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MORINPEPTIDES: *IN SILICO* IDENTIFICATION OF ANTIMICROBIAL PEPTIDES AND BIOLOGICAL ACTIVITY FROM *Moringa oleifera* LEAVES

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Abstract

The excessive use of antibiotics together with the high mutation rate at which bacteria become resistant constitute a public health issue that generates great concern. As a consequence, the prevalence of health care-associated infections (HCAIs) is increasing, especially in developing countries. In view of this, natural products from traditional plants have been envisaged as a new source of antimicrobial peptides (AMPs). This study evaluated the antibacterial activity of ethanolic extracts of *Moringa oleifera* leaves against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* responsible for the appearance of HCAIs. In parallel, we performed an *in silico* identification of AMPs using a previously published genome of Moringa. The results showed the susceptibility of the microbial isolates to different concentrations and incubation times with the plant extract. The decreasing order of growth inhibition was *C. albicans* > *S. aureus* > *P. aeruginosa* at 30 minutes and *C. albicans* > *P. aeruginosa* > *S. aureus* after 24 hours of incubation. These tendencies were also observed along a concentration gradient of 12.5%, 25%, 50%, 75% and 100% extract. Additionally, a total of 50 AMPs sequences were identified in the Moringa genome. Ten sequences were reported to display antimicrobial activity against at least one of the microorganisms evaluated here. The results of this study show that *M. oleifera* ethanolic leaf extracts have a potent antimicrobial action and retain a valuable potential as a source of AMPs. Furthermore, our findings suggest that its use could be effective against HCAIs of bacterial/fungal origin.

Key words: *antimicrobial activity, AMPs, moringa, peptides*

Introduction

In developing countries, health care-associated infections (HCAIs) can produce up to 50% of reported cases of mortality, a situation that, added to the indiscriminate use of antibiotics, generates an increase in microorganisms resistant to conventional medications (1). This represents one of the most serious problems that current health systems must face. For this reason, it is necessary to search for alternatives to the excessive use of common antibiotics through the acquisition of new drugs, especially from plants. In recent years, it has been possible to obtain new compounds with antimicrobial properties through bioprospecting (2,3). In general, plants have been continuously exposed to the attack of insect pests and pathogenic organisms such as fungi and bacteria; therefore, this condition has generated various defense mechanisms throughout the evolutionary process. For instance, the synthesis of low molecular weight compounds, such as proteins and peptides, have the capacity to inhibit, alter or block the metabolic processes of a large number of microorganisms, including those causing HCAIs. Among these small peptides, defensins are a group of basic peptides rich in cysteine, which are characterized by low molecular weights (between 3.0 to 6.0 KDa) and two to six disulfide bridges that complete the molecule making stable its structure (4).

Antimicrobial peptides (AMPs) represent an important mechanism of innate immunity and are necessary for the survival of multicellular organisms. South America has innumerable "hot spots of biodiversity" corresponding to terrestrial and marine biomes that are currently being threatened and that should be studied as potential places to find AMPs. Colombia hosts a number of previously reported plant families with potential for the production of antimicrobial peptides (AMPs) and / or secondary metabolites useful for the design of new drugs that allow facing the problem of resistance to antibiotics.

Moringa oleifera is a plant belonging to the Moringaceae flowering plant family (5). Its leaves are a rich source of vitamin C, calcium, potassium, β-carotene and natural antioxidants (6). *M. oleifera* is now incorporated into health formulations that are marketed as remedies for a variety of health disorders due to its high medicinal and nutritional value (7). Extracts of *M. oleifera* leaves are known to have anti-inflammatory, antimicrobial, antidiabetic and anti-ulcer properties. Additionally to cholesterol lowering and blood pressure stabilizing effects (8). Although several small proteins/peptides have been reported for *M. oleifera* (8,10,11,12,13), to our knowledge there are no studies reporting an *in silico* search of AMPs in Moringa. The present paper is the first systematic attempt to identify *in silico* peptides from *M. oleifera* harbouring antibacterial activities.

