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Biological Potentials of Ginger Associated *Streptomyces* Compared with Ginger Essential Oil

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Abstract: Medicinal plants and associated microorganisms are recognized to have beneficial relationship. These two organisms are well known for their ability to produce bioactive secondary metabolites which the similarity has been demonstrated in a few works. This study had for objective to assess biological potentials of actinomycetes isolated from ginger rhizomes and its rhizospheric soil and to determine their similarity and efficiency with ginger essential oil. Among the 63 actinomycetes strains isolated from the rhizomes and rhizospheric soils of two ginger countries plantations of Soavinandriana Itasy-Madagascar, biological activity tests showed that 16 strains (2 endophytes and 14 from rhizospheric soils of ginger) exhibited antimicrobial activity against at least one germ. The strains are more active against Gram+ bacteria and fungi than Gram- bacteria. Only, one strain isolated from ginger rhizospheric soil of the site n°2 (AHO 18) inhibited the development of all tests germs. The tests conducted on six representative strains selected on the basis of antimicrobial assay showed that extracts from the isolates AHO 3 and AHO 43 have strong antiproliferative activity on cells HT-20 (colon cancer) with IC50 values of 5µg/ml and 2,2µg/ml, respectively; strong antimalaria activity against the chloroquino-resistant *Plasmodium falciparum* strain (IC50=1,25µg/ml for AHO 3 extract and 2,5<IC50<5µg/ml for AHO 43 extract) and antioxidant activity (IC50=15mg/ml for AHO 3 extract and 10,6mg/ml for AHO 43 extract). The 2 isolates based on phenotypic and molecular characterization using their 16S rRNA gene were identified as *Streptomyces chrysomallus* (isolate AHO 3) and *Streptomyces sp* (isolate AHO 43). Moreover, the two essential oils of ginger tested showed antimicrobial activity against all tests germs used and antioxidant activity. Only, ginger essential oil from the site n°2 exhibited moderate antiproliferative potential (IC50=14µg/ml) on colon cancer cells and high antiplasmodial activity (2,5<IC50<5µg/ml). *Streptomyces sp* showed similar and strong biological activities than those of ginger essential oil from the site n°2. Chemical screening of the *Streptomyces sp* extract and the essential oil H2 revealed the common presence of terpens and phenolic compounds.

Keywords: Actinomycetes, Endophytes, Rhizospheric Soil, Rhizome, Ginger, Essential Oil, Antimicrobial, Antioxidant, Antimalaria, Antiproliferative

1. Introduction

Medicinal plants are well-known for their richness in natural bioactive substances. They have been used since the first civilization and the therapy has been continued to resort to these vegetal resources nowadays in two ways: the extraction of pure natural substances frequently designed for major therapeutic indications or in nature with simple or innovative family medication forms (extract, powder…), generally used in minor pathology or in appoint therapy. In some cases, the different steps of bioactive molecules extraction, the diverse screening and the clinical assays impose an important quantity of plant materials. The over-harvesting of these natural resources could make, thus, some

species in danger. One alternative for the production of bioactive compounds similar to those of plants and for the preservation of vegetal biodiversity is the valorization of microorganisms associated with the plants. Among them, endophytic fungi are well-known. *Fusarium solani* and *Entrophospora infrequens* isolated from *Apodytes dimidiata* tree and *Nothapodytes foetida* twigs, respectively, produced the same anticancer substances as the two plants: the camptothecin [1, 2]. The cajanol, an anticancer produced by *Hypocrea lixii* isolated from *Cajanus cajan* roots [3]. Another endophytic fungus (*Mucor fragilis*) isolated from *Sinopodophyllum hexandrum* rhizomes produced similar anticancer substances as the plant: the podophyllotoxin and the kaempferol [4]. However, other endophytic microorganisms belonging to actinomycetes group have been demonstrated important sources of natural bioactive substances showing comparable biological activity and producing similar bioactive substances than host plant. Caruso *et al.* [5] were isolated from different organs of *Taxus baccata* and *Taxus brevifolia* endophytic fungi and actinomycetes producing similar anticancer compounds (the taxol) than host plants. Recently, Akshatha *et al.* [6] were isolated from leaves and stems of two antidiabetic plants (*Leucas ciliata* and *Rauwolfia densiflora*) two actinomycetes (*Streptomyces longisporoflavus and Streptomyces sp.*) which extracts exhibited antidiabetic potential.

Actinomycetes are prokaryote, Gram positive and filamentous bacteria which provide 70% of actual drugs and anti-infectious [7]. Secondary metabolites produced by these microorganisms represent a wide source of compounds with broad structural diversity and are endowed of important biological potential. Then, they are used in many domains as human therapeutic and veterinary, food industry and agriculture to fight against certain pathogens and toxinogens for human, animals and vegetal. Actinomycetes are ubiquitous and can be isolated from different natural habitats as soils [8, 9], plants [10, 11], waters [12], marine organisms [13] and even extreme sites (arid area, polar site…) [14]. The present work selects ginger as plant material for its therapeutic and aromatic properties well-known for millennium, its medicinal use as ubiquitous as its culinary use and its essential oil (1 to 3% of rhizomes) rich in active compounds with different properties [15, 16].

In recent years, there has been renewed interest in ginger as a source of bioactive natural products. Research works carried out on ginger have been focused on the effects of ginger consumption on health [17, 18, 19], the isolation and characterization of ginger bioactive compounds [15, 20] and the isolation of ginger endophytes [21, 22]. In order to find natural substances with comparable biological activity as active extract of the plant, actinomycetes were isolated from rhizomes and rhizospheric soil of ginger. This study was undertaken with a view to test the potential of endophytic and telluric actinomycetes from ginger as producers of natural substances showing comparable biological activity as ginger essential oil. A chemical screening of the extracts from target isolates has been reported and compared with the chemical compounds of ginger essential oil.

