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SCREENING AND BIOASSAY-GUIDED ISOLATION OF ANTIMICROBIAL COMPONENTS FROM *Laggera mollis*

[2]Dauda, U. and [1*]Mudi, S. Y.
[1]Department of Pure and Industrial Chemistry, Bayero University Kano
[2]Department of Basic Science, Taraba State College of Agriculture, Jalingo
*Correspondence author

ABSTRACT
There are about 20 identified species in the genus Laggera and only few have been extensively investigated. The study was aimed at evaluation the antimicrobial activity of Laggera mollis and isolation of the bioactive component(s) for new potent antimicrobial agent. Four fractions each from the leaves and stem-root of the plant were sequentially extracted with petroleum ether, chloroform, ethyl acetate and ethanol using soxhlet extraction scheme, while the marcs were decocted for the aqueous extracts. Phytochemical screening of four extract fractions each from the leaves and stem-root of the plant revealed the presence of alkaloids, tannins, flavanoids, saponins, steroids, terpenoids and reducing sugar distributed across the fractions. Disc diffusion method of Antimicrobial Sensitivity Test (ATS) was adopted in testing the bioactivity of the plant extracts against seven bacteria and one fungus. Bioassay-guided isolation from the petroleum ether fraction (LM1-01) of the leaves of the Laggera mollis using column chromatography, led to the isolation of two pure compounds. The results of the antimicrobial activity of the isolated compounds indicted that LM1-1-10 was more potent against Salmonella typhi (11 mm) at 40 µg/disc followed by Streptococcus pyogens (10 mm) at 40 µg/disc and Staphylococcus aureus (10 mm) at 40 µg/disc. The spectra of the activity exhibited by the isolates signified their potency for the development of therapeutic agents against these pathogenic microorganisms.
Keywords: Bioassay-guided isolation, antimicrobial activity, Laggera mollis, clinical isolates, extracts.

INTRODUCTION

The global health system is presently facing the most important challenges in the history of medicine. The growing incidences of drug-resistant pathogens of both clinical and agricultural importance and profuse use of commercial antibiotic and synthetic pesticides for human and crop protection is harmful to human health, ecosystem and environment (Handa *et al*., 2008). The quest for solving these challenges has necessitate a search for new antimicrobial substance from other sources including screening of medicinal plants for antimicrobial activities and photochemical for finding potential new compounds for therapeutic uses (Venkatesan *et al*., 2009). Medicinal plants have their intrinsic ability to resist pathogenic microorganisms and this has led researchers to investigate their mechanisms of action and isolation of active compounds (Cos *at al*., 2006). This has enabled exploration of medicinal plants for the treatment of microbial infections of both plants and humans by developing new antimicrobial agents.

Laggera mollis (D. Don), (Hausa; Taabaa-taabaa), is an annual herb, with erect stems and very soft glandular hairs, growing 30-80 cm high and commonly found in tropical waterlogged areas. The leaves of the plant alternate with irregularly tooth. The individual flowers are composite on common receptacles. *Laggera mollis* has applications in the traditional medicine systems. Decoction from the leaves of the plant is used in the treatment of acute fever in children, malaria, rheumatism, and HIV/AIDS in the northern part Nigeria. In India, the decoction from the leaves is also used in the treatment of diarrhea (Srivastava, 2008). The genus *Laggera* (Asteraceae) is distributed mainly in tropical Africa and South-east Asia (Hoa *et al*., 2012).

This research entails extensive search of the bioactive components responsible for activity in the target plant. It is therefore imperative to follow standard methods to authenticate these claims of antimicrobial action.

MATERIALS AND METHODS

Collection and identification of the plant

Fresh plants of *Laggera mollis* were collected in the month of August 2011, at waterlogged area of Tukuntawa ward in Kano Municipal Council, Kano State. The plant was identified colloquially as "*Taabaa-taabaa*" by Malam Ali (Baba) Garko a former gardener in the department of biological sciences, Bayero University Kano. It was later confirmed and authenticated as *Laggera Mollis* (Voucher No. 2015) in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

Preparation of plant materials and extraction

The whole plant of the *Laggera mollis* was carefully screened washed and removed the unwanted. The fresh leaves were striped from the stem, collected separately and air-dried at room temperature under electric fan. The air-dried leaves and stem-root were ground separately and sieved into fine powder.