Methods

Plant material and extract preparation

M. oleifera leaves were collected from plants at Tena in the department of Cundinamarca (4°38'51.3"N 74°23'05.9"W) located at 1384 m.a.s.l. on the Western Andes mountain range in Colombia. The municipality has an annual mean temperature of 21°C, an annual mean rainfall of 1600 mm, and relative humidity of 70% and 90% in the dry and wet seasons, respectively. The extract was prepared from 20 g of fresh leaves that were washed by immersion in chlorinated water at 200 ppm. Then, distilled water was used to remove excess chlorine. The leaves were oven dried at 30°C with airflow for 48 hours, until reaching final equilibrium moisture of less than 10%. Finally, the plant material was ground and added to 80 mL of 70% ethanol. The leaf-ethanol homogenate was left for five days, mixed every two days. After, the mix was filtered and the volume was adjusted to 100 mL with 70% ethanol and stored at room temperature. We evaluated the organoleptic and quantitative characteristics of the *M. oleifera* leaf extract such as appearance, colour, odour, density, and refractive index (IR).

Physicochemical characterization of the extract

Quantification of total phenolic content. Total phenolic content was quantified using Folin-Ciocalteu's phenol reagent. Briefly, the *M. oleifera* leaf extract was centrifuged at 7500 rpm for 10 min at 10°C. Then, Folin-Ciocalteu's reagent was diluted

in water (1/10) and 7.5% NaCO3 and left to stabilize for 1 min. Fifty microliters of the extract were added to 3 mL of the previous mix and incubated for 30 min at room temperature. Finally, the absorbance was measured at 765 nm in a Beckman DU650 spectrophotometer.

Quantification of total flavonoid content. Total flavonoid content was quantified according to Ortiz et al. (2014) (13). Briefly, the extract was centrifuged as described above and 300 µL of the centrifuged extract were added to 1.2 mL of deionized water. Ninety microliters of NaNO2 were added at t0, then 90 µL AlCl3 were added after 5 min, followed by 600 µL 1M NaOH and 720 µL deionized water after 6 min. Finally, the mix was vortexed and absorbance readings were taken at 500 nm on a Beckman DU650 spectrophotometer.

Strains and inoculum preparation

We used the following strains recommended by the National Committee for Clinical Laboratory Standards: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. The inoculum was prepared by culturing in Buffered Peptone Water and incubating for 24/48 hours at 37°C under aerobic conditions for *P. aeruginosa* and *S. aureus* and for 48/72 hours at 30°C under anaerobic conditions for *C. albicans*. Suspensions of 100 CFU/mL were prepared for testing.

Antimicrobial activity assay

The antimicrobial activity assay was conducted based on the Kirby-Bauer Diffusion Susceptibility Test Protocol, according to guidelines of the Clinical and Laboratory Standards Institute (CLSI). We assessed the antimicrobial activity of five concentrations (100%, 75%, 50%, 25%, and 10%) of the *M. oleifera* ethanolic leaf extract on the assay microorganisms. The concentration gradient was obtained by 10-fold dilutions of the extract in peptone water and the first dilution (100%) corresponds to 1mL of extract in 9 mL peptone water. Briefly, each strain was independently inoculated in Buffered Peptone Water medium with different concentrations of the extract and incubated at 30°C for 24 hours. Then, *P. aeruginosa* and *S. aureus* were plated on Tryptic Soy Agar (TSA) and incubated at 37°C for 24 hours, while *C. albicans* was plated on Sabouraud Dextrose Agar (SDA) and incubated at 30°C for 48 hours under aerobic conditions. The positive and negative controls corresponded to Buffered Peptone Water medium with and without the inoculum, respectively. The activity of the leaf extract at each concentration was determined through the number of CFU/ml. Each test was performed twice and the results correspond to the mean value. The percentage of reduced microbial growth was determined according to the formula:

$$\%\ Recovery = \frac{\text{Sample Growth (CFU)}}{\text{Positive Control Growth (CFU)}}\ x\ 100$$

$$\%\ Reduction = 100 - \%\ Recovery$$

Identification of antimicrobial peptides

We performed a screening of genes and peptides involved in antimicrobial and antioxidant activities from a previously reported genome assembly of *M. oleifera* (13). Antimicrobial peptides (AMPs) were identified by a combination of three strategies. The first one used blastp (14) against a previous curated set of peptides extracted from specialized public databases on antimicrobial activity (APD, ADAM) (15,16) The second strategy used Hidden Markov Models (HMM) profiles from PFAM (17) and CAMPR3 (18) involved in APMS activity to determine the possible AMP candidates using the tool HmmSearch (19). Finally, machine learning algorithms (20) including Random Forest (RF), Discriminant Analysis (DA), Artificial Neural Networks (ANN) and Support Vector Machines (SVM) (21) were used and filtered based on a probabilistic value (P > 0.9). After the three strategies were applied, the results were cross-referenced providing a unified set of annotated peptides with potential antimicrobial activities. On the other hand, in order to identify antioxidant activities, only the first two previous strategies were used but this time extracting the reference antioxidant protein sequences from Pfam and Uniprot (22) databases. Finally, we again cross-

referenced the result of the two strategies with scores >= 100.