2. Materials and Methods

2.1. Plant and Soil Sampling

Fresh ginger rhizomes and rhizospheric soil samples were collected from two ginger rural plantations: Ampamaha (site n°1, 19°11'S46°23'E; 1132m Alt.) and Andrefanivinany (site n°2, 19°10'S46°26'E; 1335m Alt.) located in the district of Soavinandriana in Itasy Region, Madagascar [23].

2.2. Isolation of Actinomycetes Strains

Isolation of actinomycetes strains from ginger rhizomes was conducted according to Fischer *et al.*'s methods [24] with modifications [25-26, 23] while actinomycetes isolation from rhizospheric soil samples was performed by soil dilution and heat treatment techniques as reported previously [23].

2.3. Biological Activities of Actinomycetes

2.3.1. Antimicrobial Activity of the Isolates

Actinomycetes isolates were evaluated *in vitro* for their antimicrobial activity against human pathogens and phytopathogenic microorganisms: *Klebsiella oxytoca* ATCC 8724, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 13061, *Staphylococcus aureus* ATCC 11632, *Candida albicans* and *Fusarium sp*.

Tests microorganisms were inoculated onto Mueller Hinton agar (for bacteria) and on Potato Dextrose Agar (PDA) (for yeast and fungus). Antibacterial activity was investigated as described by Acar and Goldstein [27] with modifications. Disks (6mm in diameter) of Mueller Hinton agar were taken aseptically and substituted by disks of mature actinomycetes grown on starch casein agar (SCA). Antifungal activity was, however, performed according to Loqman *et al.*'s techniques [28] by aseptic transfer of mature actinomycetes disks to the inoculated PDA plates with fungal mycelial disk (6mm of diameter) in the center.

Plates were kept in a refrigerator + 4°C for at least 4 h to allow the diffusion of any antibiotics produced, then incubated at 37°C for human pathogens and at 30°C for *Fusarium sp*.

The inhibition of pathogens growth was appreciated by the measure of the diameter of inhibition zone around the actinomycetes colonies, after 1 to 2 days of incubation for human pathogens and after 4 days for phytopathogenic fungus. Only isolates showing an inhibition zone greater than 8 mm were considered as active isolates.

2.3.2. Antioxidant Activity

i. Extraction of secondary metabolites

Actinomycetes isolates showing large spectrum of antimicrobial activity were selected to extract secondary metabolites. SCA media were inoculated with pure cultures of selected isolates and incubated for 8 to 14 days at 30°C. Thirty milliliters of ethanol (95°) were, then, poured into inoculated SCA. The mixture was settled at room temperature for two

hours and filtered with a steriflip. The obtained filtrat constitutes the extract which was dried by speedvac.

ii. DPPH radical scavengening activity

In vitro antioxidant capacity of actinomycetes extracts was assessed by the measure of DPPH (1,1-diphényl-2-picrylhydrazyl) radical scavengening according to Leitao *et al*. [29]. Two milliliters of different concentrations of extract methanolic solution from 16mg/ml to 0,5mg/ml were prepared and mixed with DPPH methanolic solution (0,002%). The inhibition of DPPH radical was estimated by spectrophotometer at 517nm after incubation for 30min in dark at room temperature. Antioxidant capacity of the extracts was estimated in comparison with a natural antioxidant, the ascorbic acid. The extracts were tested in triplicate and the inhibition of free DPPH radical in percentage (I %) was calculated as follows:

I % = A blank – A sample x 100/ A blank [30]; A blank: Absorbance of the blank (DPPH in methanol), A sample: Absorbance of the extract.

The IC50 value (concentration of required antioxidant to reduce initial concentration of DPPH to 50%) was calculated by linear extrapolation of the data that lay on the either side of the 50% inhibition level.

2.3.3. Antiproliferative Activity

In vitro antiproliferative activity of extracts from actinomycetes of ginger rhizomes and rhizospheric soils was assayed on human A-2780 ovarian and HT-20 colon cancer cells at Virginia Polytechnic Institute and State University, USA according to Cao *et al*.'s methods [31].

2.3.4. Antiplasmodial Activity

This assay was also performed at Virginia Polytechnic Institute and State University, USA according to Harinantenaina *et al.*'s methods [32] on chloroquine-resistant *Plasmodium falciparum* Dd2 strain.

2.4. Biological Activities of Ginger Rhizomes Essential Oil

In view of the various properties attributed to ginger and in the purpose to compare biological activities of ginger essential oil and those of associated actinomycetes extracts, previous tests were performed with ginger essential oil. The same methods as described above were used for antioxidant, antiproliferative and antiplasmodial tests whereas antimicrobial activity was tested using aromatogram method [23].

For all activities, the results are expressed according to the average (±SE) of three replicate determinations.

2.5. Identification of Active Isolates

Isolates exhibiting activities were characterized phenotypically and identified by 16S rRNA gene sequencing as reported by Andriambeloson *et al*. [23].

2.6. Chemical Screening of Streptomyces sp Extract

As the biological activity of an organism is due to a specific chemical compound, this work aims to determine the chemical families of *Streptomyces sp* extract and the chemical compounds of ginger essential oil.