Extraction methods involved separation of medicinally active fractions of plant tissues from inactive/inert components by using selective solvents and extraction technology (Das *et al*., 2009). The powdered leaf/stem-root (100g of each) were separately extracted with 400 cm^3 each of petroleum ether, chloroform, ethyl acetate and ethanol sequentially, using soxhlet extractor, at temperature of 50^0C (Sheeba, 2009; Venkatesan and Karrunakaran, 2010, Kamboj *et al*., 2011). Time interval of 6 hours was set as the duration of the extraction. The fractions were concentrated using Rotary evaporator (R110) at 40^0C. The residues were subsequently re-extracted (decocted) with 400 cm^3 of distilled water in 1dm^3 conical flask at 50^0C for five hours and the extracts was filtered and evaporated at the same temperature (Handa *et al*., 2008). All the concentrated fractions were collected in separate weighed beakers and lyophilized into powders to be used for the study. The fractions were coded and labeled as LM1-1 to LM1-5 (leaf extracts) and LM2-1 to LM2-5 (stem-root).

Phytochemical screening of the extracts

The extracts were subjected to phytochemical screening to determine the group of secondary metabolites present in the plant materials. The tests were conducted for alkaloids, saponins, flavanoids, reducing sugars, tannins, cardiac glycoside, terpenoid and steroids**,** using standard procedures as described by Sofowora (1993); Trease and Evans (1989) and Harborne (1988).

Antimicrobial Bioassay

Preparation of stock solution and disc concentrations

The non-aqueous extracts were dissolved using di-methyl sulphoxide (DMSO), while the aqueous extracts were dissolved using distilled water. The stock solutions were formed by dissolving 100 mg of each extract in 1ml of the appropriate solvent. For each fraction, concentrations of 1000 µg/disc, 500 µg/disc, 200 µg/disc and 100 µg/disc were prepared separately in sterilized Bijou bottles containing 50 sterile improvised Whatman No. 1 filter paper discs that had absorbance potency of 0.01 cm^3. In each case, the filter papers were saturated with plant extract of the desired the concentration.

Source of bacterial and fungal isolates

The cultures of seven bacteria (two Gram positive and five Gram negative) and one fungus were used. The test microorganisms were clinical isolates obtained from Pathology Department of Aminu Kano Teaching Hospital, Kano, which were further re-identified and confirmed in the Microbiology laboratory of the Department of Biological Sciences, Bayero University Kano.

Inocula Standardization

As described by standard sensitivity test of National Committee for Clinical Laboratory Standards (NCCLS, 2000), two loops of the confirmed isolates were introduced in Peptone water in separate sterilized bottles and kept overnight in an incubator at 37^0 C. Few colonies of the overnight growth of the isolates to be tested were dispersed in sterile normal saline to form a turbid culture suspension that matched 0.5 McFarland turbidity (NCCLS, 1993).

Reference Antibiotics

Reference antibiotics were obtained from a pharmaceutical store to serve as positive controls. Levofloxacin was selected as a reference for the bacteria while ketoconazole was selected as a reference antifungal. Concentration of 10µg/disc for each of the two controls was prepared using serial dilution method as previously described.

***In vitro* antimicrobial susceptibility testing of the isolates**

Antimicrobial activity of the plant extracts was determined using disc diffusion method as described by NCCCL (NCCCL, 2000). Standardized inoculum of each of the isolates was swabbed onto the surface of the solidified and oven-dried Nutrient Agar in separate Petri-dishes under sterilized environment. The four prepared discs of the different concentrations of the extracts (LM1-01 to LM1-05 and LM2-01 to LM2-05) were then placed onto the surface of the inoculated media at intervals in a clockwise direction. The positive control disc was placed at the center of the bacteria and fungus inoculated media. The plates were incubated for 24 hours at 37^0C for bacterial and 72 hours at 25^oC for fungus, after which the antimicrobial activity was observed by measuring the width of the clear inhibition zone around the discs and the values obtained were appropriately recorded.

Isolation of Bioactive Compounds

Large Scale Extraction

Two hundred grams (200g) of the powdered leaves of *Laggera mollis* were extracted with petroleum ether using soxhlet extraction process for 6 hours at 50^oC. The extract was concentrated and labeled LM1-1. The extract was allowed to dry and stored in a refrigerator.

