Original Research Article

Chemical Composition and Antibacterial Activity of Fractions from Bridelia micrantha Stem Bark Methanol Extract

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A B S T R A C T

Bridelia micrantha commonly known as coastal golden leaf is a member of the family Phyllanthaceae. In preliminary studies, nine fractions, named F₁–F₉, were obtained by fractionating the crude methanol extract of the stem bark of Bridelia micrantha using column chromatographic techniques. The F₆ fraction was found to be the most active when tested for the antibacterial activity. This study is thus aimed at investigating the effect of fractionation on antibacterial activity of F₆ fraction. The F₆ fraction was fractionated by adsorption chromatography on silica gel into eight sub-fractions designated F’₁–F’₈. A product was isolated from the dichloromethane/methanol (10%) fraction and the structure was determined on the basis of spectroscopic data. The antibacterial activity of the F₆ fraction, sub-fractions and the product was evaluated by broth microdilution method against two reference strains and eighteen clinical bacterial strains. The chemical analysis of F₆ and three sub-fractions F’₃, F’₄ and F’₅ was done using HPLC-MS. The fraction F₆ exhibited strong activity on all the tested bacteria with MIC values of 128 µg/ml on nine strains, including Pseudomonas aeruginosa, methicillin-resistant Staphylococcus aureus, and Salmonella sp. The sub-fractions F’₄ and F’₅ exhibited the best activities on all the tested bacteria with MIC values of 32 to 256 µg/ml. The chemical analysis by HPLC-MS of F₆, F’₁, F’₄ and F’₅ revealed the presence of almost 180 identified compounds from various classes of secondary metabolites including alkaloids, flavonoids, steroids and terpenoids. The product obtained although inactive was elucidated as Daucosterol.

Keywords
Bridelia micrantha, Methanol extract, Fractionation, Antibacterial activity, Chromatography

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Introduction

The importance of medicinal plants in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the treatment of many intractable diseases (Umar et al., 2018). Plant chemistry is the basis of the therapeutic uses of herbs. A good knowledge of the chemical composition of plants leads to a better understanding of its possible medicinal value (Hussein and El-Anssary, 2018). Plants produce a good deal of secondary metabolites that have variously been shown to exhibit interesting biological and pharmacological activities (Verpoorte, 1998). Secondary plant metabolites are classified according to their chemical structures into several classes. They are expected to form new sources of antimicrobial drugs, especially against bacteria (Namita and Mukesh, 2012). Antimicrobial resistance in bacterial pathogens is a worldwide challenge leading high morbidity and mortality in clinical settings (WHO, 2014). A selected group of bacteria described by the acronym of “ESCAPE” are the most frequent bacterial agents causing severe infections with significant MDR mechanisms. The term refers to Enterococcus faecium, Staphylococcus aureus, Clostridium difficile, Acinetobacterbaumannii, Pseudomonas aeruginosa, and Enterobacteriaceae (covering all gram-negative enteric bacteria including E. coli, K. pneumonia, Proteus spp. and Enterobacter spp.) (Peterson, 2009).

A crude plant extract is a complex mixture in which compounds may interact antagonistically interfering with or masking the activity of one another (Nwodo et al., 2010). One approach to solving this problem has been to separate the compounds to greater purity and to concentrate them into fractions by various processes, including by chromatography (Jean et al., 2001). It is generally believed that fractionation of plant extracts and purification of the active principles would optimize their potencies. However, in some cases fractionation has been found to extend the spectrum of activity of plant extracts (Etame et al., 2018; Etame et al., 2019; Aboudi et al., 2019), while in others it was found to reduce the spectrum of activity (Nwodo et al., 2010), depending on whether certain constituents of the crude extract interact antagonistically, synergistically or additively when used in combination. Among the several medicinal plants distributed worldwide, Bridelia micrantha (Phyllanthaceae) is commonly used to treat several ailments including amoebic dysentery, cough, diarrhoea, gastric ulcer, eye diseases, infertility and tapeworms (Ngueyem et al., 2009; Maroyi, 2017).

Preliminary studies from our research team highlighted the antibacterial activity of the stem bark methanol extract and a significant increase of this activity achieved with an active fraction F6 following a partition of this methanol extract and column chromatography on silica gel of the dichloromethane (DCM) portion (Aboudi et al., 2019). As a continuation to this previous work, the current study was initiated to investigate the effect of further fractionation of the active fraction F6 of B. micrantha stem bark methanol extract on its antibacterial activity and to analyse its chemical composition.

Materials and Methods

Materials

Plant material

Fresh barks of B. micrantha used in this experiment were collected in January 2017 in the Centre Region of Cameroon at Mount
Kalla. The plant was identified at the Cameroon National Herbarium where a voucher specimen N° 5714 HNC (YA) was deposited.

**Chemicals**

Ciprofloxacin (Sigma-Aldrich, Germany) was used as reference antibiotic. p-Iodonitrotetrazolium chloride (Mouokeu et al., 2014) was used as microbial growth indicator.

**Microorganisms and Culture Media**

The antibacterial activity was carried out on two reference strains (ATCC 27853, CIP 76110) and eighteen clinical strains. The clinical strains of *Escherichia coli* (EC 96, EC 99, EC 136, EC 137), *Enterobacter aerogenes* (ENT 119, ENT 144, ENT 167), *Klebsiella pneumonia* (KL 111), and *Staphylococcus aureus* (ST 9, ST 113, ST 120) were obtained from patient suffering from gastroenteritis at the Bafang ADLUCEM hospital. Those of *Salmonella enterica* serovar *typhi* (SAL 9), *Salmonella enterica* serovar *paratyphi B* (SPB), and *Salmonella enterica* serovar *typhimurium* (STM) were obtained from the Laboratory of Bacteriology and Mycology of the “Centre Pasteur” Yaounde-Cameroon.

Methicillin-resistant *Staphylococcus aureus* strains (MRSA 3, MRSA 9, MRSA 12) were obtained from the culture collection of the Laboratory of Microbiology, Graduate School of Pharmaceutical Sciences, University of Tokyo, Japan. Multidrug resistant *Providencia stuartii* strain (PSNEA 16) was obtained from the culture collection of the University of Mediterranean, France. The characteristics of these bacteria were reported earlier (Aboudi et al., 2019).