The screening method used for the determination of *Streptomyces sp* extract chemical families was the same as used in chemical phytoscreening which is a qualitative analysis based on coloration or precipitation reaction of the compounds of the chemical families in the crude extract [33]. The tested families are: the terpenoides, the tannins, the leucoanthocyanes, the flavonoides, the alkaloids, the saponosides, the anthraquinones, the polysaccharides and the polyphenols.

2.7. Determination of the Chemical Composition of Ginger Essential Oil

Chemical compounds of the essential oil were analyzed qualitatively and quantitatively by Gas Chromatography at Virginia Polytechnic Institute and State University, USA.

3. Results and Discussions

3.1. Actinomycetes Isolation

From the 2 ginger rhizomes samples and the 2 ginger rhizospheric soil samples collected in two ginger plantations localized in the middle-west region of Madagascar, a total of 63 actinomycetes strains were isolated on SCA medium. Among them, 8 strains were obtained from ginger rhizomes samples (AHO 1- AHO 8) and 55 from the rhizospheric soils (AHO 9- AHO 63) (figure 1). Cultural characters of the isolates are summarized in the table 1 and some varieties of isolated strains are shown in the figure 2. According to these results, the number of isolated endophytes is largely lower than actinomycetes from rhizospheric soils'number. In our knowledge, any comparative work of the number of endophytic actinomycetes and rhizospheric soil actinomycetes isolated from the same plant has been cited in the literature. However, our results confirm those obtained by Intra *et al.* [34] who reported that the rhizospheric soil is an important source of actinomycetes. Crawford *et al.* [35] showed also that soils associated with the rhizosphere contain twice as many actinomycetes as soils non- associated with the rhizosphere.

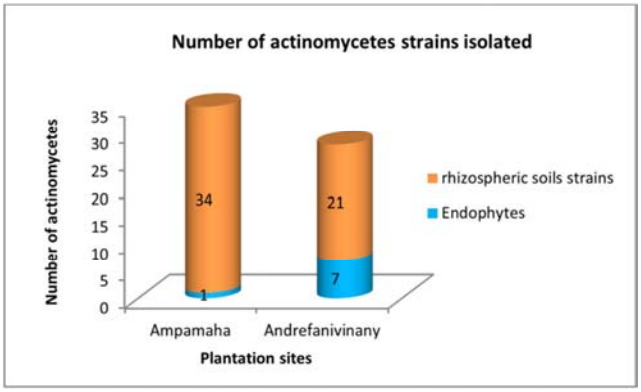


Figure 1. *Number of actinomycetes strains isolated according to the plantation sites.*

For plants, many works have been demonstrated that many

endophytic actinomycetes could be isolated from a plant. However, they are especially abundant in the root [36, 37, 38] confirming, thus, their isolation from the rhizomes in the present work.

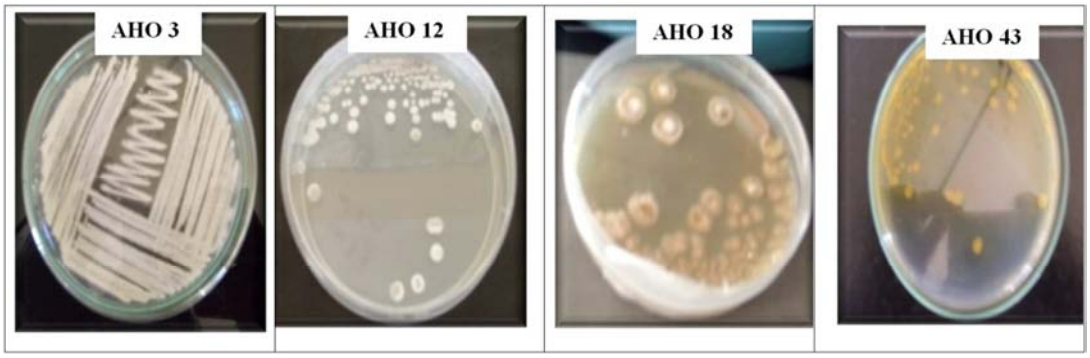


Figure 2. *Varieties of actinomycetes isolates.*

Table 1. *Cultural characters of actinomycetes isolates on SCA medium.*

Characteristics	Actinomycetes isolates
Aspects of the colonies	Opaque, powdery, embedded in agar
Color of substrate mycelia	White, grey, yellow, brown
Color of aerial mycelium	White, grey, yellow, brown, orange
Diffusible pigment	Brown, yellow
Size of the colonies	1mm-5mm

3.2. Biological Activities

3.2.1. Antimicrobial Activity of Actinomycetes Isolates

Modified method used for antimicrobial test of the isolates leads to good results, the diameters of inhibition zone in Acar and Goldstein's method were low than those obtained in modified method (figure 3). Out of the 63 isolates, 16 actinomycetes strains were active against one or more of the tested pathogens, especially against Gram positive bacteria (10 isolates) and fungi (13 isolates). These results were in agreement with those of some works demonstrating that antagonistic reaction of actinomycetes against Gram positive bacteria was much higher than Gram negative bacteria [39, 8, 40]. It could be explained by the difference in the composition of the cell membrane which is complex in Gram negative bacteria offering their resistance to diverse metabolites [41]. One isolate (AHO 18) from ginger rhizospheric soil of Andrefanivinany showed inhibition of all tested microorganisms growth. Nevertheless, this strain was less active than nalidixic acid (30µg) against Gram negative bacteria but more active than fusidic acid (10µg) and nystatin (100UI) against *Staphylococcus aureus* and *Candida albicans*, respectively (table 2). In addition, other isolates as AHO 12, AHO 14 and AHO 43 exhibited high inhibition of pathogens growth than standard antibiotics used.