Column Chromatography of LM1-1

Column chromatography of the leaf petroleum ether extract *Laggera mollis* LM1-1 was carried out. 300g of washed silica gel (50-200) (with petroleum ether and then chloroform) was packed into a glass column (135x2cm) in slurry of petroleum ether. 12g of the concentrated and dried petroleum ether fraction of the leaves extract (LM1-1) was thoroughly mixed with silica gel until a non-sticky homogenous powder was form and then loaded into the silica gel column. Additional silica gel (10g) was added on top to serve as a protective layer. The column was eluted by one Liter of each solvents of increasing polarity in the following stepwise gradient: petroleum ether (100%), petroleum ether-chloroform (1:1), chloroform (100%), chloroform-ethyl acetate (1:1), chloroform-ethyl acetate (2:3), ethyl acetate (100%), ethyl acetate-methanol (1:1) and methanol (100%).

The eluetes were collected in bottles of approximately 100ml from the beginning to the end of the elution, in each case the eluetes were labeled accordingly (from LM1-1-01 to LM1-1-107) and allowed to evaporate to dryness at room temperature.

The eluetes were analyzed on TLC (Sharma and Achaya, 1988) using different solvent system of petroleum ether-chloroform in five step-wise gradients. From the chromatograms developed, fractions with similar R_f values, colour and shape of the spots were pooled together and coded accordingly.

Further bioassay revealed that pooled eluates fractions LM1-1-05, LM1-1-10 and LM1-1-22 showed considerable bioactivity. The eluates were subjected to column chromatography using a solvent system of petroleum ether-chloroform in a burette (50ml) for further separation. Twenty milliliter (20ml) of the eluetes from each elution were collected separately and allowed to evaporate at room temperature. Three Semi-pure isolates were obtained.

Purification of the semi-pure isolates

Re-crystallization and preparative TLC methods were adopted for the purification of the semi-pure compounds isolated (Sharma and Achaya, 1988). Preparatory TLC was used in purifying isolate LM1-1-05 using 1.0 cm thick silica gel coated chromatoplates, developed in an eluant of 50% petroleum ether: 50% chloroform. LM1-1-10 and LM1-1-22 were purified by re-crystallization, where n-Hexane was used as the solvent at a low temperature. The purity of the isolates were confirmed by conducting TLC using three different eluates (4:1, 1:1,and 2:3) of Petroleum ether: chloroform.

Bioassay of the pure compounds

The bioactivity of the pure isolates (LM1-1-05B, LM1-1-10A and LM1-1022) was tested against the eight human pathogens using disc diffusion antimicrobial sensitivity test. In this case, 10 µg/disc, 20 µg/disc and 40 µg/disc were taken as the disc concentrations for the test (Das *et al*., 2010).

RESULTS

The plant parts were extracted using five solvents of different polarity. Among all the solvents used, water extract was found to have the maximum extractive yield followed by petroleum ether extract. Extracts from petroleum ether and chloroform retain their natural appearance. The amount and appearance of each fraction are presented in Table 1.

Table 1: Physical characteristics of fractions obtained of *Laggera mollis*

Leaf extracts				
FRACTION	**CODE**	**WEIGHT(g)**	**TEXTURE**	**COLOUR**
Pet. Ether	LM1-01	5.10	Gummy	Dark-green
Chloroform	LM1-02	2.10	Gummy	Dark-green
Ethyl acetate	LM1-03	1.22	Oily	Greenish
Ethanol	LM1-04	2.11	Oily	Brown
Distilled Water	LM1-05	17.20	Solid	Dark-brown
Stem-root extracts				
Pet. Ether	LM2-01	2.52	Gummy	Dark-green
Chloroform	LM2-02	1.01	Gummy	Dark-green
Ethyl acetate	LM2-03	1.12	Oily	Greenish
Ethanol	LM2-04	2.13	Oily	Brown
Distilled Water	LM2-05	12.51	Solid	Dark-brown

Preliminary phytochemical screening was conducted on fractions obtained from both the leaves and stem-root extracts of the plant. The results showed the presence of alkaloids, tannins, flavonoids, Saponins, steroids and reducing sugar in the plant. Table 2 revealed the groups of secondary metabolites detected in each fraction.

