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ANTI-BACTERIAL ACTIVITY OF *GUIZOTIA SCABRA L.* AND *MAESA LANCEOLATA L.* EXTRACTS ON *ERWINIA CAROTOVORA L.* RESPONSIBLE FOR IRISH POTATO SOFT ROT IN RWANDA

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ABSTRACT

Food security is handicapped by the postharvest losses caused by pathogenic bacteria such as *Erwinia carotovora L*. (L: is a Swedish botanist called Linnaeus). Nowadays in Rwanda as well as in the different developing countries, harvested Irish potatoes undergo losses due to rotting caused by different pathogenic microorganisms found in soil, water, and other vectors. In Musanze District, Irish potato seeds or small tubers and table potatoes are spoiled by the bacterial species called *Erwinia carotovora.* Curative chemicals imported from developed countries are very expensive and not available in all regions of rural areas. There is a need to find local alternatives in terms of traditional treatment methods practiced by traditional healers for animals and human body. Medicinal plants roots, barks, leaves and flowers were collected from the Institute of Higher Education (called INES-Ruhengeri) botanical garden established near Volcanoes of Rwanda. Dried crude extracts were prepared in the laboratory for anti-microbial activity assay on identified *Erwinia carotovora* from infected samples of rotten Irish potatoes collected from Musanze District. Targeted medicinal plants were *Guizotia scabra L.* and *Maesa lanceolata L.* with a good reputation in traditional healing of human diseases. The objective of this research work was to assess or evaluate the anti-bacterial activity of crude extracts of *Guizotia scabra L.* and *Maesa lanceolata L.* on *Erwinia carotovora L.* identified from rotten Irish potatoes from Musanze District in Rwanda. Crude extracts were used in order to determine the minimum volume in micro-litres (minimum dose) to be used in order to inhibit *Erwinia carotovora L.* isolated from rotten Irish potatoes. It was found that crude extracts of *Maesa lanceolata L.*, especially from leaves and roots exhibit a better antimicrobial effect on identified *Erwinia carotovora L.* For *Maesa lanceolata L.*, the minimum dose was 5 µl with 3 mm of inhibition zone found on antibiogram, while for *Guizotia scabra L.*, the minimum volume was 10 µl with 5mm of inhibition zone diameter. It was concluded that both medicinal plants were similarly active against *Erwinia carotovora* L. responsible for Irish potato soft rot in Musanze conditions. Thus, the two endangered medicinal plants species should be protected, multiplied and promoted at industrial level for the contribution to sustainable development of the country.

Key words: Anti-bacterial activity, Crude extract, *Erwinia carotovora*, *Guizotia scabra*, *Maesa lanceolata*, Irish potato, pathogenic bacteria, rot

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INTRODUCTION

Irish potato (*Solanum tuberosum L.)* belongs to the family of Solanaceae and is consumed worldwide, processed at industrial level for chips, flour, starch, glucose, different chemicals, pharmaceuticals and animal feeds. It is the most economically important food widely used in Rwandan dishes such as French fries (strips of potato fried in deep fat), traditional peeled Irish potatoes mixed with cooked legumes (beans and peas), chips, Sauté, and flakes.

Potatoes are prone to different diseases like soft rot caused by *Erwinia carotovora L.* species. In tropical countries like Rwanda, blackleg and soft rot are commonly occurring diseases in the field as well as during transit and storage of potatoes. Due to its endemic nature, blackleg disease caused by *Erwinia carotovora atroseptica* is prevalent in cool and temperate regions of Canada, the US, Western Europe, India and Pakistan [1].