Results

Physicochemical characterization of the *M. oleifera* ethanolic leaf extract

The organoleptic and quantitative characteristics of the *M. oleifera* leaf extract are shown in Table 1. The refraction index results showed that the extract can behave like a sacarose solution at 50% Brix, which can lead to an alcohol percentage of 34.1%. The polyphenols content in the extract was 240.47 ± 4.86 ppm of the gallic acid equivalent and the flavonoid content was 96.27 ± 2.50 ppm of the catechin equivalent. These values indicate low contents of polyphenols and flavonoids in the leaf extract of *M. oleifera*.

Antimicrobial activity

The antimicrobial activity assay showed that the leaf extract of *M. oleifera* inhibits the growth of *P. aeruginosa*, *S. aureus* and *C. albicans* at all of the test concentrations. The antimicrobial effect was similar on the three microorganisms, mainly at high concentrations of the extract. After 30 minutes of incubation, the growth of *P. aeruginosa* was reduced by 65.8% - 73.7% compared to the positive control (Figure 1, Table 2). Likewise, *S. aureus* showed a growth reduction of 75.6% - 80.2% and *C. albicans* showed the highest reduction with 91.7% - 95.8% (Figure 1, Table 2). After 24 hours of incubation, growth was reduced by 44.0% - 92.8% for *P. aeruginosa*, 12.5% - 91.2% for *S. aureus*, and 69.4% - 93.3% for *C. albicans* (Figure 2, Table 2).

Identification of antimicrobial peptides

After the individual execution of machine learning classifiers like Support Vector Machines (SVM), Random Forest (RF), Artificial Neural Network (ANN) and Discriminant Analysis (DA), 115 peptides out of 19,465 proteins were selected as potential In-silico AMPS candidates, then a cross-reference analysis between all strategies were filtering using a P-value > 0.9, that generates a final 50 AMPs set that is shown on Table 3.

These 50 AMPs were annotated according to the CAMP3 (http://www.camp.bicnirrh.res.in/) and APD (http://aps.unmc.edu/AP/main.php) databases for AMPs families classification and the corresponding target microorganisms (Table 3). More than 50% of the peptides were not found in plants which means that are potential new AMPs sources in plants but taking in count that share active sites, domains and motifs with some other organisms (orthologs). We found AMPs against Gram-negative and Gram-positive bacteria, fungi, and specific biological activity against *S. aureus*, *P. aeruginosa*, *E. coli*, *Klebsiella* sp., *S. cerevisiae*, *C. albicans*, and *A. brassicicola*.

Discussion

The therapeutic value of *M. oleifera* has been widely acknowledged due to a wide variety of phytochemical constituents in its seeds, roots, leaves, pod husks, and bark that display pharmacological actions (23). In this study, we confirm the antimicrobial activity of the ethanolic extract of *M. oleifera* leaves, which has also been by reported by several (15,23–26). Similar to our findings, Ajayi & Fadeyi (2015) reported susceptibility of *S. aureus* to different concentrations of leaf extracts (0.2, 0.4, 0.6 mg/mL), in which the reduction in growth was greater at lower concentrations (25). This contrasts our results since we found greater inhibition at higher concentrations of the extract. Rockwood et al. (2013) found that *S. aureus* was inhibited by seed extracts but not by leaf extracts, whereas our study found that leaf extracts are also effective in reducing the growth of this pathogen (26). Bukar et al. (2010) also reported antimicrobial activity of various extracts of *M. oleifera* on *S. aureus* and *P. aeruginosa*, finding that the bacteria were mostly sensitive to the ethanolic leaf extract at high concentrations (200mg/ml) (24), in agreement with our results. Finally, Kheir et al. (2014) reported that the extract of leaf alcohol was active at high concentrations (500, 250, 125 mg/mL) against gram-positive bacteria, including *S. aureus*, while gram-

negative bacteria such as *P. aeruginosa* showed inhibition mainly by petroleum extracts with variable results (27). Regarding *C. albicans*, our results confirm the antifungal value of *M. oleifera* extracts and provide new evidence about their inhibitory potential, as it has not been reported before. Altogether our results contribute new layers of information and demonstrate the antimicrobial potential of *M. oleifera* ethanolic leaf extracts against *S. aureus*, *P. aeruginosa*, and *C. albicans*.