Table 2: Phytochemical constituents detected in leaf and root-stem extracts of *Laggera mollis*

Plant Parts	**Fractions**	**Phytochemical**					
		Tannins	**Alkaloid**	**Flavonoids**	**Saponins**	**Steroids**	**Red. Sugar**
Leaves	LM-01	-	-	+	-	+	+
	LM-02	-	+	-	-	+	-
	LM-03	+	+	+	-	+	+
	LM-04	+	+	+	+	+	+
	LM-05	+	-	+	+	+	+
Stem-root	LM-01	-	-	-	+	+	-
	LM-02	-	-	-	+	-	-
	LM-03	-	+	-	+	-	+
	LM-04	+	+	+	+	+	+
	LM-05	+	-	+	+	+	+

KEYS: + = Positive (Present); - = Negative (Absent); LM-01-petroleum ether fraction, LM-02-chloroform fraction, LM-03-ethyl acetate fraction, LM-04-ethanol fraction, LM-05-aqueous fraction

The antimicrobial activities of petroleum ether, chloroform, ethyl acetate, ethanol and aqueous fractions of both the leaves and stem-root parts of the plant are shown in Table 3. Among all the extracts, only petroleum ether and the aqueous extracts were found to be active against some of the human pathogens. Petroleum ether extract of the leaves showed activity against four bacteria (*Streptococcus pyogens*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Proteus Vulgaris*) while only three (*Streptococcus pyogens*, *Klebsiella pneumonia* and *Staphylococcus aureus*) were susceptible to the petroleum ether extract of the stem-root. The aqueous extract of the leaves showed activity against three bacteria (*Streptococcus pyogens*, *Klebsiella pneumoniae*, and *Proteus vulgaris*) while the stem-root aqueous extract was found to be active against two bacteria (*Streptococcus pyogens* and *Klebsiella pneumoniae*). Petroleum ether extracts was found to have maximum zone of inhibition against *Streptococcus pyogens* (23 mm) and the minimum (07 mm) against *Proteus vulgaris.* The aqueous extracts showed maximum zone of inhibition against *Klebsiella pneumonia*e (21 mm) and the minimum against *Streptococcus pyogens* (10 mm).

Table 3. Antimicrobial activity of leaf and stem-root fractions of *Laggera mollis*

Plant parts	Fractions	Conc. (µg/disc)	Zones of inhibition (mm) against microorganisms							
			SP	KP	SA	PV	ST	PA	EC	CA
Leaves	LM-01	1000	23	21	10	10	-	-	-	-
		500	13	19	10	10	-	-	-	-
		200	10	10	08	07	-	-	-	-
		100	09	08	07	07	-	-	-	-
	LM-02	1000	-	-	-	-	-	-	-	-
		500	-	-	-	-	-	-	-	-
		200	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
	LM-03	1000	-	-	-	-	-	-	-	-
		500	-	-	-	-	-	-	-	-
		200	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
	LM-04	1000	-	-	-	-	-	-	-	-
		500	-	-	-	-	-	-	-	-
		200	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
	LM-05	1000	20	10	10	-	-	-	-	-
		500	19	08	07	-	-	-	-	-
		200	18	10	06	-	-	-	-	-
		100	18	-	06	-	-	-	-	-
	Control	10	30	18	23	23	18	18	18	18
Stem-root	LM-01	1000	15	21	-	14	+	-	-	-
		500	13	09	-	13	+	-	-	-
		200	09	10	-	08	-	-	-	-
		100	08	-	-	-	-	-	-	-
	LM-02	1000	-	-	-	-	-	-	-	-
		500	-	-	-	-	-	-	-	-
		200	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
	LM-03	1000	-	-	-	-	-	-	-	-
		500	-	-	-	-	-	-	-	-
		200	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
	LM-04	1000	-	-	-	-	-	-	-	-
		500	-	-	-	-	-	-	-	-
		200	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
	LM-05	1000	15	13	-	-	+	-	-	-
		500	13	12	-	-	-	-	-	-
		200	10	11	-	-	-	-	-	-
		100	-	10	-	-	-	-	-	-

Zones of inhibition (mm). C=control drug (Levofloxacin for bacteria, Ketoconazole for fungus) Microorganisms: SP=*Streptococcus pyogens*, KP=*Klebsiella pneumoniae*, SA=*Staphylococcus aureus*, PV=*Proteus vulgaris*, ST=*Salmonella typhi,* PA=*Pseudomonas aeruginosa*, EC=*Escherichia coli* , CA= *Candida albicans,* Conc. = Concentration

Separation of the constituents of the petroleum ether extract of the leaves was achieved using column chromatography which yielded 107 eluates. The eluates obtained were pooled to 26 fractions based on their TLC analysis. The fractions pooled, amount and the appearance of the eluates is shown in Table 4. The dried eluates fractions appeared in various physical forms; as waxy solid, yellow to yellowish oils, yellow to yellowish solids, crystalline solids, greenish to pale-green solids, dirty green, etc.