**Methods**

**Plant extraction**

*B. micrantha* barks were collected and dried for 21 days in an ambient environment under shade and ground into powder. The powdered plant material (2.5 kg) was soaked in 10L of methanol for 3 days. The mixture was filtered using a Whatman N°1 filter paper and the residue was re-extracted four times as previously described. The total methanol extract was concentrated using rotatory evaporator (Heidoph). The extract was further dried in an oven (VENTI-Line) at 45°C for 24 hours.

**Fractionation of the crude extract**

The extract (600 g) was dissolved in a mixture of methanol (2000mL), distilled water (1000mL) and dichloromethane (2000mL). The DCM phase and the methanol/water phase were separated. Each phase was concentrated using a rotatory evaporator. The DCM phase (150g) was chromatographed through a silica gel as described previously (Aboudi et al., 2019). Nine fractions labeled F₁ to F₉ were obtained.

**Fractionation of F₆ fraction**

The F₆ fraction (24 g) was chromatographed through a silica gel (250-300 Mesh) column (2 cm internal diameter and 30 cm height) using DCM-MeOH (100:0; 95:5; 90:10; 80:20) as eluent. Seventy-three fractions of 150 mL each were collected and concentrated using rotary evaporator at 45°C under reduced pressure; then they were combined on the basis of their thin layer chromatography (TLC) profiles into eight major sub-fractions labelled F’₁ to F’₈ (F’₁: 1-7; F’₂: 8-15,17; F’₃: 18-25, F’₄: 26-35, F’₅: 36-47;F’₆: 48-59; F’₇: 60-69; F’₈: 70-73). Crystals were isolated
from the fraction 16 by recrystallizing with DCM/MeOH (v/v) followed by filtration. Ethyl acetate (100%) was used to wash crystals and revelation was done with UV (254-350 µm) first, then by using sulfuric acid 30%. The compound obtained was labeled CF16 (111mg).

**Antibacterial activity assay**

The *in vitro* antibacterial activity of the F6 fraction, sub-fractions, and the purified compound was evaluated by determining the Minimum Inhibitory Concentrations (MIC) using broth microdilution method (CLSI, 2015). Briefly, the stock solution of F6 fraction, sub-fractions, and the purified compound was prepared with 5% dimethylsulfoxide (DMSO) in broth culture medium. A bacterial suspension of about 1.5 x 10^8 CFU/ml following N° 0.5 McFarland standard turbidity was prepared from an 18 hours old bacterial culture. These suspensions were further diluted in Mueller Hinton broth to give 1.5 x 10^6 CFU/ml. The antibacterial susceptibility tests were performed in 96-well microtiter plates. A serial two-fold dilution of the F6 fraction, sub-fractions was performed to obtain final concentrations ranging from 1024 to 8 µg/ml in a total volume of 100 µl/well (the final concentrations of the purified compound were ranging from 256 to 2 µg/ml). These wells were finally inoculated with 100µl inoculum. The plates were incubated at 37°C for 18h. Following incubation, bacterial growth was monitored colorimetrically using p-iodonitrotetrazolium chloride (INT). Viable bacteria change the yellow dye of p-iodonitrotetrazolium violet into a pink colour. MIC value was recorded as the lowest concentration of the test substance that completely inhibited bacterial growth (Mouokeu et al., 2014). The antibacterial activity was classified as *strong* if the extract displayed a MIC value less than 500 µg/ml, *moderate* from 500 to 1500 µg/ml and *weak* when over 1500 µg/ml (Aligiannis et al., 2001).

The Minimum Bactericidal Concentrations (MBC) were determined by adding 50µl aliquots of the preparations which did not show any growth after incubation during MIC assays to 150 µL Mueller Hinton broth medium. These preparations were incubated at 37°C for 24h. The MBC values were regarded as the lowest concentration of extracts which did not produce any color change after addition of INT as mentioned above (Kuete et al., 2009).

The experiments were performed in duplicate and repeated three times. Ciprofloxacin (Cipro) was used as positive control while 5% DMSO was used as negative control.

**HPLC-MS analysis**

HPLC was undertaken to assess the various components present in the fraction F6 and sub-fractions F’3, F’4, F’5. High resolution mass spectra were obtained with a Q-TOF Spectrometer (Bruker, Germany) equipped with a HESI source. The spectrometer was operated in positive mode (mass range: 100-1500, with a scan rate of 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements within 2 ppm deviation using Na-Formate as calibrant. The following parameters were used for experiments: spray voltage of 4.5 kV, capillary temperature of 200°C. Nitrogen was used as sheath gas (10 L/min). The spectrometer was attached to an Ultimate 3000 (Thermo Fisher, USA) HPLC system consisting of LC-pump, Diode Array Detector (DAD) (λ: 215, 254, 280, 330 nm), auto sampler (injection volume 5 µl) and column oven (50°C). The separations were performed using a synergic MAX-RP 100A (50x2mm, 2.5µm particle size) with a H2O (+0.1 % HCOOH) (A)/acetonitrile (+0.1 % HCOOH)
(B) gradient (flow rate 500 µL/min). Samples were analyzed using a gradient program as follows: 95 % A isocratic for 1.5 min, linear gradient to 100 % B over 6 min, after 100 % B isocratic for 2 min, the system returned to its initial condition (90 % A) within 1 min, and was equilibrated for 1 min.

**Compound structural analysis**

The chemical structure of CF$_{16}$ was elucidated using spectroscopic data such as NMR 1D ($^1$H, $^{13}$C, APT) and NMR 2D (COSY, HMBC). NMR $^{13}$C data were set using HMQC experiments while fragment arrangements were done using COSY.

**Results and Discussion**

The increasing prevalence of antimicrobial drug-resistant microorganisms recovered from hospitalized patients is a major concern worldwide (WHO, 2014). Many strains of *Staphylococcus aureus* and many strains of Gram negative bacteria display multi-drug resistance (GNPIN, 2018). Because of their safety and low cost as well as their impact on a large number of microbes, medicinal plants may have the ability to treat bacterial resistance to many types of antibiotics (Hassawi and Kharma, 2006). The antimicrobial effects of extracts from a large number of plants have been evaluated and reviewed (Mouoikeu et al., 2011; Ngono et al., 2011) and the mechanisms that enable the natural ingredients of herbs to resist microbes have been discussed (Montanari et al., 2012; Etame et al., 2018). The results show that these mechanisms vary greatly depending on the components of the extract (Holley and Patel, 2005); that can actually be concentrated by the means of fractionation for optimal activity.