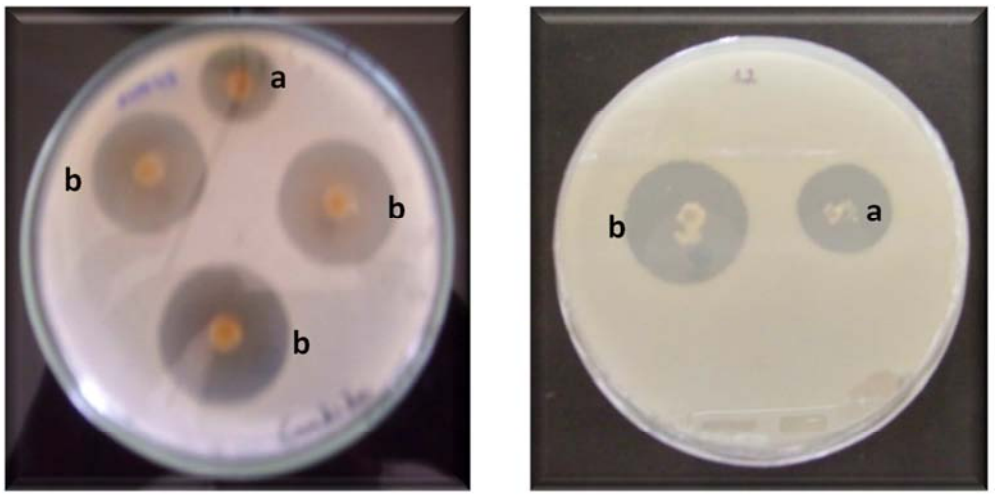
Table 2. *Antimicrobial activity of active actinomycetes isolates.*

Diameter of the inhibition zone (mm)							
Strains	**Sites**	***Escherichia coli* ATCC 25922**	***Klebsiella oxytoca* ATCC 8724**	***Bacillus cereus* ATCC 13061**	***Staphylococcus aureus* ATCC 11632**	***Candida albicans***	***Fusarium* sp.**
AHO 1	2	-	-	-	-	-	$13,5 \pm 0,7^{d}$
AHO 3	2	-	-	-	-	-	$17,5 \pm 0,7^{bc}$
AHO 12	1	-	-	$*19 \pm 1,4^{b}$	$25 \pm 0,0^{b}$	$10 \pm 0,0^{ef}$	$21 \pm 1,4^{a}$
AHO 13	2	-	-	-	-	$14 \pm 1,4^{de}$	-
AHO 14	2	-	-	-	-	$43,5 \pm 2,1^{b}$	$20 \pm 0,0^{ab}$
AHO 16	1	-	-	$20 \pm 0,0^{b}$	$19 \pm 1,4^{c}$	-	0
AHO 18	2	$12,5 \pm 0,0^{a}$	$12 \pm 0,0^{a}$	$12,5 \pm 0,7^{c}$	$28,5 \pm 2,1^{b}$	$59 \pm 2,9^{a}$	$18 \pm 0,0^{bc}$
AHO 24	1	-	-	$20 \pm 0,0^{b}$	$19,5 \pm 0,7^{c}$	$14,5 \pm 0,7^{d}$	$17 \pm 1,4^{c}$
AHO 31	1	-	-	$12 \pm 0,0^{c}$	$18 \pm 1,4^{c}$	$10 \pm 0,0^{ef}$	-
AHO 32	1	-	-	$9 \pm 0,0^{cd}$	-	-	-
AHO 36	1	-	-	-	-	-	$10,5 \pm 0,7^{ef}$
AHO 38	2	-	-	-	-	$18 \pm 1,4^{d}$	-
AHO 41	1	-	-	$9,5 \pm 0,7^{cd}$	-	-	$13 \pm 1,4^{de}$
AHO 43	2	-	-	$52,5 \pm 3,5^{a}$	$38 \pm 2,8^{a}$	$33,5 \pm 2,1^{c}$	$11 \pm 0,0^{def}$
AHO 44	1	-	-	$13 \pm 0,7^{c}$	-	-	-
AHO 51	1	-	-	$14 \pm 0,7^{c}$	-	-	$10 \pm 0,0^{f}$
NA 30	-	18 (S)	23 (S)	-	-	-	-
NET 30	-	-	-	27 (S)	-	-	-
FA 10	-	-	-	-	25 (S)	-	-
NY 100	-	-	-	-	-	25 (S)	-

(-): no inhibition; (S): sensitive, NA: Nalidixic acid, NET: Netilmicin, FA: Fusidic acid, NY: Nystatin

* The data in the same column followed by the same letter don't show significant difference according to Anova test ($P<0,05$)

For the 8 endophytic actinomycetes, 2 isolates were active against one of the 2 tested fungi, *Fusarium sp* (table 2). This result concurs with the finding of Taechowisan and Lumyong [21] who reported that endophytic actinomycetes isolated from *Zingiber officinale* and *Alpnia galanga* exhibited fungicide activity against phytopathogens (*Colletotrichum musae* and *Fusarium oxysporum*). Furthermore, previous works showed antagonistic effect of endophytic actinomycetes against many varieties of fungi such as *Alternaria*, *Rhizoctonia*, *Verticillium*, *Fusarium*, *Phytophthora* and *Phytium spp* [42, 43, 44, 45, 11, 46].