Erwinia carotovora subsp. atroseptica and Erwinia carotovora subsp. carotovora are considered the main source of primary inoculum for blackleg and soft rot of potato. They are responsible for losses both quantitatively and qualitatively. Both subspecies are commonly associated with Irish potato tuber soft rot, but *Erwinia carotovora subsp. atroseptica* usually causes rot in the basal part of the stem (blackleg disease). *Erwinia carotovora subsp. atroseptica* occurs in both temperate and warm climates but mostly during storage. The rotting of mother tubers during the growing season has been reported as the major source of inoculum for contaminating progeny tubers, which later during storage, when conditions are favorable, could lead to losses due to soft rot of tubers [2]. An infected tuber has cream to tan colored tissues that are very soft and watery. The diseased area often has a black border separating it from a healthy one. The soft rot decay is generally odorless but becomes foul and slimy when other secondary bacteria invade the infected tissues. The affected area becomes soft and mushy and generally turns dark in colour [3].

Imported industrial chemicals are expensive in the treatment of Irish potato soft rot. Hence there is a need to assay local medicinal traditional plants crude extracts in order to control Irish potato soft rot caused by *Erwinia carotovora* in tropical conditions.

MATERIALS AND METHODS

Extracts of medicinal plants (*Guizotia scabra L.* and *Maesa lanceolata L.)* from fresh leaves, stem barks, leaves and roots were dried at room temperature in the laboratory at INES-Ruhengeri. Decomposing Irish potatoes tubers collected from Musanze District in Rwanda were used for further *Erwinia carotovora* isolation and testing.

Isolation of the pathogen associated with blackleg disease of Irish potato (*Solanum tuberosum L.*) plants showing typical symptoms of blackleg disease were collected in polythene bags and brought to the laboratory for isolation of bacterium. Infected portions of the stem blackened at collar region were cut into small pieces and disinfected by dipping in 0.5% Mercuric Chloride ($HgCl_2$) to reduce surface microflora. Then two washings were given with distilled water to reduce the injurious effects of $HgCl_2$, before

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drying by placing on filter paper. The disinfected bits of potato plants from diseased portions were then plated on solidified nutrient agar medium in Petri plates and incubated at 30 ± 2^{o}Cfor one day. Grayish-white bacterial growth appeared around the cut stem tissues of the diseased plant [4].

Classical biochemical tests were used for identification of pectinolytic *Erwinia carotovora*. The culture medium MH included violet bile lactose agar, Simmons citrate agar, Chapman medium, Mac Konkey medium, and blood agar. Biochemical tests were done using standard methods [5, 7].

Control tests of plants extracts sterility were done before use for bacterial inhibition purpose. Antibacterial effects were tested using medicinal crude extracts (for example, natural occurring phenolic substances, flavonoids, terpenes and alkaloids) from local medicinal plants such as *Guizotia scabra* and *Maesa lanceolata* in order to determine the minimum inhibitory concentration (minimum lethal dose of studied plant) that could stop the growth of *Erwinia carotovora* inoculated on culture medium in Petri dishes.

RESULTS

Erwinia carotovora was isolated and identified from rotten Irish potatoes and the shape of the Gram negative stain (results of microbiological analysis of cultured bacterium) is shown in the Plate 1:

Plate 1: Soft rot of potatoes and *Erwinia carotovora* identification in laboratory of INES

Plate 1 shows Irish potatoes spoilage (first pictures), microscopy of samples by the author (second photos at the left) and image of *Erwinia carotovora L.* isolated from rotten Irish potatoes samples at the right.

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Two local plants which are extinct in Rwanda were used in order to extract active ingredients considered as antibacterial chemical compounds naturally synthesised by plant (also called secondary metabolites produced during plant metabolism process) and the results are presented in the Table 1.

Crude extracts were obtained from different organs of the both plants. The yield of different parts for each plant species is presented in Figure 1.