The F$_6$ fraction from methanol extract of *B. micrantha* stem bark, its sub-fractions, and compound were evaluated for their antibacterial activities on a panel of bacteria strains including two reference strains and eighteen clinical strains (Table 1). These results showed strong activity of the F$_6$ fraction on all the tested bacteria with MIC values of 128 µg/ml on nine strains, including *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, *Enterobacter aerogenes*, and *Salmonella sp* strains.

Previous authors reported the antibacterial activity of this plant (Steenkamp et al., 2007; Gangoué-Piéboji et al., 2009; Adefuye et al., 2011). Adefuye *et al.*, (2011) revealed MIC$_{50}$ values of ethyl acetate and acetone stem bark extract of *B. micrantha* ranged from 78 to 1250 µg/ml and 78 to 625 µg/ml respectively on *Staphylococcus aureus*, *Shigella sonnei*, *Salmonella Typhimurium*, and *Helicobacter pylori* strains. Gangoué-Piébojjet *et al.*, (2009) evaluated the antibacterial activities of *B. micrantha* methanol stem bark extract against *E. coli*, *P. aeruginosa*, and *S. aureus* by using agar-dilution assay. The MIC values of methanol extract against the tested bacteria were of 1250 µg/mL.

Steenkamp *et al.*, (2007) using broth micro-dilution method found that the methanol bark extract showed MIC value of 4000 µg/mL against *S. aureus*. These results line up with those obtained in this work, and point out the *B. micrantha* barks as a source of antibacterial compounds.

After the fractionation process of the F$_6$ fraction, eight sub-fractions were obtained. Among them, two were (F’$_1$ and F’$_2$) found to be inactive on all the tested bacteria; while the six others (F’$_3$, F’$_4$, F’$_5$, F’$_6$, F’$_7$, F’$_8$) exhibited antibacterial activity with MIC values ranging from 32 to 1024 µg/ml. F’$_3$ showed strong activity on fifteen tested bacteria strains with MIC value of 64 to 256 µg/ml. F’$_4$ and F’$_5$ were the most active considering their MIC value. They showed strong activity on all the twenty tested bacteria strains with MIC value...
of 64 to 256 µg/ml for F’₄, while MIC values of 32 to 256 µg/ml were obtained with F’₅. This latter was found to be more efficient on S. aureus sensitive clinical strains (ST9, ST113, and ST120) with MIC value of 32 µg/ml (Table1). Thus from the fraction F₆ to sub-fractions F’₃, F’₄ and F’₅ the antibacterial activity increases. Increase antibacterial activity with fractionation reveals that the active principles of the stem barks of this plant are concentrated during fractionation in some fractions and highlights the fractionation as alternative to ameliorate plant extracts antimicrobial activity. Similar approach was reported by several authors (Khan et al., 2011; Adefuye and Ndip, 2013; Etame et al., 2019).

The sub-fractions F’₄ and F’₅ were found to be the most active with this activity extended to methicillin-resistant Staphylococcus aureus (MRSA strains). Infections caused by MRSA are generally severe with the highest mortality rate (Cosgrove et al., 2003). Actually very few antibiotics as Lysocin E are efficient against MRSA (Hamamoto et al., 2015). The activity of these sub-fractions was extended to ESCAPE pathogens, particularly S. aureus, K. pneumonia, P. aeruginosa, E. aerogenes strains. The ESCAPE pathogens are differentiated from other pathogens due to their increased resistance to commonly used antibiotics. This increased resistance, combined with their clinical significance in the medical field, results in a necessity to combat them with novel antibiotics (Terra et al., 2018). Therefore, the sub-fractions F’₄ and F’₅ could be used directly as antibacterial or could provide molecules which could be useful as antibacterial or substrates for the synthesis of new broad spectrum antibiotics to overcome gastrointestinal tract infection bacteria.

Regarding the MBC values of the fractions F₆ to sub-fractions F’₃, F’₄ and F’₅, it was seen that MBC/MIC ratio was less than 4 in many cases meaning that they all exerted a bactericidal activity on many of the tested organisms (Marmonier, 1990; Djeussi et al., 2013).

Figure 1
### Table 1 MIC/MBC of F<sub>6</sub> fraction and sub-fractions from the methanol extract of *B. micrantha* stem bark (μg/mL)