The identification of AMPs indicated that definsin and gamma-thionin families show antimicrobial activity against Gram-positive bacteria. We found that lamu_GLEAN_10008342 and lamu_GLEAN_10002588 have anti-*S. aureus* activity and both peptides share gamma-methionin sites; in addition, these peptides have been reported by (28,29). On the other hand, lamu_GLEAN_10012786 showed antimicrobial activity against *E. coli* and *Klebsiella* sp. and it has been demostrated that colisins are weapons against several bacterias (28). The peptides against *P. aeruginosa* are cystein-rich and this could be related with their antimicrobial activity (30). Furthermore, we found metallothionein, gamma-thionin and defensin families involved in antifungal activity. In previous studies, it has been shown that plant defensins with a gamma core motif exhibit antifungal activity (30). Some of the antimicrobial peptides have not been reported in plants; therefore, we provide the first report of these peptides in *M. oleifera* which can be associated with the antimicrobial activity displayed by the leaf extract of *M. oleifera.*

Conclusions

The antibacterial activity of *Moringa oleifera* extracts have been tested in vitro and i*n silico*. Ethanolic leaf extracts showed high antimicrobial activities against *Candida albicans, Staphylococcus aureus and Pseudomonas aeruginosa,* even 24 hours after incubation and with concentrations as low as 12.5%. Additionally, 50 antimicrobial peptides have been identified in the previously reported genome (31), confirming the biological activity against the evaluated microorganisms and suggesting the prominent use of Moringa extracts to prevent or treat health care-associated infections (HCAIs). *In silico* approaches can become an important tool to explore antimicrobial activities within the biodiversity hot spots in South America and to optimize in vitro evaluations of plant extracts.

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Table 1. Physicochemical parameters of the ethanolic extract of *Moringa oleifera* leaves.

Parameter	Value
Acidity	15.9 pK
Refractive index	1.3642
pH	5
Odor	alcohol-like
Redox Potential	0.67 %
Color	Green
Density	0.8626 g/mL

Table 2. Effect of different concentrations of the ethanolic leaf extract of *Moringa oleifera* on the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* after 30 minutes and 24 hours of incubation. The concentration gradient was obtained by 10-fold dilutions of the extract in peptone water and the first dilution (100%) corresponds to 1 mL extract: 9 mL peptone water. The percentage of reduced growth is calculated compared to the positive controls (0% extract concentration).

	Percentage of reduced growth after 30 minutes		
Concentration of *Moringa oleifera* leaf extract	*P. aeruginosa*	*S. aureus*	*C. albicans*
0%	-	-	-
100%	73.7%	77.8%	95.8%
75%	71.1%	80.2%	95.4%
50%	65.8%	80.0%	93.3%
25%	68.4%	77.8%	92.2%
12.5%	68.4%	75.6%	91.7%
	Percentage of reduced growth after 24 hours		
Concentration of *Moringa oleifera* leaf extract	*P. aeruginosa*	*S. aureus*	*C. albicans*
0%	-	-	-
100%	92.8%	91.2%	93.3%
75%	90.4%	72.5%	92.5%
50%	76.4%	56.2%	87.2%
25%	62.4%	50.6%	78.3%
12.5%	44.0%	12.5%	69.4%