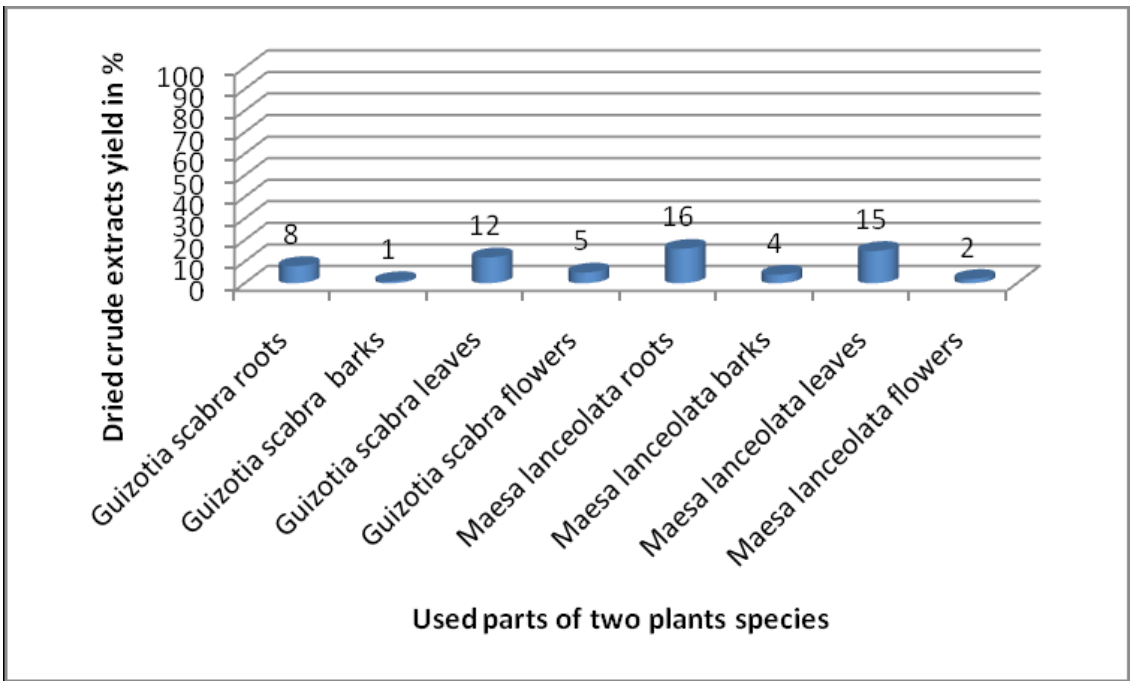

Figure 1: Yield of dried crude extracts from the two medicinal plants species

Figure 1 shows higher production of dried crude extracts from roots and leaves of *Maesa lanceolata L.* than in parts of *Guizotia scabra L.* According to results of Figure 1, leaves and roots of medicinal plants should be used for better production of crude extracts in big quantities, which will be used for antimicrobial activity on identified bacteria causing Irish potato soft rot.

Yield of dried crude extracts from different parts of *Guizotia scabra* and *Maesa lanceolata* were utilized in order to evaluate their anti-microbial activity on isolated and cultivated *Erwinia carotovora* causing potato soft rot as shown in Table 2.

Table 2 shows the different doses used for inhibition of bacterial species of *Erwinia carotovora* identified from rotting Irish potato in Musanze region. The *Maesa lanceolata* extract had a higher anti-bacterial activity on potato bacterium at initial dose of 5 microliters while *Guizotia scabra* demonstrated an antimicrobial effect at 10 microliters as shown in Figure 2.