| Bacteria            | F<sub>6</sub> | R  | F<sub>3</sub> | R  | F<sub>4</sub> | R  | F<sub>5</sub> | R  | F<sub>6</sub> | R  | F<sub>7</sub> | R  | F<sub>8</sub> | R   | Cipro | R   |
|---------------------|--------------|----|--------------|----|--------------|----|--------------|----|--------------|----|--------------|----|--------------|-----|-------|------|
| *K. pneumoniae*     |              |    |              |    |              |    |              |    |              |    |              |    |              |     |       |      |
| KL111               | 256/256      | 1  | 512/512      | 1  | 256/512      | 2  | 256/256      | 1  | 512/1024     | 2  | 512/1024     | 2  | 512/1024    | 2    | 1/16  | 16   |
| *E. aerogenes*      |              |    |              |    |              |    |              |    |              |    |              |    |              |     |       |      |
| ENT167              | 128/512      | 4  | 512/1024     | 2  | 128/1024     | 8  | 128/1024     | 8  | 512/-        | -  | 512/-        | -  | 512/-       | 8/128 | 16    |
| ENT144              | 256/256      | 1  | 512/512      | 1  | 256/512      | 2  | 128/512      | 4  | 256/1024     | 4  | 512/1024     | 2  | 512/1024    | 2    | 1/-   | -    |
| ENT119              | 256/512      | 2  | 256/512      | 2  | 128/256      | 2  | 64/512       | 8  | 512/1024     | 2  | 256/1024     | 4  | 512/512    | 1    | 1/-   | -    |
| *S. aureus*         |              |    |              |    |              |    |              |    |              |    |              |    |              |     |       |      |
| ST9                 | 128/512      | 4  | 128/512      | 4  | 128/256      | 2  | 32/256       | 8  | 128/1024     | 8  | 256/1024     | 4  | 512/1024    | 4    | 1/64  | 64   |
| ST113               | 128/512      | 4  | 128/512      | 4  | 128/512      | 4  | 32/512       | 16 | 128/-        | -  | 128/1024     | 8  | 128/-       | -    | 1/64  | 64   |
| ST120               | 256/512      | 2  | 128/512      | 4  | 64/512       | 8  | 32/256       | 8  | 256/1024     | 4  | 512/1024     | 2  | 512/1024    | 2    | 8/128 | 16   |
| MRSA9               | 256/256      | 1  | 256/512      | 2  | 128/512      | 4  | 128/256      | 2  | 512/-        | -  | 256/1024     | 4  | 256/-       | -    | 2/4   | 2    |
| MRSA3               | 128/256      | 2  | 256/1024     | 4  | 256/512      | 2  | 256/256      | 1  | 512/-        | -  | 512/-        | -  | 512/-       | -    | 32/-  | -    |
| MRSA12              | 256/512      | 2  | 256/1024     | 4  | 128/512      | 4  | 128/512      | 2  | 1024/-       | -  | 1024/-       | -  | 2/16  | 8    |
| *E. coli*           |              |    |              |    |              |    |              |    |              |    |              |    |              |     |       |      |
| EC96                | 128/512      | 4  | 256/1024     | 4  | 128/1024     | 8  | 64/1024      | 16 | 512/-        | -  | 512/-        | -  | 512/-       | -    | 1/128 | 128  |
| EC99                | 256/-        | -  | 128/512      | 4  | 128/512      | 4  | 128/512      | 4  | 256/1024     | 4  | 256/1024     | 4  | 256/1024    | 4    | 4/16  | 4    |
| EC136               | 256/256      | 1  | 256/512      | 2  | 128/512      | 4  | 64/512       | 8  | 512/1024     | 2  | 256/1024     | 4  | 256/1024    | 4    | 1/8   | 8    |
| EC137               | 128/256      | 2  | 256/512      | 2  | 128/256      | 2  | 64/128       | 2  | 256/1024     | 4  | 512/1024     | 2  | 512/1024    | 2    | 16/128 | 8    |
| *P. stuartii*       |              |    |              |    |              |    |              |    |              |    |              |    |              |     |       |      |
| PSNEA16             | 256/-        | -  | 512/512      | 1  | 128/512      | 4  | 128/512      | 4  | 512/1024     | 2  | 256/1024     | 4  | 256/1024    | 4    | 1/16  | 16   |
| *S. Typhi*          |              |    |              |    |              |    |              |    |              |    |              |    |              |     |       |      |
| SAL 9               | 256/-        | -  | 256/512      | 2  | 128/512      | 4  | 256/512      | 2  | 256/1024     | 4  | 128/1024     | 8  | 256/1024    | 4    | 1/16  | 16   |
| *S. typhimurium*    | 128/512      | 4  | 64/1024      | 16 | 64/128       | 8  | 128/512      | 4  | 256/-        | -  | 128/-        | -  | 128/-       | -    | 32/64 | 2    |
| *S. paratyphi B*    | 128/512      | 4  | 128/512      | 4  | 64/1024      | 16 | 64/512       | 8  | 512/1024     | 2  | 1024/1024    | 1  | 512/1024    | 2    | 1/128 | 128  |
| *P. aeruginosa*     |              |    |              |    |              |    |              |    |              |    |              |    |              |     |       |      |
| ATCC 27853          | 256/512      | 2  | 512/512      | 1  | 256/512      | 2  | 256/256      | 1  | 512/1024     | 2  | 512/1024     | 2  | 512/-       | -    | 1/64  | 64   |
| CIP 76110           | 128/512      | 4  | 64/512       | 8  | 64/512       | 8  | 128/256      | 2  | 256/1024     | 4  | 256/1024     | 4  | 128/1024    | 8    | 1/64  | 64   |

- = MIC or MBC that was greater than 1024
Table 2: Isolated compounds by the HPLC-MS