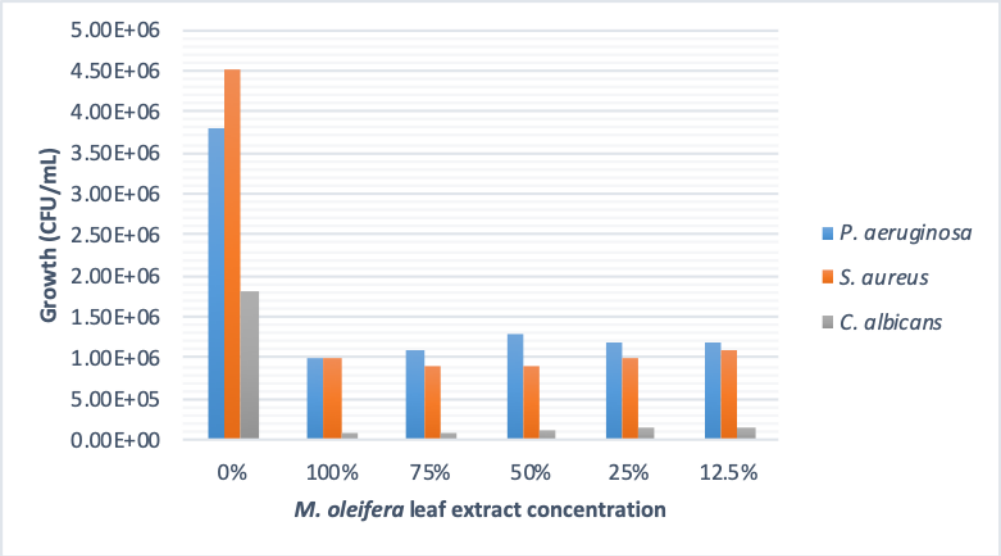

Figure 1. Effect of different concentrations of the ethanolic leaf extract of *Moringa oleifera* on the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* after 30 minutes of incubation. The concentration gradient was obtained by 10-fold dilutions of the extract in peptone water and the first dilution (100%) corresponds to 1 mL extract: 9 mL peptone water. The positive controls correspond to 0% extract concentration.

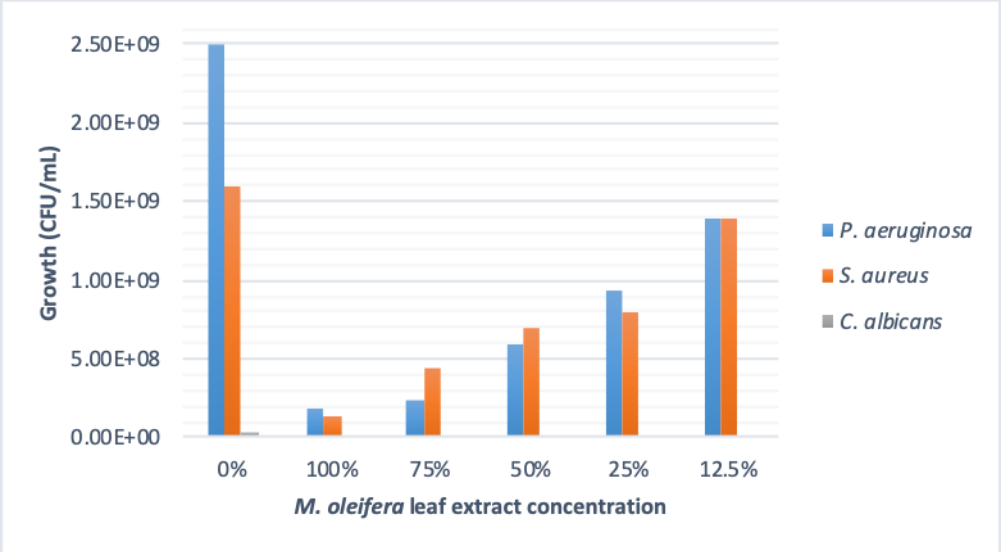

Figure 2. Effect of different concentrations of the ethanolic leaf extract of *Moringa oleifera* on the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* after 24 hours of incubation. The concentration gradient was obtained by 10-fold dilutions of the extract in peptone water and the first dilution (100%) corresponds to 1 mL extract: 9 mL peptone water. The positive controls correspond to 0% extract concentration.