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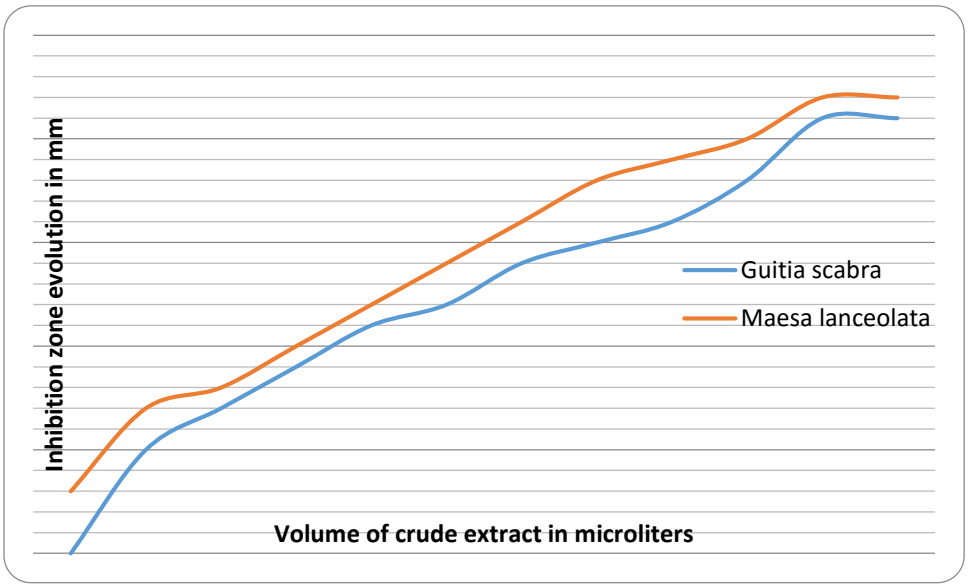

Figure 2: Inhibition zone versus volume of crude extract from roots

Figure 2 shows that the minimum volume for *Erwinia carotovora* inhibition was 1 ml considered as minimum dose for *Maesa lanceolata* roots extract having an inhibition zone of 3 mm, while at the same dose *Guitia scabra* has 0 mm. There was an exponential increase of inhibition zone when the dose was also gradually increased in microliters. Therefore, above 10µl of both crude extracts, the inhibition of *Erwinia carotovora* growth was in steady state. It was found that *Maesa lanceolata* roots extracts exhibited a better antimicrobial activity than extracts from *Guitia scabra*.

Leaves and flowers of crude extracts were also used to evaluate the antimicrobial effect on bacterium causing soft rot of Irish potatoes in Musanze District of Rwanda. The results are given in Table 3.

Table 3 shows the inhibition zone diameter of cultured bacteria (*Erwinia carotovora L.*) in mm is gradually increasing proportionally to exponential increase of applied crude extracts volume in microliters for antimicrobial growth effect. The leaves of both plants species seemed to be similar in terms of antimicrobial activity. The similarities of leaves for the two medicinal plants are presented in Figure 3.

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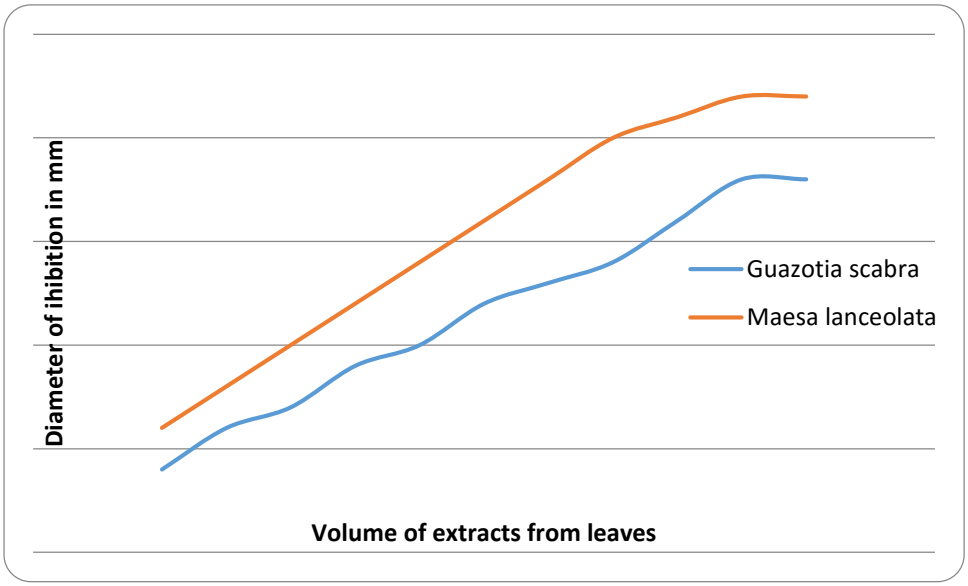