| Compound name                        | Chemical formula | F<sub>6</sub> Fraction | F'<sub>3</sub> Fraction | F'<sub>4</sub> Fraction | F'<sub>5</sub> Fraction | Compound Class |
|--------------------------------------|------------------|------------------------|------------------------|------------------------|------------------------|----------------|
| Sacranoside A                        | C<sub>21</sub>H<sub>34</sub>O<sub>10</sub> | ✓                      | x                      | x                      | x                      |                 |
| Verbenol                             | C<sub>10</sub>H<sub>16</sub>O          | ✓                      | x                      | x                      | x                      |                 |
| p-Cymene                             | C<sub>10</sub>H<sub>14</sub>           | ✓                      | x                      | x                      | x                      |                 |
| Schizonepetoside C                   | C<sub>16</sub>H<sub>26</sub>O<sub>7</sub> | ✓                      | x                      | x                      | x                      |                 |
| Neohancoside B                       | C<sub>21</sub>H<sub>36</sub>O<sub>11</sub> | ✓                      | x                      | x                      | x                      |                 |
| Neohancoside A                       | C<sub>21</sub>H<sub>36</sub>O<sub>10</sub> | ✓                      | x                      | x                      | x                      |                 |
| Thujopsadiene                        | C<sub>15</sub>H<sub>22</sub>           | ✓                      | x                      | x                      | x                      |                 |
| Widdrol                              | C<sub>15</sub>H<sub>26</sub>O          | ✓                      | x                      | x                      | ✓                      |                 |
| Dendroside E                         | C<sub>21</sub>H<sub>36</sub>O<sub>8</sub> | ✓                      | x                      | x                      | x                      |                 |
| Dendroside E                         | C<sub>21</sub>H<sub>36</sub>O<sub>8</sub> | ✓                      | x                      | x                      | x                      |                 |
| Trilobolide                           | C<sub>27</sub>H<sub>38</sub>O<sub>10</sub> | ✓                      | x                      | x                      | x                      |                 |
| Pterosin E                           | C<sub>14</sub>H<sub>16</sub>O<sub>3</sub> | ✓                      | x                      | x                      | x                      |                 |
| Ursiniolide A                        | C<sub>22</sub>H<sub>28</sub>O<sub>7</sub> | ✓                      | x                      | x                      | x                      |                 |
| 5alpha-Acetyl-5alpha-decinnamoyltaxagifine | C<sub>30</sub>H<sub>40</sub>O<sub>13</sub> | ✓                      | x                      | x                      | x                      |                 |
| Homofukinolide                       | C<sub>25</sub>H<sub>34</sub>O<sub>6</sub> | ✓                      | x                      | x                      | x                      |                 |
| Vernodalin                           | C<sub>19</sub>H<sub>20</sub>O<sub>7</sub> | x                      | x                      | x                      | ✓                      |                 |
| Roseoside                            | C<sub>19</sub>H<sub>30</sub>O<sub>8</sub> | ✓                      | x                      | x                      | x                      |                 |
| Valerenic acid                       | C<sub>15</sub>H<sub>22</sub>O<sub>2</sub> | x                      | x                      | x                      | ✓                      |                 |
| Turmerone                             | C<sub>15</sub>H<sub>20</sub>O          | ✓                      | x                      | x                      | x                      |                 |
| Marioside                             | C<sub>22</sub>H<sub>34</sub>O<sub>10</sub> | ✓                      | x                      | x                      | x                      |                 |
| Psilostachyin                        | C<sub>15</sub>H<sub>20</sub>O<sub>5</sub> | x                      | ✓                      | ✓                      | ✓                      |                 |
| Oriediterpenol                       | C<sub>20</sub>H<sub>32</sub>O<sub>2</sub> | ✓                      | ✓                      | x                      | ✓                      |                 |
| 2,5,7-Trihydroxy-6,8-dimethyl-3-(4' -methoxybenzyl)chroman-4-one | C<sub>19</sub>H<sub>20</sub>O<sub>6</sub> | ✓                      | x                      | x                      | x                      |                 |
| Taxezopidine B                       | C<sub>26</sub>H<sub>38</sub>O<sub>10</sub> | ✓                      | x                      | x                      | x                      |                 |
| Compound                  | Molecular Formula | S  | 1  | 2  | 3  |
|--------------------------|-------------------|----|----|----|----|
| Taxuspine W              | C_{26} H_{36} O_{9} | ✓  | x  | x  | x  |
| Taxuyunnanine E          | C_{33} H_{42} O_{12} | ✓  | x  | x  | x  |
| Taxumairol B             | C_{28} H_{40} O_{12} | ✓  | x  | x  | x  |
| Shikokianin              | C_{24} H_{32} O_{8}  | ✓  | x  | x  | x  |
| Taxumairol C             | C_{28} H_{38} O_{11} | ✓  | x  | x  | x  |
| Lungshengenin G          | C_{26} H_{34} O_{9}  | ✓  | x  | x  | x  |
| Yadanzioside M           | C_{33} H_{40} O_{15} | ✓  | x  | x  | x  |
| 10-Hydroxyacetyl baccatin VI | C_{37} H_{46} O_{15} | ✓  | x  | x  | x  |
| Taxuspine U              | C_{28} H_{40} O_{11} | ✓  | x  | x  | x  |
| Baccatin VI              | C_{37} H_{46} O_{14} | ✓  | x  | x  | x  |
| Taxchin B                | C_{41} H_{52} O_{14} | ✓  | x  | x  | x  |
| 9(betaH)-9-Dihydro-19-acetoxy-10-deacetyl baccatin III | C_{31} H_{40} O_{12} | ✓  | x  | x  | x  |
| 13-Deacetoxy-13,15-epoxy-11(15-->1)-abeo-13-epi-baccatin VI | C_{35} H_{42} O_{12} | ✓  | x  | x  | x  |
| cis-Neoabienol           | C_{20} H_{34} O     | ✓  | x  | x  | ✓  |
| 9-Deacetyl-9-benzoyl-10-debenzoyltaxchinin A | C_{31} H_{40} O_{10} | ✓  | x  | x  | x  |
| Taxuspine O              | C_{26} H_{36} O_{10} | ✓  | x  | x  | x  |
| Forskoditerpenoside C    | C_{28} H_{44} O_{11} | ✓  | x  | x  | x  |
| Ganolactone              | C_{27} H_{36} O_{6}  | x  | x  | x  | ✓  |
| 3beta-Acetyl ursa-14-en-16-one | C_{32} H_{50} O_{3}  | x  | x  | x  | ✓  |
| Nigakilactone I          | C_{21} H_{28} O_{6}  | x  | x  | ✓  | ✓  |
| Lup-20(29)-ene-3alpha-acetoxy-24-oic acid | C_{32} H_{50} O_{4}  | x  | ✓  | x  | ✓  |
| 3-Hydroxy-25-norfriedel-3,1(10)-dien-2-one-30-oic acid | C_{29} H_{42} O_{4}  | x  | ✓  | x  | ✓  |
| Camellin                 | C_{18} H_{30} O_{7}  | ✓  | x  | x  | x  |
| Sobrerol                 | C_{10} H_{18} O_{2}  | ✓  | ✓  | x  | ✓  |
| Hypolidemethylether      | C_{21} H_{26} O_{3}  | ✓  | x  | x  | x  |
| Isovaleroxy-hydroxy dihydrovaltrate | C_{27} H_{40} O_{11} | ✓  | x  | x  | x  |
| Alkaloids                                                                 | Formula       | Yes| Yes| No | Yes |
|--------------------------------------------------------------------------|---------------|----|----|----|-----|
| Taxuspine F                                                              | C_{28}H_{38}O_{10} | ✓  | ✓  | x  | ✓   |
| Valerosidatum                                                            | C_{21}H_{34}O_{11} | ✓  | x  | x  | x   |
| 8-(O-Methyl-p-coumaroyl)harpaside                                        | C_{25}H_{32}O_{12} | ✓  | x  | x  | x   |
| Riddelline                                                               | C_{18}H_{23}N_{6}O | ✓  | x  | x  | x   |
| Serratinidine                                                            | C_{18}H_{28}N_{2}O_{2} | ✓  | x  | ✓  | ✓   |
| Securinol C                                                              | C_{13}H_{17}N_{3}O_{3} | ✓  | x  | x  | x   |
| Argentine                                                                | C_{23}H_{26}N_{4}O_{3} | ✓  | x  | x  | x   |
| Ervadivaricatine A                                                       | C_{43}H_{56}N_{4}O_{5} | x  | x  | x  | ✓   |
| 1beta,2beta,5alpha,11-Tetraacetoxy-8alpha-benzoyl-4alpha-hydroxy-7beta-nicotinoyl-dihydroagarofuran | C_{36}H_{41}N_{14}O_{14} | x  | x  | x  | ✓   |
| 3-O-Tetradecanoyl-1-cyano-2-methyl-1,2-propene                            | C_{19}H_{33}N_{2}O_{2} | ✓  | ✓  | x  | ✓   |
| Wilsonine                                                                | C_{20}H_{25}N_{4}O_{4} | x  | x  | x  | ✓   |
| Nor-orixine                                                              | C_{16}H_{19}N_{6}O_{6} | ✓  | x  | x  | x   |