Effect of AHO 43 on *Candida albicans* Effect of AHO 12 on *Staphylococcus aureus*

a: Acar and Goldstein's method; b: modified method

Figure 3. *Inhibition of pathogens growth by actinomycetes strains.*

3.2.2. Antioxidant Activity of Selected Actinomycetes Extracts

Six actinomycetes isolates (AHO 1, AHO 3, AHO 12, AHO 18, AHO 24 and AHO 43) with large spectrum of antimicrobial activity were kept for metabolites extraction. The extracts were, then, screened for their antioxidant, antiplasmodial and antiproliferative activities. Among the 6 extracts, 2 extracts from the isolates AHO 3 and AHO 43 showed antioxidant activity with IC50 values of 15±1,5mg/ml and 10,6±0,7mg/ml, respectively. It was reported that the majority of actinomycetes extracts possessed weak antioxidant activity than ascorbic acid [47, 48]. This result is consistent with our findings in which antioxidant activity of the isolates AHO 3 and AHO 43 was 1,53 times and 1,08 times, respectively, lower than ascorbic acid activity which IC50 value is 9,8±0,7mg/ml.

3.2.3. Antiproliferative of Actinomycetes Extracts

Of the 6 actinomycetes extracts screened for their antiproliferative activity, the two same extracts from the isolates AHO 3 and AHO 43 exhibited a strong cytotoxic activity on colon cancer cells HT-20 with IC50 values of 5±0,5µg/ml and 2,2±0,2µg/ml, respectively according to the criteria of National Cancer Institute [49]. These results corroborate those of many studies which indicate that actinomycetes constitute an important source of antiproliferative compounds [5, 50, 51]. The two extracts were, yet, less active than taxol (standard) which IC50 value is 0,0082±0,003µg/ml).

3.2.4. Antiplasmodial Activity of Actinomycetes Extracts

Even though, the study on the evaluation of actinomycetes antiplasmodial activity is yet scarce, the 2 extracts from the isolates AHO 3 and AHO 43 showed very strong activity against *Plasmodium falciparum* Dd2 chloroquino-resistant. The IC50 values were 1,25±0,04µg/ml for the extract AHO 3 and 2,5<IC50<5µg/ml for the extract AHO 43. The two extracts were also less active than artemisinin (standard) which IC50 value is 0,7±0,02µg/ml.

3.3. Biological Activities of Ginger Essential Oil

3.3.1. Aromatogram

The results of aromatogram assay showed that all tests-pathogens were sensitive to both essential oils extracted from ginger rhizomes of Ampamaha and Andrefanivinany plantations (figure 4). Bacillus cereus is very sensitive to both essential oils H1 and H2 while Candida albicans is extremely sensitive to essential oil H1 and very sensitive to essential oil H2 according to the criteria of Ponce *et al*. [52]. These results were in agreement with Oussalah *et al.* [53] who demonstrated in their work on a large range of essential oils screened for their potential antimicrobial that ginger essential oil is one of the most efficient essential oils against fungi.

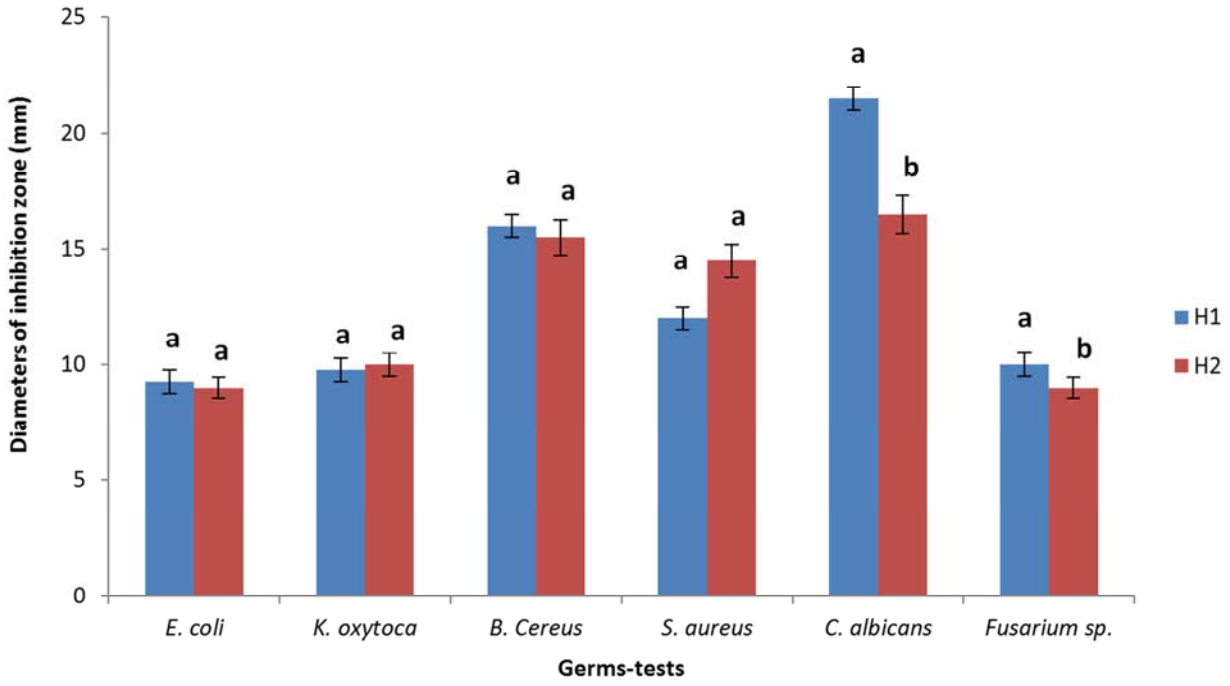


Figure 4. *Antimicrobial activity of essential oils extracted from ginger rhizomes samples.*

The bar indicates the meanstandard error for each essential oil
H1: essentiel oil extracted from Ampamaha ginger rhizomes
H2: essentiel oil extracted from Andrefanivinany ginger rhizomes

3.3.2. Antioxidant Activity of Ginger Essential Oil

The two essential oils H1 and H2 showed antioxidant activity with IC50 values of 19,5mg/ml and 19mg/ml, respectively. It emphasis what Kikuzaki and Nakatani [54] reported, about forty antioxidant compounds were identified in ginger.