Table 4: Eluates obtained from Petroleum ether (LM1-1) Fractions of leaf of *Laggera mollis*

Eluant	Fractions Pooled	New Code	Weight (mg)	Appearance
P.E. (100%)	1	LM 1-1-1	03	Colourless
P.E. (100%)	2 & 3	LM 1-1-2	82	Waxy solid
50% P.E.:50%$CHCl_3$	4 – 6	LM 1-1-3	72	Yellow
" "	7 & 8	LM 1-1-4	22	Yellowish
" "	9 – 13	LM 1-1-5	95	Yellowish
" "	14 – 16	LM 1-1-6	32	Pale – yellow
" "	17 – 22	LM 1-1-7	87	Pale – yellow
" "	23 – 25	LM 1-1-8	3,487	Crystalline
$CHCl_3$ (100%)	26 – 34	LM 1-1-9	109	Dark – green
" "	35 – 39	LM 1-1-10	102	Dark – green
" "	40 & 41	LM 1-1-11	32	Dark – green
50%$CHCl_3$: 50% E.A.	42 – 44	LM 1-1-12	342	Dark – green
" "	45 – 47	LM 1-1-13	33	Dark – green
" "	48 – 55	LM 1-1-14	38	Greenish
40%$CHCl_3$: 50% E.A.	56 – 65	LM 1-1-15	50	Greenish
" "	66 – 70	LM 1-1-16	19	Greenish
" "	71 & 72	LM 1-1-17	07	Greenish
E.A. (100%)	73 – 78	LM 1-1-18	47	Pale - green
"	79	LM 1-1-19	04	Pale – green
"	80 – 82	LM 1-1-20	15	Pale – green
"	83 & 84	LM 1-1-21	19	Pale – green
"	85 – 87	LM 1-1-22	19	Pale – green
50% E.A.: 50%$CHCl_3$	88	LM 1-1-23	438	Dirty – green
" "	89 – 91	LM 1-1-24	22	Yellow – green
" "	92 – 99	LM 1-1-25	136	Yellow – green
CHOH(100%)	100 - 107	LM 1-1-26	70	Pale – yellow

Bioassay of the pooled fractions against the pathogens showed that only fractions LM1-1-10 and LM1-1-22 have activities against some of the test microorganisms. The results of the TLC analysis presented in Table 5, revealed the presence of two components as impurities in each fraction

Table 5: Results of TLC and physical characteristics of bioactive Fractions of LM1-1 Eluetes

Pooled Fractions	R_f values	Appearance	Weight
LM1-1-10	0.43, 0.33, 0.32	Yellowish solid	95mg
LM1-1-22	0.71, 0.38, 0.28	Greenish solid	87mg

Further separations of the bioactive fractions using burette yielded semi-pure isolate from each fraction. Purification of the semi-pure fractions yielded 83 mg of white crystalline solid from LM1-1-10A and 78 mg of colourless waxy solid from LM1-1-22. The single R_f values in the respective solvent ration from the TLC indicated very high degree of purity of the pure isolated compounds (Table 6).

Table 6. Results of TLC the pure isolates developed in Pet. Ether: Chloroform

	R_f values				
Isolates	4:1	1:1	2:3	**Appearance**	**weight**
LM1-1-10A	0.28	0.67	0.69	White crystalline solid	83mg
LM1-1-22	0.12	0.70	0.73	Colourless waxy solid	78mg

The confirmatory bioassay was conducted on the test micro-organisms and the results obtained are shown in Table 7. The Pure isolates have showed activity against *Streptococcus pyogens*, *Klebsiella pneumonia* and *Staphylococcus aureus.* Isolate LM1-1-10A was found to be the most active with highest zone of inhibition of 11 mm at 40 µg/disc against *Salmonella typhi* (which showed resistance to the impure plant fractions).

The activity increases with increased in concentration of the isolates. None of the isolates was found to be active against the test microorganisms at the concentration of 10 µg/disc.