Figure 3: Determination of minimum inhibition volume in microliters

Figure 3 shows the minimum inhibition zone (from the centre of used disc set on cultured medium) of crude extracts from plants leaves of *Guizotia scabra* and *Maesa lanceolata*. Both plants leaves extracts exhibited almost identical antimicrobial activity on *Erwinia carotovora* causing soft rot of Irish potatoes. *Maesa lanceolata* roots and leaves extracts were more active than those of *Guizotia scabra* roots and leaves. Probable conditions that predispose Irish potatoes seeds to infection leading to soft rot include warm, humid weather conditions; dipping of tubers before planting; cutting of tubers and washing of young harvested potatoes seeds.

DISCUSSION

Erwinia carotovora is the major cause of soft rot in potatoes leading to spoilage and food insecurity in Musanze District of Rwanda. Disinfection of Irish potatoes was achieved by the application of green crude extracts of local medicinal plants in order to solve the problem of seeds storage and commercialisation. Eradication of *Erwinia carotovora* was done by using industrial chemicals in developed countries, which are not safe for human consumption [5, 6].

Local medicinal plants such as *Guizotia scabra* and *Maesa lanceolata* which have good antibacterial activity can be used to treat *Erwinia carotovora L.* However, the results from these local medicinal plants are similar to industrial chemicals use. But imported ones are very expensive and not safe for human health [1,2].

CONCLUSION

According to the present findings, *Guizotia scabra L.* and *Maesa lanceolata L.* crude extracts are a promising alternative for the safe eradication of *Erwinia carotovora L.* responsible for Irish potato soft rot.

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Symptoms of soft rot include rotten tissues of Irish potato tubers that are wet, cream to tan in colour, and soft. Soft rot begins on the tuber surface and progresses inward. Infected tissues of Irish potato tubers are sharply delineated from healthy tissue by dark brown or black margins.

Rotting tissue is usually odourless in the early stages of decay, but develops a foul odour as secondary organisms invade infected tissue. Soft rot can also infect wounded seeds, table potatoes, stems and roots. In this regard, there is a need to eradicate completely the pathogens from potato seeds and soil using safe green extracts from studied medicinal plants.

In areas where soft rot is particularly prevalent, it is important to plant cultivars that show resistance to the disease and respect agricultural rotation methods. After harvesting, potato seeds should be selected, cleaned and disinfected in order to avoid any propagation of pathogens.

The use of local medicinal plants extracts is a low-cost technology which is affordable to rural people. It would be better to investigate the toxicity of *Guizotia scabra L.* and *Maesa lanceolata L.* extracts in a well-equipped laboratory in order to protect consumers and the environment.

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Table 1: Yield of crude extracts from *Guizotia scabra L.* and *Maesa lanceolata L.*

Scientific name of medicinal plant used	Yielded crude extracts from medicinal plants in %			
	Root	Bark	Leaves	Flower
Guizotia scabra L.	8	1	12	5
Maesa lanceolata L.	16	4	15	2

Table 2: Diameter of inhibition zone of *Erwinia carotovora L.* sensibility for different plants parts used

Volume in Microliters (µl)	Diameter of inhibition zone (mm)	
	Guizotia scabra L.	*Maesa lanceolata L.*
5	0	3
10	5	7
15	7	8
20	9	10
25	11	12
30	12	14
35	14	16
40	15	18
45	16	19
50	18	20
55	21	22
60	21	22

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Table 3: Diameter of Inhibition zone for each used volume of crude extracts

Volume of Extracts in µl	Inhibition zone Diameter in mm for:	
	Guizotia scabra Leaves	*Maesa lanceolata* Leaves
5	0	0
10	4	6
15	6	8
20	7	10
25	9	12
30	10	14
35	12	16
40	13	18
45	14	20
50	16	21
55	18	22
60	18	22

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