Table 3. Antimicrobial peptides identified by the *in silico* approach

AMP ID	AMP Sequence	size	Annotation	Bacteria Target
lamu_GLEAN_10010810	MARKGTTLIALAWLLVLIFTVICANATAAARLQKPEDVLHPQGCRCCWFIWQPMIRCGKACCGDDCCTLP	70	PF00711 : (Beta defensin)	
lamu_GLEAN_10014849	MLKRGLRKVVRLSIAQVLTVISQKQKAALREAYKNKKYLPLDLRPKKTRAIRRRLTKHQAERHISVRNRAQQ	72	Not found/Not found in plants	
lamu_GLEAN_10002282	MTPVTSKVKKIKMKSYSSYKSRFKTLSDGTIRRWREGKRHNAHLKSKKSKRRLRQPALVPAAYAKVMKKLNFCG	74	PF00304 : (Gamma-thionin family)	
lamu_GLEAN_10019309	MANSKAASLSQQSIMGILLFVLVLTSSEVAGPVAALRFSPFYTCIGVCSPLCGEACVAKGFPKGGECMGPNCCCN	75	PF03784 : (Cyclotide family	
lamu_GLEAN_10008342	MMEKRSLGFFLLLLIVLASQEMVMPSEARLCQSKSHKFKGACMGDHNCGLVCRTEGFTGGKCRGFRRRCFCTKRC	75	PF00304 : (Gamma-thionin family)	Escherichia coli, Staphylococcus aureus, pseudomonas aeruginosa, Candida albicans
lamu_GLEAN_10005071	MSASRFIKCVTVGDGAVGKTCLLISYTSNTFPTVDKKVADGLLRLILLKGPLGNCVFTGDYDRKALDDTLRTFCKS	76	cgUbiquitin	
lamu_GLEAN_10010635	MSSCGGSCGCGSGCKCGGGCNGCGMYPQLGYAEKATTETIVAGVAPVKMFHEGSKMSFASEGCKCGSNCSCDPCNC	76	Bacteriocin	
lamu_GLEAN_10002588	MGRSMPLFSAAFVLLLLLFATEMGPKVAEARACESRSHHFRGMCVRKSNCATICRMEGFHGGRCRGFRRRCFCTKHC	77	PF00304 : (Gamma-thionin family)	Escherichia coli, Staphylococcus aureus, pseudomonas aeruginosa
lamu_GLEAN_10014655	MYRKQHKKDIAQEAVKKRRRTTKKPYSREIKERIKKTKDEKKAKKAEVMAKQQKTQGKSNVPKGGAQKGPKIGGGGGKR	79	PF13841 : (Beta defensin)	

lamu_GLEAN_10015178	MSCCGGNCGCGSGCKCGNGCGGCKMYPDMSFAEKTATETLLLGVGPEKAHYEGSAEMGVWAENGGCKCGDNCTCNPCNCK	80	Bacteriocin	
lamu_GLEAN_10014199	MSKKNSLARRKKQHEFDLKREKEEKEKKAKKLQAKKNKMKVDGSDKKKKGSGFQVGKRKVKTRLTAMAKAKASQAMELDK	80	PF13841 : (Beta defensin)	
lamu_GLEAN_10005033	MQVPLKTCEDCGGSGICPECKGEGFVLKKLSEESAERARLTAKNMATRYTAGLPKKWSYCTKCSSARSCTTCGGRGKLGY	80	Snakin-2	
lamu_GLEAN_10003437	MTVTLKVSVHFLGFKVHVSAQFRFIRVPASVRTNPRSAERLGPLQWVQGKRVIKDCDSHGQKGKWGILDGSIWAHNVLDG	80	Not found/Not found in plants	
lamu_GLEAN_10010830	MVGLVCNADNGNDPKQIHEIKDFLLTARRKDARSVKIKRSRDVVKFKVRCSKYLYTLSVFDSEKADKLKQSLPPGLSVQDL	81	Not found/Not found in plants	
lamu_GLEAN_10006047	MKSFPVSTVLFVLILSAISVNEVASAIRAGSSVICTVVLHRGNCSIDECDAGCKQKYEADAHGFCFQLDAPNDSCICRNPNC	82	defensin-like beta structure	Staphylococcus aureus
lamu_GLEAN_10012786	MLVNRVRSRDRNHFIDNNNWGHSIRSIKSRGIQGTGAGKRRGDVSSSLGIRKVDHSIIRLLVRRLMTTRTLYQVMSDSSGGA	82	Domain colisin A	Domain colicin A, klebsiella sp, E.coli
lamu_GLEAN_10000623	MARFSCINVCLVFLIVLSGVFPVMGSRENKKVCQFEVPGDGHCDPKRCQAECKTWSPSGKGSCVKTKSKFLHCLCKFCHTSS	82	Defensin	Saccharomyces cerevisiae, Alternaria brassicicola
lamu_GLEAN_10001903	MTQIIVKALEKTGQRGIINKGWGGLGDLAEPKDFVYLLDNCPHDWLFLRCVAVVHHGGAGTTAAGLKAGVVYCAFPHLSLPML	83	Not found/Not found in plants	
lamu_GLEAN_10016980	MASSSRSAPFLPFTASQIPCNTLSLSLFAAAASNKNTKPNSVICADCDGNGAVLCSQCKGTGVNAIDLFNGQFKAGDSCWLCG	83	Hepcidin	
lamu_GLEAN_10001849	MASGWGVNGTKGRCYDFWVDFSECMSRCREPKDCALLREDYLECLHHSKEFQRRNRIYKEEQRKLRAAARKAKEGGDEVAGHH	83	Beta-defensin	
lamu_GLEAN_10016190	MGKRKSRAKPPPKKRMDKLDTVFCCPFCSNNSSVECRIDMKNLIGEATCRVCQESFSTTVTGGSYSKKKKKLGPWFCEYSETR	83	Hepcidin	
lamu_GLEAN_10013226	MTANMGCAHCRRRVSHLISKMTGIKEYTVDAHNKQVIAKGDLGFQWSANDRSPGRKVKKDSYPLKFLSSLLAACFSKQFVDRLN	84	Coleoptericin	
lamu_GLEAN_10002769	MVLQNDIDLLNPPAELEKRKHKLKRLVQSPNSFFMDVKCQGCFNITTVFSHSQTVVVCGNCQTVLCQPTGGRARLTEGCSFRRKGD	86	thaumatin-like	

