



## PHYTOCHEMICAL IDENTIFICATION OF THE ETHANOLIC EXTRACT OF SABA SENEGALENSIS LEAVES BY LIQUID CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY

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### ABSTRACT

The study focused on the phytochemical analysis of the ethanolic extract of *Saba senegalensis* (Apocynaceae) leaves using liquid chromatography coupled with mass spectrometry (LC-MS). Positive mode analysis revealed the presence of twelve compounds, including major flavonoids such as myricetin, myricitrin, hyperoside, afzelin, taxifolin, and avicularin. In negative mode, nine compounds were identified, including astilbin, naringin, and (+)-syringaresinol. These metabolites have recognized biological properties: antioxidant, anti-inflammatory, antimicrobial, cardioprotective, and antidiabetic. These results provide a solid scientific basis for the potential pharmaceutical applications of this medicinal species.

**KEYWORDS:** *Saba senegalensis*, LC-MS, flavonoids, antioxidant and ethnopharmacology.

### 1. INTRODUCTION

Natural substances derived from plants are a prime source of bioactive molecules, the exploration of which plays a central role in natural product chemistry and pharmacognosy. The search for new bioactive substances derived from traditional African pharmacopoeia is a promising avenue for therapeutic innovation. Indeed, many plant species used in traditional medicines contain specialized metabolites with various biological activities, including flavonoids, tannins, alkaloids, lignans, and other polyphenols.<sup>[1]</sup> Among these molecules, flavonoids represent a major class of secondary metabolites, widely distributed in the plant kingdom and characterized by remarkable structural diversity. They are known to play a key role in plant defense against biotic and abiotic

stresses, but also for their beneficial effects on human health. Numerous studies have highlighted their pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and cardioprotective effects.<sup>[2,3]</sup>

Flavonoids exert their antioxidant effects mainly through their ability to trap reactive oxygen species (ROS), chelate pro-oxidant metal ions, and modulate the activity of enzymes involved in oxidative processes. These mechanisms help reduce oxidative stress, a factor involved in the etiology of many chronic diseases such as cardiovascular disease, cancer, and neurodegenerative diseases.<sup>[2]</sup> The interest of their study therefore lies in elucidating the relationships between their chemical

structure and their biological effects; this opens up prospects for therapeutic applications.

*Saba senegalensis* (A. DC.) Pichon, belonging to the Apocynaceae family, is a plant widely used in traditional African medicine. Its leaves, roots, and fruits are used in the treatment of various disorders, including gastrointestinal infections, inflammatory conditions, fever, and metabolic disorders.<sup>[4,5]</sup> However, despite its empirical uses, scientific data on its phytochemical composition remain limited.

The advent of modern analytical techniques, particularly liquid chromatography coupled with mass spectrometry (LC-MS), has greatly improved researchers' ability to identify and characterize complex molecules present in plant extracts.<sup>[6]</sup> This approach allows for qualitative and semi-quantitative analysis of compounds while offering high sensitivity and specificity.

In this context, the aim of this study is to perform a detailed chemical analysis of the ethanolic extract of *Saba senegalensis* leaves using High Performance Liquid Chromatography coupled with Mass Spectrometry (LC-MS), in positive and negative modes. The identification of bioactive compounds, particularly flavonoids and other specialized metabolites, will not only enrich our knowledge of this species, but also provide scientific arguments to justify its traditional uses and support future pharmacological applications.

## 2. MATERIALS AND METHODS

### 2.1. Materials

#### 2.1.1. Plant material

The plant material consists of leaves from *Saba senegalensis*. The leaves were harvested in October 2022 in the town of Korhogo (9° 27' 28" North, 5° 37' 46" West). They were dried for 10 days away from sunlight in a room at room temperature. Finally, the dry leaves were crushed in a mortar and then sieved to obtain a fine powder which was used to prepare the extract for testing.

#### 2.1.3. Laboratory equipment and solvents

The laboratory equipment includes standard glassware, an electronic balance (DENVER INSTRUMENT SI-234), a hot plate (Rommelsbacher), an oven (Mettler), and a spectrometer (Thermo Scientific Orbitrap Exploris 480). Distilled water and methanol were used as solvents.

### 2.2. Methods

#### 2.2.1. Extraction

A mass of 7 g of *Saba senegalensis* leaves powder was macerated in 70 mL of ethanol (96%) for 24 hours under constant agitation in an air-conditioned room. After filtration, the filtrate was placed in an oven at 50°C for 3 days. The ethanol extract of *S. senegalensis* obtained was used to identify the different compounds by LC-MS analysis.

#### 2.2.2. LC-MS analysis

The sample was analyzed in nanoLCMS (nLCMS) mode on a Thermo Scientific Orbitrap Exploris 480 spectrometer in positive and negative electrospray modes. The ethanolic extract from *S. senegalensis* leaves was solubilized in HPLC Grade H<sub>2</sub>O/CH<sub>3</sub>OH (95/5) solvent mixture. For the analyses, 500 ng of material was deposited on the PepMap Neo C18 pre-column, 5 µm, 300 µm x 5 mm / EASY - Spray column PepMap Neo 1500 bars, 75 µm x 150 mm, C18, 2 µm, 100 Å.

Over a 40-minute run, the following instrumental and gradient parameters were used for positive and negative mode analyses: CRMPO-PJ-P-480k-40min-100-1500-2000V-SL75-Gradient 2 - Trap and Elute – BackFlush. Using the workflow Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mzLogic.cdProcessingWF\_AMBEU\_241113, the Compound Discoverer 3.3 SP3 (3.3.3.200) software enabled the processing of the various acquisitions.

