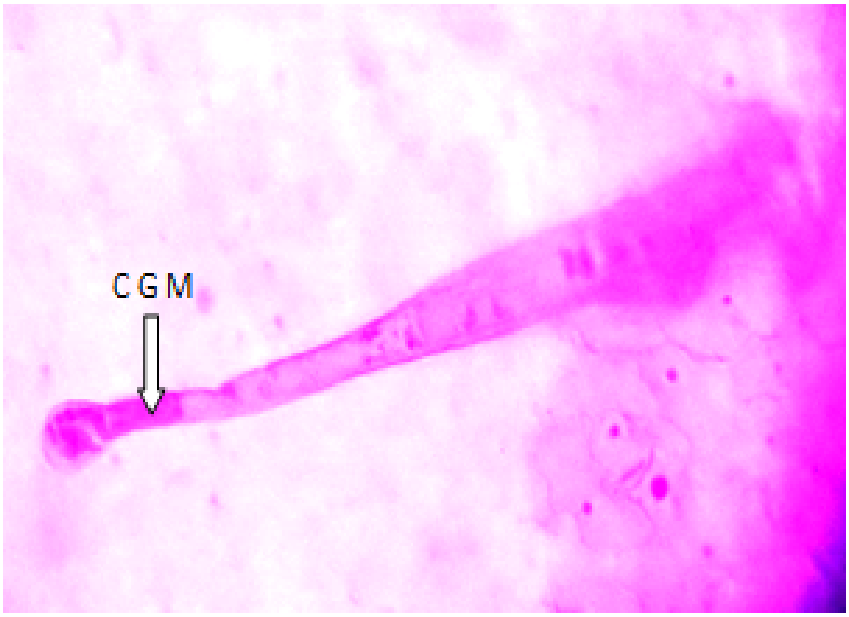

Figure 8. Capitate glandular multiseriate trichome in Adaw-ting Improved

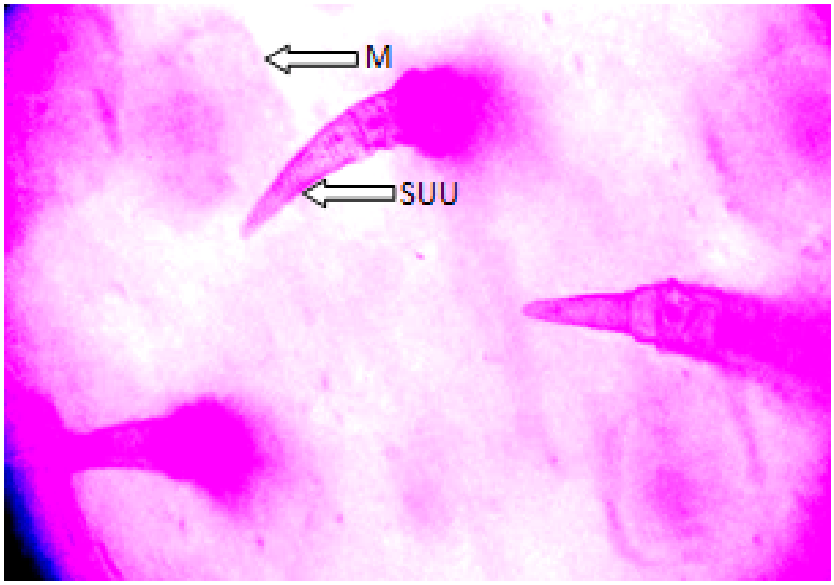

Figure 9. Multicellular and short unicellular uniseriate trichome in Ex-Gombe 3

Asteraceae. Glandular trichomes have in common the capacity to produce, store and secrete large amounts of different classes of secondary metabolites (Fahn, 2000; Schilmiller et al., 2008). Many of the specialized metabolites that can be found in glandular trichomes have become commercially important as natural pesticides, but also have been found to be used as food additives or pharmaceuticals (Duke et al., 2000; Aharoni et al., 2006). For instance, plants of the Lamiaceae, comprising species such as Mint (Mentha × piperita), Basil (*Ocimum basilicum*), Lavender (*Lavandula spica*), Oregano (*Origanum vulgare*) and Thyme (*Thymus vulgaris*), are cultivated for their glandular trichome-produced essential oils (Schilmiller et al., 2008). Moreover, artemisinin, a sesquiterpene lactone that is produced in the glandular trichomes of annual wormwood (*Artemisia annua*), is used for the treatment of malaria (Weather et al., 2011). In addition, gossypol and related compounds, which are dimeric disesquiterpenes produced by cotton (*Gossypium hirsutum*) trichomes,