3.3.3. Antiproliferative and Antiplasmodial Activities of Ginger Essential Oil

Among the two essential oils screened for their antiproliferative activity, only the essential oil H2 exhibited a moderate activity on colon cancer cells HT-20 with IC50 value of 14±4,0µg/ml. In spite of this, anticancer potential of ginger essential oil has been demonstrated in several works [55, 56]. In addition, this essential oil showed a very strong antiplasmodial activity with IC50 value included between 2,5 and 5µg/ml. In our knowledge, antiplasmodial potential of ginger was demonstrated for the first time.

From these results, it could be deduced that the endophytic actinomycete AHO 3 and the ginger rhizospheric soil actinomycetes AHO 43 showed comparable biological activities as the essential oil H2 of ginger rhizomes. However, the isolate AHO 43 possessed the closest biological activities as ginger essential oil. Moreover, the biological activities of these 2 isolates were more accentuated than those of ginger essential oil. For the antimicrobial activity, the diameters of inhibition zone of the isolate AHO 43 were 2 or 3 times higher than those of ginger essential oil. For the antioxidant, antiproliferative and antiplasmodial activities, all the IC50 values of the two actinomycetes extracts were higher than those of ginger essential oil (table 3). Zhao *et al.* [3] obtained a comparable result in which cytotoxicity level of the cajanol produced by the endophytic fungus (*Hypocrea lixii*) of *Cajanus cajan* roots against lung cancer cells A-549 was higher than the cajanol produced by the host plant.

Compared to the standards used for each activity (ascorbic acid, taxol and artemisinin), the activity of the two extracts from the isolates AHO 3 and AHO 43 were low. Nevertheless, it is important to emphasize that the tested extracts were yet crude extracts whereas all standards used were pure compounds.

Table 3. *Recapitulation of the biological activities of actinomycetes isolates (AHO 3, AHO 43) and ginger essential oil H2 of Andrefanivinany plantation.*

Isolates/Extracts	Antimicrobial activity (diameters of inhibition zone in mm)						Antioxidant activity (IC50 mg/ml)	Antimalaria activity (IC50 µg/ml)	Antiproliferative activity (IC50 µg/ml)
	E. cloacae	*K. oxytoca*	*B. cereus*	*S. aureus*	*C. albicans*	*Fusarium sp.*			
AHO 3 (site 2)	6	6	6	6	6	17,5	15	1,25	5
AHO 43 (site 2)	6	6	38	52,5	33,5	11	10,6	1,25<x<2,5	2,2
H2	9	10	15,5	14,5	16,5	9	19,00	2,5<x<5	14

E: *Enterobacter*, K: *Klebsiella*, S: *Staphylococcus*, B: *Bacillus*, C: *Candida*, -: no activity

For the case of Ampamaha ginger plantation; none of endophytes or telluric actinomycetes isolated showed comparable biological activities to the essential oil H1. This could be due to the isolation method where the diversity of actinomycetes isolated is widely dependent on [57].

3.4. Identification of Active Isolates

Phenotypic and molecular characterization using 16S rRNA gene sequence of the active isolates showed that they belong to the genus *Streptomyces* as reported previously [23]. Thus, the isolate AHO 3 was identified as *Streptomyces chrysomallus* and the isolate AHO 43 as *Streptomyces sp* (figure 5).