| Chelirubine                                                              | C_{21}H_{16}N_{5}O_{5} | x  | ✓  | x  | ✓   |
| Dihydrokoumine                                                           | C_{20}H_{24}N_{2}O | x  | x  | x  | ✓   |
| N-Methyltyramine-O-alpha-L-rhamnopyranoside                              | C_{15}H_{23}N_{5}O_{5} | ✓  | x  | x  | x   |
| Pseudobrucine                                                            | C_{23}H_{26}N_{2}O_{5} | ✓  | x  | x  | x   |
| Euoverrine A                                                             | C_{48}H_{51}N_{18}O_{18} | ✓  | ✓  | ✓  | ✓   |
| Subaphyllin                                                              | C_{14}H_{20}N_{2}O_{3} | ✓  | x  | x  | x   |
| Aldohypaconitine                                                         | C_{33}H_{43}N_{11}O_{11} | ✓  | x  | x  | x   |
| Ergocornine                                                              | C_{31}H_{39}N_{5}O_{5} | ✓  | x  | x  | x   |
| Geniculine                                                               | C_{34}H_{47}N_{11}O_{11} | ✓  | x  | x  | x   |
| Teixidol                                                                 | C_{28}H_{40}O_{10} | ✓  | x  | x  | x   |
| Thalicmine                                                               | C_{21}H_{23}N_{2}O_{5} | ✓  | x  | x  | ✓   |
| Lysergamide                                                              | C_{16}H_{17}N_{3}O | ✓  | x  | x  | ✓   |
| Compound                      | Formula          | Phenol Content | Benzoate Content | Cinnamate Content | Phenolic Compounds |
|-------------------------------|------------------|----------------|------------------|-------------------|-------------------|
| 12-Methoxyaffinisine         | C$_{21}$H$_{36}$N$_{2}$O$_{2}$ | ✓              | x                | x                 | x                 |
| Voacamine                     | C$_{43}$H$_{52}$N$_{4}$O$_{5}$  | ✓              | x                | x                 | x                 |
| Terminaline                   | C$_{23}$H$_{41}$N O$_{2}$    | ✓              | ✓                | x                 | x                 |
| Camptothecin                  | C$_{20}$H$_{16}$N$_{2}$O$_{4}$ | ✓              | ✓                | ✓                 | ✓                 |
| 11-Deoxojervine               | C$_{27}$H$_{41}$N O$_{2}$    | ✓              | ✓                | x                 | x                 |
| Germerine                     | C$_{37}$H$_{59}$N O$_{11}$   | ✓              | ✓                | x                 | x                 |
| Buxbodine D                   | C$_{28}$H$_{46}$N$_{2}$O$^*$  | ✓              | x                | x                 | x                 |
| Parasorbicacid               | C$_{6}$H$_{8}$O$_{2}$       | ✓              | ✓                | x                 | ✓                 |
| Danshensu                     | C$_{6}$H$_{10}$O$_{5}$      | ✓              | ✓                | x                 | ✓                 |
| 2-Hexenyl benzoate            | C$_{13}$H$_{16}$O$_{2}$     | x              | x                | x                 | ✓                 |
| Phenethylcaffeate             | C$_{17}$H$_{16}$O$_{4}$     | ✓              | x                | x                 | ✓                 |
| 6'-O-Methylhonokiol           | C$_{19}$H$_{20}$O$_{2}$     | ✓              | x                | x                 | ✓                 |
| Protocatechuoylcalleryanin    | C$_{20}$H$_{22}$O$_{11}$    | ✓              | ✓                | x                 | ✓                 |
| 6- Shogaol                    | C$_{17}$H$_{24}$O$_{3}$     | x              | x                | x                 | ✓                 |
| 4-Prenyl dihdgdropinosylvin   | C$_{19}$H$_{22}$O$_{2}$     | x              | ✓                | ✓                 | ✓                 |
| Thelephantin C                | C$_{32}$H$_{30}$O$_{9}$     | x              | ✓                | x                 | ✓                 |
| Salicylic acid                | C$_{7}$H$_{6}$O$_{3}$       | x              | ✓                | x                 | ✓                 |
| Tropolone                     | C$_{7}$H$_{6}$O$_{2}$       | x              | ✓                | x                 | ✓                 |
| Vanililly alcohol             | C$_{6}$H$_{10}$O$_{3}$      | x              | ✓                | x                 | ✓                 |
| Pyrogallol                    | C$_{6}$H$_{6}$O$_{3}$       | ✓              | x                | x                 | x                 |
| Phenyl-2-propanone            | C$_{9}$H$_{10}$O$_{3}$      | x              | ✓                | ✓                 | ✓                 |
| alpha-Thujaplicin             | C$_{10}$H$_{12}$O$_{2}$     | x              | x                | x                 | ✓                 |
| 9,12-Dihydroxy-15-nonadecenoic acid | C$_{19}$H$_{36}$O$_{4}$ | ✓              | x                | ✓                 | ✓                 |
| Palmitoleic acid              | C$_{16}$H$_{30}$O$_{2}$     | ✓              | ✓                | x                 | ✓                 |
| Valerenolic acid              | C$_{16}$H$_{24}$O$_{2}$     | ✓              | x                | ✓                 | ✓                 |
| 9,10-Dihydroxystearic acid    | C$_{18}$H$_{36}$O$_{4}$     | ✓              | ✓                | x                 | ✓                 |
| Methyl 9-octadecenoate        | C$_{19}$H$_{36}$O$_{2}$     | ✓              | ✓                | x                 | ✓                 |
| Trichosanacacid              | C$_{18}$H$_{30}$O$_{2}$     | ✓              | ✓                | ✓                 | ✓                 |
| Hydnocarpicacid              | C$_{16}$H$_{28}$O$_{2}$     | x              | ✓                | x                 | ✓                 |
| Ethyloctadecanoate            | C$_{20}$H$_{40}$O$_{2}$     | x              | ✓                | x                 | ✓                 |
| Chemical Name                  | Molecular Formula | C, H, O  |   |   |   |   |   |   |   |   |   |
|-------------------------------|-------------------|---------|---|---|---|---|---|---|---|---|---|
| **Fatty acids**               |                   |         |   |   |   |   |   |   |   |   |   |
| Nilic acid                    | C_5 H_{10} O_3    | x       | x | x | x | x | ✓ |   |   |   |   |
| 4,8,12-Trimethyl tridecanoic acid | C_{16} H_{32} O_2 | x       | ✓ | x | ✓ |   |   |   |   |   |   |
| Coronaric acid                | C_{18} H_{32} O_3 | x       | x | x |   | x | ✓ |   |   |   |   |
| Docosandioic acid             | C_{22} H_{42} O_4 | x       | x | x | x |   | ✓ |   |   |   |   |
| Tetradecenoic acid C          | C_{14} H_{26} O_2 | x       | ✓ | x |   |   | ✓ |   |   |   |   |
| Gadoleic acid                 | C_{20} H_{38} O_2 | x       | x | x | x |   | ✓ |   |   |   |   |
| Eucalyptus wax                | C_{33} H_{64} O_2 | ✓       | x | x | x | x |   |   |   |   |   |
| Sarcostin                     | C_{21} H_{34} O_6 | ✓       | x | x | x |   |   |   |   |   |   |
| Strophanthidin                | C_{23} H_{32} O_6 | ✓       | x | x | x |   |   |   |   |   |   |
| Bufotalin                     | C_{25} H_{34} O_7 | ✓       | x | x | x |   |   |   |   |   |   |
| Taccalonolide H               | C_{36} H_{44} O_{14} | ✓ | x | x |   |   |   |   |   |   |   |
| β-sitosterol 3-O-β-D-glucopyranoside | C_{35} H_{60} O_6 | ✓       | x | x | x |   |   |   |   |   |   |
| Cinobufagin                   | C_{26} H_{34} O_6 | ✓       | ✓ | ✓ | ✓ | ✓ |   |   |   |   |   |
| 1,4-Epoxy-16-hydroxyheneicos-13,12,14-tetraene | C_{21} H_{34} O_2 | ✓       | ✓ | x | x | x |   |   |   |   |   |
| 5beta-Cholanic acid           | C_{24} H_{40} O_2 | ✓       | ✓ | ✓ |   | x | ✓ |   |   |   |   |
| Sengosterone                  | C_{20} H_{44} O_9 | ✓       | x | x | x |   |   |   |   |   |   |
| 4-Methyl-7-ergosta-8,24(28)-diene | C_{29} H_{48}    | ✓       | ✓ | x | x | x |   |   |   |   |   |
| Stigmasta-4,25-dien-3beta,6beta-diol | C_{29} H_{48} O_2 | ✓       | x | x | x |   |   |   |   |   |   |
| 25R-Spirost-4-en-3,12-dione   | C_{27} H_{38} O_4 | x       | x | x | x |   | ✓ |   |   |   |   |
| Flavaspidin                   | C_{23} H_{30} O_8 | ✓       | x | x | x | x |   |   |   |   |   |
| 3,5-Diacetyltambulin          | C_{22} H_{20} O_9 | x       | ✓ | ✓ | ✓ | ✓ |   |   |   |   |   |
| Retusine                      | C_{16} H_{25} N O_5 | ✓ | x | x | x | x |   |   |   |   |   |
| Agastachin                    | C_{47} H_{44} O_{22} | ✓ | ✓ | x | x | x |   |   |   |   |   |
| Triacetylhispidulin           | C_{22} H_{18} O_9 | x       | x | x |   | x | ✓ |   |   |   |   |
| Ergochrysin                   | C_{31} H_{28} O_{14} | x       | x | x | x |   | ✓ |   |   |   |   |
| Mulberrofuran Q               | C_{34} H_{24} O_{10} | x       | ✓ | x | x | x |   |   |   |   |   |
| Theasinensin A                | C_{44} H_{34} O_{22} | x       | x | x | x | x |   |   |   |   |   |
| **Steroids**                  |                   |         |   |   |   |   |   |   |   |   |   |
| **Flavonoids**                |                   |         |   |   |   |   |   |   |   |   |   |