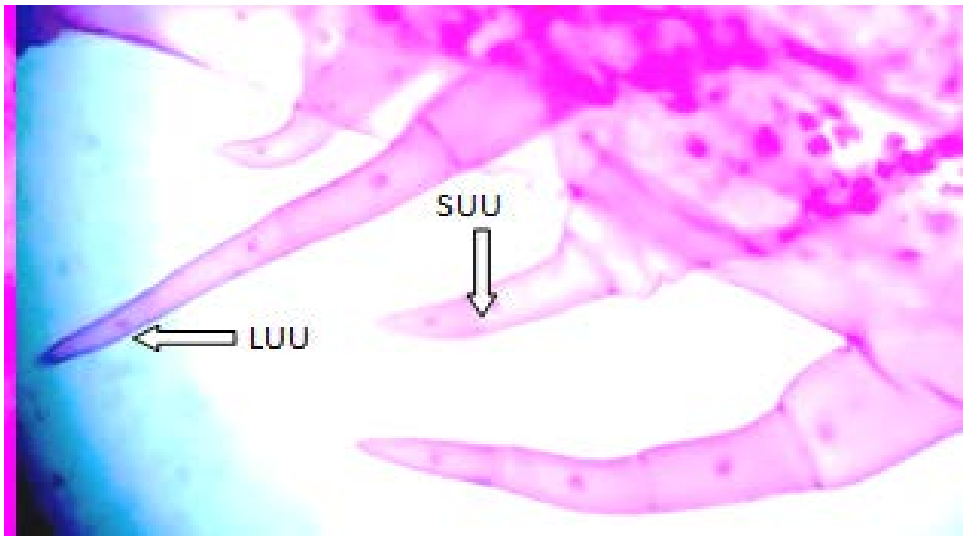

Figure 10. Long and short uniseriate unicellular trichomes in Lale-duk

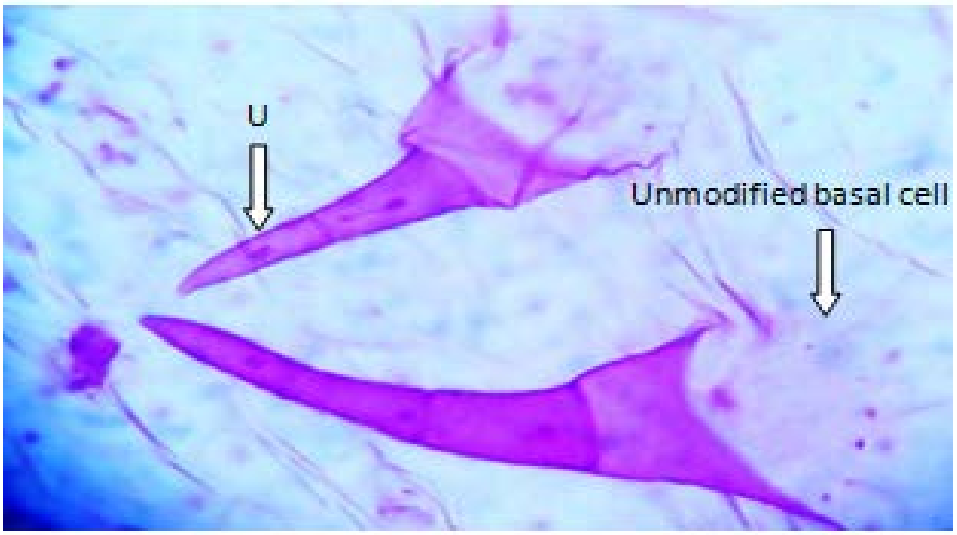

Figure 11. Unicellular with unmodified basal cell form in Ex-Sudan

have strong antifungal activity (Mellon et al., 2012) and are potential natural pesticides Dayan and Duke (2003). It is for these kinds of specialized metabolic properties, and for the opportunities to modify these properties via genetic engineering (Lange et al., 2011), that trichomes have received increased attention over the past years (Tissier, 2012). According to Aschenbrenner et al. (2013), glandular trichomes secrete substances characteristics of a species, including essential oils, gums, mucilages or resins.