lamu_GLEAN_10008337	MKGNNGSKEDESDDLLIMEEGQKEGEGLEDGKGDEAIRADNDVAIVANIVIKATKRVEEVVEHDVEVVRGVEEVVAPVEFRSCQFI	86	Not found/Not found in plants	
lamu_GLEAN_10008374	MSWRGKRKDEEGHASDVHSEDHAPPKKISKNRRVSVRNWQGKIWVDIREFYVKDGKQLPGKKGISLNVEQWNVLRDHVEEIDKALA	86	Not found/Not found in plants	
lamu_GLEAN_10018302	MGRGRVQLRRIENKISRQVTFSKRRTSLLKKAHEISVLCDAEVALIVFSTKGKLYEYATDSRLRDLGLFPGLQAKYISLSFVPYGA	86	Not found/Not found in plants	
lamu_GLEAN_10001551	MGHSNVWNSHPKNYGPGSRACRVCGNPHGLIRKYGLMCCRQCFRSNAKEIGFIKGLHPFLTVVERLIEHLHSTRSLLNGIFLAIHP	86	Not found/Not found in plants	Escherichia coli, Pseudomonas aeruginosa
lamu_GLEAN_10015692	MAGVGAFWGARVMEIVKKHDSGGLVWKRIKLTSTRKANAKKRLLRVWQNEAVLRACAEPPHSKTSGAGPDGIGEKDSANSQVGGQNE	87	Not found/Not found in plants	
lamu_GLEAN_10007311	MEGNRRSGSRAAKLLCLVLVVAFVLNAATVKALTGAQCKQERKLLVMPASQCWHLAALIDVNYAVKVIRSCGRHLPRHFKCGSITTP	87	Snakin-1	
lamu_GLEAN_10019310	MAISKVSVTPKCLFKIGFFCLLILLISGNKGGAEIPPGSRLKPVGTCVQFPACNQHCIDIGFPLGGLCGKQSLTSPPEPLTCLCKVL	87	Not found/Not found in plants	Staphylococcus aureus
lamu_GLEAN_10003697	MKAFLVALLLVSLVLTSSVFEVAVAGSSFCGSKCEVRCSKAGYKDRCMKYCGICCEKCNCVPSGTYGNKHECPCYMRLKNSKNKPKCP	88	Snakin-1	
lamu_GLEAN_10013414	MATVRSALLRTAIRGGSKISAPPKRGFASSGHHDDAYECAKWEKITYLGIATCTVLAIVNLSKGHHHFDEPPAYEYLHIRNKEFPWVR	88	Not found/Not found in plants	
lamu_GLEAN_10016509	MAISQLIPCQPFLVGSGAAQPSLACCSGVQAVNNAATTPEARRQLCPCLVNAAKSLGVNAEKAKQLPQLCHVQVPVPIDPNVNCSSIQ	88	Cy-AMP3	
lamu_GLEAN_10001550	MGRSPCCEKAHTNKGAWTKEEDQRLIDYIRAHGEGCWRSLPKAAGLLRCGKSCRLRWINYLRPDLKRGNFTEEEDELIIKLHSLLGNK	88	Vicin-like antimicrobial peptide 2c-3	Escherchia coli
lamu_GLEAN_10006726	MGKRKSRAKPPPKKRMDKLDTVFCCPFCNHGTGVECRIDMKNLIGEAVCRICHESFSTTITALTEPIDIYSEWIDECERVNNLEDDGA	88	Not found/Not found in plants	
lamu_GLEAN_10015650	MCRRNLLMAEVMKPTKVEIQQSREKLHKIRITLCSKNVKNIEKVCKDLINAAKEKGVTVKGPARMPTKILRITTRKSPCGEGIIRKNNK	89	Not found/Not found in plants	
lamu_GLEAN_10015668	MAISKFSPACLALFAVLLLTHLAFHECIRDNIDLSISESKVDWSNEQKINLRGCVQQKCVEDICWCCMNIPEGTCWSDTVSCVAHCPHP	89	Sonorensin	