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| Compound                                      | Formula        | C  | H  | O  | N  | P  |    |
|-----------------------------------------------|----------------|----|----|----|----|----|----|
| Methyl-3-O-beta-D-glucopyranosyl polygalacate  | C\textsubscript{37} H\textsubscript{60} O\textsubscript{11} | x  | x  | x  | x  |    | ✓  |
| Coelovirin A                                  | C\textsubscript{21} H\textsubscript{30} O\textsubscript{12} | ✓  | x  | x  | x  |    |    |
| 1,1'-Dibenzene-6',8',9'-trihydroxy-3-allyl-4-O-beta-D-glucopyranoside | C\textsubscript{24} H\textsubscript{30} O\textsubscript{9} | ✓  | x  | x  | x  |    |    |
| Tetracentronside B                            | C\textsubscript{26} H\textsubscript{32} O\textsubscript{11} | ✓  | x  | x  | x  | x  |    |
| Magnoshinin                                   | C\textsubscript{24} H\textsubscript{30} O\textsubscript{6} | ✓  | x  | ✓  | ✓  | ✓  | ✓  |
| Sterekunthal B                                | C\textsubscript{20} H\textsubscript{18} O\textsubscript{4} | ✓  | x  | x  | x  |    |    |
| Theaspirone                                   | C\textsubscript{13} H\textsubscript{20} O\textsubscript{2} | ✓  | ✓  | x  | x  | ✓  | ✓  |
| Senkyunolide K                                | C\textsubscript{12} H\textsubscript{16} O\textsubscript{3} | ✓  | ✓  | ✓  | x  | x  |    |
| Senkyunolide M                                | C\textsubscript{16} H\textsubscript{22} O\textsubscript{4} | x  | ✓  | x  | ✓  | ✓  |    |
| Adenine                                       | C\textsubscript{5} H\textsubscript{5} N\textsubscript{5}  | ✓  | ✓  | ✓  | ✓  | x  | x  |
| Hypoxanthine                                  | C\textsubscript{5} H\textsubscript{4} N\textsubscript{4} O | x  | x  | x  | x  | ✓  |    |
| Zeatin                                        | C\textsubscript{10} H\textsubscript{13} N\textsubscript{5} O | x  | x  | x  | x  | ✓  |    |
| Prenylcaffeate                                | C\textsubscript{14} H\textsubscript{16} O\textsubscript{4} | ✓  | x  | x  | x  | x  |    |
| 6-Hydroxy-7-methylesculetin                   | C\textsubscript{10} H\textsubscript{8} O\textsubscript{3} | ✓  | x  | x  | x  | x  |    |
| Quassimarin                                   | C\textsubscript{26} H\textsubscript{34} O\textsubscript{11} | ✓  | ✓  | ✓  | x  | x  |    |
| Picrasinoside C                               | C\textsubscript{28} H\textsubscript{42} O\textsubscript{11} | ✓  | x  | x  | x  |    |    |
| alpha:beta-Diolein                            | C\textsubscript{39} H\textsubscript{72} O\textsubscript{5} | x  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Phycocyanobilin                               | C\textsubscript{33} H\textsubscript{36} N\textsubscript{4} O\textsubscript{6} | ✓  | x  | x  | x  | x  |    |
| Biliverdin                                    | C\textsubscript{33} H\textsubscript{34} N\textsubscript{4} O\textsubscript{6} | ✓  | x  | x  | x  | x  |    |
| 3-Methylcyclotripdecan-1-one                  | C\textsubscript{14} H\textsubscript{26} O | x  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Civetone                                      | C\textsubscript{17} H\textsubscript{30} O | x  | x  | ✓  | ✓  | ✓  |    |
| Maesaquinone                                  | C\textsubscript{26} H\textsubscript{42} O\textsubscript{4} | ✓  | x  | x  | x  |    |    |
| Methylenetanshinquinone                       | C\textsubscript{18} H\textsubscript{14} O\textsubscript{3} | ✓  | x  | x  | x  |    |    |
| Isoallylbenzene                               | C\textsubscript{9} H\textsubscript{10} | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 1-Propenyl-cyclohexane                        | C\textsubscript{9} H\textsubscript{16} | x  | ✓  | ✓  | x  | ✓  |    |
| Compound                                | Molecular Formula | Properties          | Type                        |
|-----------------------------------------|-------------------|---------------------|-----------------------------|
| Exaltolide                              | C_{15}H_{28}O_{2}  | ✓                   | Macrolide                   |
| Adenosine                               | C_{10}H_{13}N_{5}O_{4} | ✓ ✓ ✓ ✓ ✓        | Nucleoside                   |
| Muricatacin                              | C_{17}H_{32}O_{3}  | ✓ ✓ x               | Acetogenin                   |
| (2S)-1-O-(9Z,12Z-Octadeca-dien-noylo)-3-O-beta-D-galactopyranosyl-glycerol | C_{27}H_{48}O_{9}  | ✓ x x x            | Glycerolipid                 |
| Suaveolol                               | C_{20}H_{34}O_{2}  | ✓ x x x            | Polycyclic compound          |
| Wilforonide                             | C_{13}H_{16}O_{3}  | x x x ✓            | Keto-ester                   |
| Deoxymorellin                           | C_{33}H_{38}O_{6}  | ✓ ✓ x ✓            | Miscellaneous                |
| Yonogenin                               | C_{27}H_{44}O_{4}  | ✓ x x ✓            | Saponin                      |
| Urushiol III                            | C_{21}H_{32}O_{2}  | x ✓ ✓ x            | Cathecol                     |
| 3beta-Methoxy-9beta,19-cyclolanost-23(E)-en-25,26-diol | C_{31}H_{52}O_{3}  | x ✓ ✓ x            | Vitamin (Vit E)              |
| 8-Methyl-5-isopropyl-6,8-nonadiene-2-one | C_{12}H_{22}O_{2}  | x x x ✓            | Ether                        |
| 6-Phenylundecane                        | C_{17}H_{28}O     | x x x ✓            | Alkylbenzene                 |
| Sandaracopimarol                        | C_{20}H_{32}O_{2}  | x x x ✓            | Phenanthrene                 |
| 5-Methoxy-1,7-diphenyl-3-heptanone      | C_{20}H_{24}O_{2}  | x x x ✓            | Diarylheptanoid              |
| Spatheliabischromene                    | C_{20}H_{20}O_{4}  | ✓ x x x            | Benzopropyranoid             |
| Icaride A2                              | C_{22}H_{28}O_{9}  | ✓ x x x            | Phenylpropanoid              |
| Margaspidin                             | C_{24}H_{30}O_{8}  | ✓ x x x            | Phloroglucinol derivative    |
| Shikonofuran C                          | C_{21}H_{26}O_{5}  | ✓ x x x            | Shikonin derivative          |
Identification of isolated compound