Table 7. Antimicrobial Activity of the Pure Isolates against the test microorganisms

Fractions	Concentration (µg/disc)	Zones of inhibition (mm) against micro-organisms							
		SP	KP	SA	PV	ST	PA	EC	CA
LM1-1-10A	10	-	-	-	-	-	-	-	-
	20	07±1.00	06±0.58	06±0.58	-	08±0.58	-	-	-
	40	10±0.58	07±0.58	10±0.58	-	11±0.58	-	-	-
LM1-1-22	10	-	-	-	-	-	-	-	-
	20	-	06±1.00	-	-	-	-	-	-
	40	070±0.58	07±0.58	06±0.58	-	-	-	-	-
Control	10	30	18	23	23	18	18	18	18

Values are expressed as the mean ± SD of triplicate
Zones of inhibition (mm). C=control drug (Levofloxacin for bacteria, Ketoconazole for fungus) Microorganisms: SP=*Streptococcus pyogens*, KP=*Klebsiella pneumonia*, SA=*Staphylococcus aureus*, PV=*Proteus vulgaris*, ST=*Salmonella typhi,* PA=*Pseudomonas aeruginosa*, EC=*Escherichia coli*, CA= *Candida albicans*

Phytochemical screening of the fractions revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, reducing sugar and steroids distributed across the fractions (Table 2). The usefulness of plants medicinally is due to the presence of these biological active secondary metabolites (Wink, 1990) therefore, the antimicrobial activity can be attributed to the presence of compound(s) belonging to one or of more these Phytochemical. The presence and biological activities of Phenolics (Ahmed *et al*., 1998 and Wu *et al*., 2006, 2009), Terpenoids (Asfaw *et al*., 1999) and steroids (Kuljanabhagavad *et al*., 2009) were reported from the other species of *Laggera* family. So far, the reported activities of these plants were attributed to these secondary metabolites.

Petroleum ether fraction of the leaves extract of *L*. mollis was found to have highest biological activity compared to that of the other fractions from the plant. Different extracts from same plant can show different constituents and antimicrobial activities on the same organism (Gunnar *et al*., 1991). The preliminary Phytochemical screening revealed the presence of flavonoids, steroids and reducing sugar in the petroleum ether fraction of the plant. Isolation of two steroids (β-sitosterol and stigmasterol) from the n-Hexane extract the leaf of *L. pterodonta* have been reported (Kuljanabhagavad *et al*., 2009). The antimicrobial activity of steroids have been reported (Taleb-contini *et al*., 2003)

Pure isolates (LM1-1-10A and LM1-1-22) from the petroleum ether extract of *l. mollis* have showed activity against *Streptococcus pyogens*, *Klebsiella pneumonia* and *Staphylococcus aureus* including *Salmonella typhi***.** It is however interesting, the inhibitory effects were on both gram positive and gram negative bacteria which have been implicated in several human and animal infections. The most active isolate, LM1-1-10A was more potent to *Salmonella typhi* (11 mm) at 40 µg/disc followed by *Streptococcus pyogens* (10 mm) at 40 µg/disc and Staphylococcus *aureus* (10 mm) at 40 µg/disc, respectively. The activity increases with increase in the concentration of the isolates. The *Salmonella typhi* that showed highest susceptibility (11 mm) to the isolate, LM1-1-10A was found to have initially resisted the plant extracts. This may be due to the effect of other components in extracts that might have inhibited its activity on the organism. The activities of the isolates were significant as compared to that of positive control. Characterization of the isolates is necessary revealed the chemical structure of Phytochemical responsible for the antibacterial activity.

The significant of this study is the inhibition of pathogenic microbial growth by the isolates form *L. mollis.* Few investigations on the antimicrobial properties of plants from *Laggera* species have been reported (Mothana *et al*., 2011). The spectra of the activity exhibited by the isolates signify their potency for the development of therapeutic agents against these pathogenic microorganisms.

CONCLUSION

The results obtained from this research have established the potency of *Laggera mollis* against some diseases (typhoid fever, pneumonia and other disease conditions cause by *Streptococcus pyogens* and *Staphylococcus aureus*) as it contains some potent antimicrobial agents supporting it traditional usage in the Northern part of Nigeria as medicinal plant. The above findings suggest that further investigations and isolations from *Laggera mollis* are required for the identification of chemistry of the potent biologically active components. This may lead to establishment of novel chemical structures or their analogues for more effective antibiotics for clinical usage.

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