lamu_GLEAN_10005083	MVRVSRALLKDAGSGFSKTLSDVLVCPLSKQPLRLCEETNSLISDVIGVSFPIKDGIPCLVPKDGKILDDEDEKLKADGVSGSTATNKE	89	Not found/Not found in plants	
lamu_GLEAN_10005889	MCCGKSRREVTSEAHRNSGAQVKQGRPRRIQNKSGGKKKKRCVYCYLSAHDKLKDKTYRKEKRYTLKEGSQREEELVLVLVPLLLGLR	89	Not found/Not found in plants	
lamu_GLEAN_10009510	MPSAGNDKRERVVKASRDITMNSKKVIFQVHRISKGNKEEVLEKAEKDLAVVRDQYISRLVKELQGTDFWKLRRAYSPGVEPCSPTTFRL	90	Not found/Not found in plants	
lamu_GLEAN_10000083	MATTSKEGSRRTNYPILTRGERKLGAGRRSDTVLVSTINDADQGSADVAFRTPLAHYEKSKSLSFGGSMVARLKLKGIDGKAAPRVEPAT	90	Not found/Not found in plants	
lamu_GLEAN_10012034	MRGEEFKPVLGPGLRSEKHCLAYVSLDLFEIKRKNVLIKGKSLHPSKVGISRKIRPTIESIPDSKLSRRKLKLKGPSLSYRHAEEERKPSP	91	Not found/Not found in plants	
lamu_GLEAN_10012514	METVELKVEMVGIHEKRLRKSLSKLKGIEKVEVDANSQKVAVTGYAHRNKILKAIRRGGLKADFWSAQNELLSAYASASYGSLKFNNFSFF	91	Pathogenesis-related thaumatin (Fragment)	
lamu_GLEAN_10008030	MLKTFSLIAKEANSKRKREKNVYDHCLARKGFRLESELALRHNKNGDYINDASRMCGEKISFKLLQIINESSNRCIPKSILTKLVDIDTYT	91	Not found/Not found in plants	
lamu_GLEAN_10008340	MTSDQYSTVPIHLRLTNICGRDGACEQILPALLNARFKDDMTEARVCQSQSHGFHGACVSNHNCALVCRNEGFSGGRCRGFRRRCFCTKLC	91	Defensin-like protein 6	Escherichia coli, Staphylocccus aureus, pseudomonas aeruginosa
lamu_GLEAN_10004027	MDNKKPKKKIWGCGKSIVPSAAVLVSDGVILDDGDVVLNDGGCTITAGDKDGPMKAGGANAVSDVIETLQVVFGRYRILGGLSGIIVALGG	91	Not found/Not found in plants	
lamu_GLEAN_10015666	MAVSKFSSARLALLGVFLLTLLALHECVRDPGGAKYDFKYEEKLNLTGCTRAQCGGDARCWCCIYNPVKGCWFSEAVCLEHCPPPQNLTQGT	92	Not found/Not found in plants	Staphylococcus aureus, pseudomonas aeruginosa
lamu_GLEAN_10006163	MTKRTKKAGIVGKYGTRYGASLRKQIKKMEVSQHSKYFCEFCGKYAVKRKAVGIWGCKDCGKVKAGGAYTLNTASAVTVRSTIRRLREQTES	92	Subtilosin A.	
lamu_GLEAN_10000333	MARGDWGYGGGRGSGCSYKRITIIVCTVNIIIALYVLHSLYASLYVKKLKQELSSEEAAIELSQVVKQRMADEILVRLRRLADSANATERQA	92	Not found/Not found in plants	
lamu_GLEAN_10019326	MCPPLPRLCQVCNEARSKYKCPSCLTPYCSLVCFKKHKASSSEIHEALEDEHIRKLICDIDNSSDAMKELDKAMGVEAFHIFTDKVLSAINP	92	Not found/Not found in plants	