Essiett et al. (2012) reported that trichome features can be reasonably employed for the delimitation of plant species. Also Ogundipe and Pereira-sheteolu (2006) reported that the presence and types of trichomes are useful diagnostic features in the Pedaliaceae family. Thus, the non-glandular trichomes observed in accessions Adaw-ting and Ex-Gombe 3 dinguished them from other accessions which recorded glandular trichomes (Table 1). The basal cell shape of the trichomes also is good systematic evidence in the diagnosis of these accessions. Ex-gombe 3 and Kenana were observed to have radial basal cells, whereas Adaw-ting had unmodified basal shape. The basal cell shapes were heterogeneous in the rest of the accessions.

Apart from the taxonomic and pharmaceutical significance of trichomes, leaf trichomes contribute to

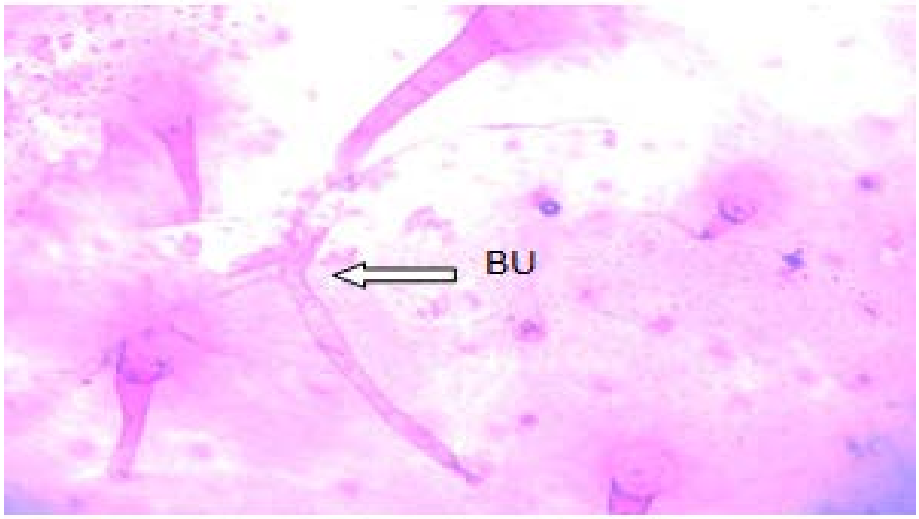

Figure 12. Branched Unicellular trichome in Adaw-wula

plant resistance against herbivory (Dalin et al., 2008; Gopfert et al., 2010; Aschenbrenner et al., 2013). The hairs generally are thought to help keep the leaf cool and also prevent rapid wind current from passing close to the surface and thus removing water vapour from transpiring areas thereby reducing the rate of transpiration (Fahn, 1990). The number of trichomes produced and trichome density vary genetically within several species (Dalin et al., 2008); but in most empirical studies, the abundance and effectiveness of natural enemies were found to be negatively correlated with the density of plant trichomes (Lovinger et al., 2000; Fordyce and Agrawal, 2001; Stavrinides and Skirvin, 2003; Mulatu et al., 2006; Olson and Andow, 2006). Thus, Ex-Gombe 3 which recorded high trichome density could be drought-tolerant accession because Abdulraham and Oladele (2004) reported that plants with high trichome density have high capacity to conserve water.

Conclusion

It is suggested based on this study that sesame trichomes in general and glandular trichomes in particular should be harvested and the bioactive compounds be identified and isolated as these trichomes may be of potential chemical factories for pharmaceutical, flavour, fragrance, pesticide and insecticide industries. This is because Covello (2008), Weathers et al. (2011) and Alain (2012) stated that secretions of glandular trichomes have been used for their medicinal properties, and in some cases active ingredients have been marketed as drugs. Artemisinin is as esquiterpene lactone produced in glandular trichomes of *A. annua* are used for the treatment of malaria as an alternative to quinine drugs, which face increasing resistance from emerging strains of the malaria parasite. Pharmaceutical companies have developed derivatives of artemisinin (artemeter, artesunate) which are now widely marketed (Shanks, 2006).

Conflicts of interest

Authors declare that there are none.

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