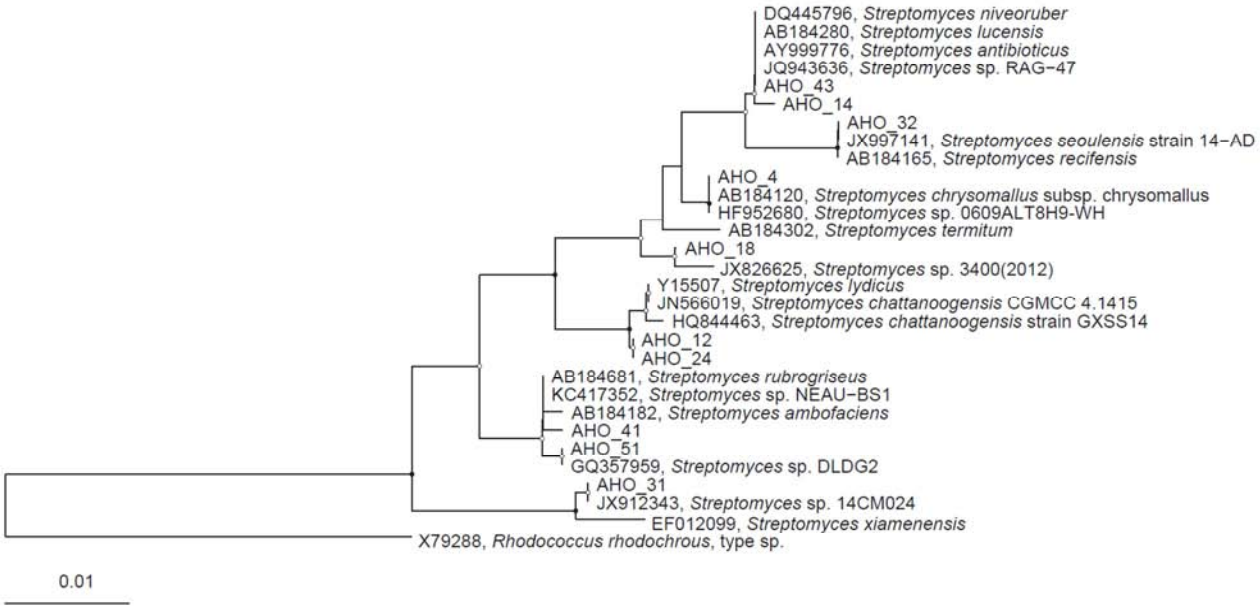


Figure 5. *Neighbor-joining tree based on partial 16S rRNA gene sequences of active isolates.*

3.5. Chemical Screening of Streptomyces sp Extract

As the essential oil H2 and the ethanolic extract of *Streptomyces* sp were closely comparable in term of biological activity, this work suggested determining the chemical family of the active compounds in the extract. Thus, the results revealed the presence of sterols, triterpens, tannins, leucoanthocyanes, flavonoids and alkaloids (table 4). According to several studies, these compounds have been demonstrated to possess important biological potentials. The alkaloids exhibit particular pharmacological activities as antitumor and antiplasmodial [58, 59, 60]. The triterpens have been demonstrated to have antiplasmodial and anticancer activities [61, 62]. The sterols are endowed of antitumoral properties [63].

Tannins and flavonoids are phenolic compounds with various biological activities. The flavonoids display antiplasmodial activity [64] whereas the tannins present antibacterial, antifungal, antioxidant and antitumor activities [65, 66, 67]. Concerning the leucoanthocyanes, these compounds have been demonstrated to have bactericide activity [68].

Table 4. *Chemical profile of Streptomyces sp extract.*

Chemical families	Tests	Observations	Results
ALKALOIDS	Mayer	Abundant precipitation	++
	Wagner	Weak precipitation	+
	Dragendorff	Weak precipitation	+
FLAVONOIDES	Willstätter	Red purple coloration	+
	Willstätter modified	Red purple coloration	+
LEUCOANTHOCYANES	Bate-Smith	Red purplish coloration	+
TERPENOIDES	Liebermann Burchard	Pink coloration	++
STEROIDES	Salkowski	Red partition ring	++
	Badjet-Kedde	Green coloration	-
ANTHRAQUINONES	Borntrager	No change of coloration	-
SAPONOSIDES	Height of the foam	At t=0 (h=5cm); at t=30min (h=2cm)	-
POLYPHENOLS	Gelatine 1%	No precipitation	-
TANNINS	Salted gelatine	Formation of precipitate	++
	$FeCl_3$	Black blue solution	++
POLYSACCHARIDES	-	No precipitation	-

-: absence, +: presence, ++: abundance

3.6. Chemical Composition of the Essential Oil H2

The results from Gas Chromatography revealed that 96,28% of the compounds of the essential oil H2 were identified: 60,88% are terpenic compounds and 35,40% oxygenated compounds. The terpenic compounds are zingiberene (14,48%), β-phellandrene (8,36%), camphene (7,98%) and β-sesquiphellandrene (6,45%). However, the majority compounds of oxygenated compounds are geranial or citral a (9,35%), neral or citral b (6,23%), eucalyptol (3,57%) and borneol (2,09%). The percentage of α-terpineol, elemol and zingiberenol is 1-2%. Unknown products are 3,72% and don't show any majority products (table 5).

Table 5. *Components of ginger rhizomes essential oil from Andrefanivinany by Gas chromatography.*