The structure of the compound CF₁₆ was determined on the basis of spectral data. This structure was confirmed by comparing with those described in literature (Moradkhani et al., 2014). By comparison of the data with those reported in the literature, the compound was identified as Daucosterol (β- sitosterol 3-O- β- D- glucopyranoside) (Fig. 1). It has a molecular weight of 576g/mol, corresponding to the empirical formula C₃₅H₆₀O₆.

The isolated compound was found to be inactive on all the tested bacteria. These results are similar to those reported by Bayor et al., (2009) who evaluated β-sitosterol-3-O-D-glucopyranoside on S. aureus, B. subtilis, E. coli, P. aeruginosa and it had no antibacterial effect. Njinga et al., (2016) reported a good activity of the molecule on a set of bacteria including S.aureus and E.coli, with MIC ranged from 25 to 50 µg/ml. This different result could be due to the fact that Njinga et al., used just a loop of a 0.5 McFarland bacterial solution as the inoculum; this is very low comparing to that we used in our work.

HPLC-MS analysis

The chemical analysis of the F₆ fraction, the sub-fractions F₃',F₄' and F₅'revealed the presence of almost 180 identified compounds from various classes of secondary metabolites including alkaloids, flavonoids, phenols, quinones, steroids and terpenoids (Table 2). These phytochemicals may explain their antibacterial capacity, since the inhibitory properties of these secondary metabolites against different pathogens have been reported (Cowan, 1999). For example, flavonoids inhibit the activity of enzymes by forming complexes with bacterial cell walls, extracellular and soluble proteins. More lipophilic flavonoids disrupt cell wall integrity (Kurtz et al., 1994). The chemical analysis revealed that many compounds that had not been detected in the F₆ fraction were found in the sub-fractions, suggesting that their concentration increased during the fractionation process. This may explain the increased antibacterial ability of sub-fractions F₃', F₄' and F₅' compared to the F₆ fraction.

In conclusion, the results revealed an increased activity with fractionation, the sub-fractions F₄' and F₅' being the most active. These sub-fractions could be used as sources of antibacterial compounds.

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