Compounds	Retention time (min)	% (w/w)	Compounds	Retention time (min)	% (w/w)
2-Heptanone	4,498	0,01	Cyclosativene	13,879	0,15
2-n-Butyl furan	4,539	0,01	Geranyl acetate	14,025	0,58
2-Heptanol	4,622	0,07	α-Copaene	14,038	0,19
Tricyclene	5,103	0,15	iso-β-Elemene	14,181	0,02
α-Thujene	5,157	0,01	cis-β-Elemene	14,312	0,67
α-Pinene	5,3	2,79	7-epi-Sesquithujene	14,507	0,12
Camphene	5,588	7,98	β-Caryophyllen	14,882	0,05
Cosmene	5,829	0,02	γ-Elemene	15,061	0,17
Sabinene	6,011	0,17	Sesquisabinene A (RI 1454)	15,212	0,02
β-Pinene	6,096	0,38	(E) Isoeugenol	15,309	0,07
6-Methyl-5-heptene-2-one	6,193	0,21	(E-β-Farnesene	15,387	0,22
Myrcene	6,284	1,21	Sesquisabinene B (RI 1467.3)	15,441	0,13
Octanal	6,505	0,08	α-Humulene	15,496	0,01
α-Phellandrene	6,594	0,54	allo-Aromadendrene	15,632	0,18
3-Carene	6,717	0,03	Selina-4,11-diene	15,862	0,20
α-Terpinene	6,831	0,02	Ar-Curcumene	15,926	3,88
p-Cymene	6,987	0,14	Germacrene D	15,984	1,16
Limonene	7,115	0,87	Tridecan-2-one	16,08	0,01
β-Phellandrene	7,112	8,26	Zingiberene	16,171	14,48
Eucalyptol	7,148	3,57	α-Selinene	16,228	0,70
2-Heptanol, acetate	7,246	0,03	α-Farnesene	16,32	3,60
cis-β-Ocimene	7,407	0,00	β-Bisabolene	16,381	3,30
Melonal	7,528	0,01	γ-Cadinene	16,53	0,25
(E)-2-Octen-1-al	7,607	0,03	epi-Cubebol	16,574	0,20
γ-Terpinene	7,661	0,04	β-Sesquiphellandrene	16,664	6,45
trans-Linalool oxide	7,943	0,00	(E)-γ-Bisabolene	16,791	0,20
Terpinolene	8,271	0,32	Unknown	17,005	0,21
2-Nonanone	8,277	0,20	Elemol	17,117	1,43
Inconnu	8,399	0,04	trans-Sesquisabinene hydrate	17,142	0,38
2-Nonanol	8,432	0,26	trans-Nerolidol	17,262	0,82
Linalool	8,447	0,92	Germacrene B	17,331	0,38
Perillene = Furan, 3-(4-methyl-3-pentenyl)	8,49	0,01	Germacrene D-4-ol	17,608	0,11
(E)-4,8-Dimethylnona-1,3,7-triene	8,788	0,06	cis-Sesquisabinene hydrate	17,769	0,96
Bicyclo [2.2.1] heptane, 2-methoxy-1, 7,7-trimethyl	8,814	0,05	Zingiberenol	18,16	1,45
cis-2-Menthenol	8,952	0,09	epi-γ-Eudesmol	18,376	0,21
Inconnu	8,984	0,04	Zingibernol isomer	18,433	0,67
trans-2-Menthenol	9,311	0,04	Unknown	18,499	0,41
Camphor	9,475	0,09	γ-Eudesmol	18,537	0,11
Citronellal	9,543	0,39	α-Cadinol	18,685	0,06
Isobornéol	9,71	0,05	β-Eudesmol	18,868	0,73
cis-β-Terpineol	9,81	0,03	Unknown	18,983	0,08
Borneol	9,89	2,09	Intermedeol	19,03	0,08
Terpinen-4-ol	10,107	0,21	β-Bisabolol	19,08	0,08
p-Cymen-8-ol	10,23	0,03	Unknown	19,296	0,08
Cryptone	10,31	0,03	Unknown	19,361	1,24
α-Terpineol	10,365	1,22	Unknown	19,416	0,09

Compounds	Retention time (min)	% (w/w)	Compounds	Retention time (min)	% (w/w)
cis-Piperitol	10,466	0,01	Unknown	19,495	0,45
Myrtenol	10,493	0,09	Unknown	19,525	0,48
n-Decanal	10,592	0,08	Unknown	19,756	0,17
trans-Piperitol	10,694	0,04	Unknown	20,01	0,10
Citronellol	11,033	0,64	trans, trans-Farnesal	20,164	0,16
Nerol	11,064	0,11	Xanthorrhizol	20,291	0,06
3-Methyl-3-(4-methylpent-3-en-1-yl) oxirane-2-carbaldehyde	11,18	0,04	Unknown	20,355	0,22
Citral b=Neral	11,364	6,23	Unknown	20,904	0,05
Geraniol	11,564	1,09	Unknown	20,956	0,02
2-Decenal, (2E)-	11,722	0,06	Unknown	21,191	0,16
Citral a=Geranial	11,949	9,35	Unknown	22,081	0,04
Borneol, acetate	12,276	0,17	Unknown	22,42	0,02
2-Undecanone	12,328	0,48	Geranyl-p-cymene	23,304	0,02
2-Undecanol	12,451	0,05	[6]-Gingerone	26,931	0,01
Myrtenyl acetate	13,013	0,00	[6]-Shogaol	27,661	0,04
δ-Elemene	13,278	0,10	[6]-Gingerol	28,805	0,00
Citronellyl acetate	13,452	0,08	[8]-Shogaol	30,086	0,00
Neryl acetate	13,669	0,00			

These different constituents have been also demonstrated to show a large biological activity spectrum. For examples, β-sesquiphellandrene is antitumor [69]; the citrals (geranial and neral) and camphene possess bactericide and antioxidant effects [70, 71]. Moreover, it would be emphasized that biological activity of the essential oils are not only from their majority compounds. Other minority compounds can develop an efficient system with synergic or antagonistic reaction against target strains [72].

Thus, biological activities of *Streptomyces sp* extract and the essential oil H2 which were at the same time antimicrobial, antioxidant, antiplasmodial and antiproliferative, are linked to the chemical components of their secondary metabolites. One or several chemical components of the extract and the essential oilcan display, therefore, one or several activities demonstrated above. Accordingly, it was observed that *Streptomyces sp* extract and the essential oil H2 contained together as common chemical components the terpens and the phenolic compounds.

4. Conclusion

From this work, it would be concluded that two actinomycetes associated to ginger plant (*Streptomyces chrysomallus*, endophyte and *Streptomyces sp*, from ginger rhizospheric soil) present high pharmacological potential and great therapeutic value as the active extract (essential oil) of the medicinal plant. These bacteria showed accentuated antimicrobial, antioxidant, antiplasmodial and antiproliferative activities than ginger essential oil which prove that actinomycetes constitute important sources of secondary metabolites with wide biological and structural diversity. Further investigation is however, required for isolation, identification, *in vitro* and *in vivo* assays of the active compounds produced by the two isolates. Likewise, this work reports the presence of rhizospheric soil actinomycetes showing comparable biological activities as those of plant active extract. Anyway, these findings provide evidence that plant associated actinomycetes could be taken as sustainable alternatives for drugs production and for plant preservation.

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