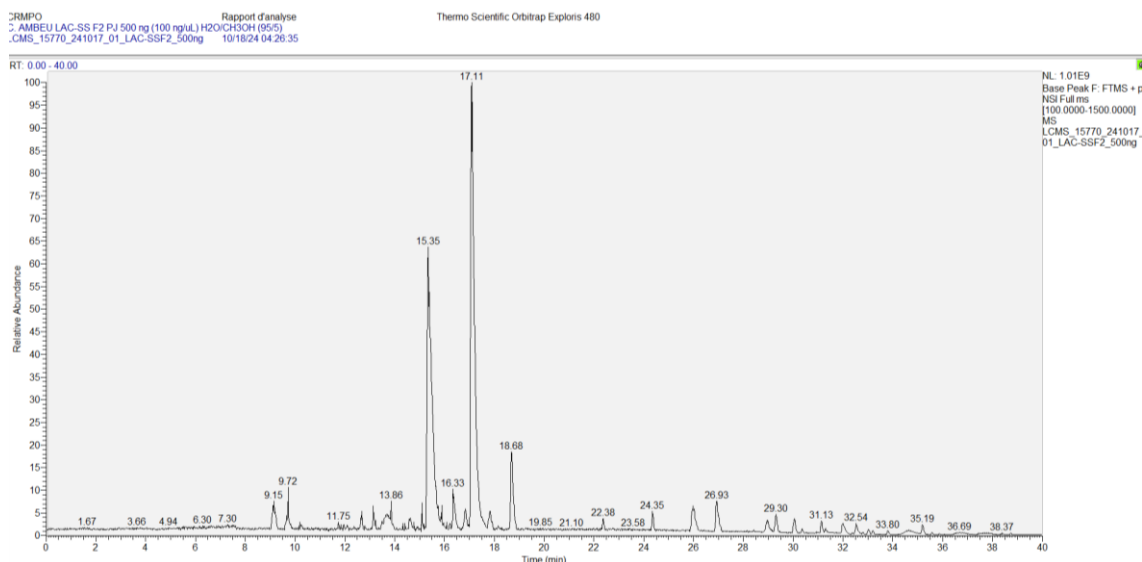
## 3. RESULTS ET DISCUSSION

### 3.1. Results

As part of the evaluation of the phytotherapeutic potential of *Saba senegalensis*, LC-MS analysis of the ethanolic extract of the leaves was performed to identify the bioactive compounds present. This analysis was carried out in positive and negative electrospray modes.

#### 3.1.1. LC-MS analysis of the ethanolic extract of *S. senegalensis* in positive electrospray mode

The chromatographic profile of the ethanolic extract of *Saba senegalensis* leaves in positive mode is shown in Figure 1.



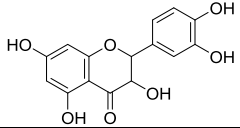
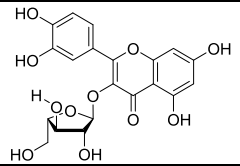
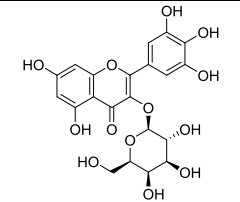
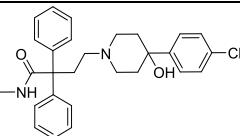
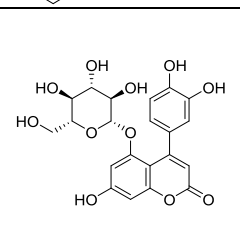
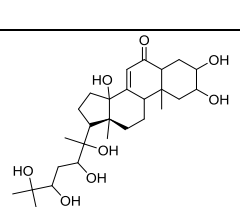
**Fig. 1: Chromatographic profile of the ethanolic extract of *S. senegalensis* leaves in positive mode.**

LC-MS analysis of the ethanolic extract of *S. senegalensis* in positive electrospray mode highlighted twelve (12) compounds (Table 1). Table 1 shows the nomenclatures, structures, retention times, molecular

formulas, calculated molar masses, and mzCloud values, which are the physicochemical parameters of the compounds.

**Table 1: Physicochemical parameters of molecules identified in positive mode.**

| Compound nomenclature                                     | Structure | Retention time (min) | Molecular formula                               | Calculated molar mass | mzCloud Best Match* |
|-----------------------------------------------------------|-----------|----------------------|-------------------------------------------------|-----------------------|---------------------|
| Myricetin                                                 |           | 15.704               | C <sub>15</sub> H <sub>10</sub> O <sub>8</sub>  | 318.03747             | 97.3                |
| 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one |           | 17.424               | C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>  | 302.04252             | 97.3                |
| Myricitrin                                                |           | 15.721               | C <sub>21</sub> H <sub>20</sub> O <sub>12</sub> | 464.09539             | 95.0                |
| Hyperoside                                                |           | 15.245               | C <sub>21</sub> H <sub>20</sub> O <sub>12</sub> | 464.09545             | 91.6                |
| Afzelin                                                   |           | 19.020               | C <sub>21</sub> H <sub>20</sub> O <sub>10</sub> | 432.10566             | 99.3                |
| Trifolin                                                  |           | 16.544               | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> | 448.10057             | 99.0                |

|                                                                                                                         |                                                                                     |        |                                                                 |           |      |
|-------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------|-----------------------------------------------------------------|-----------|------|
| Taxifolin                                                                                                               |    | 16.435 | C <sub>15</sub> H <sub>12</sub> O <sub>7</sub>                  | 304.05836 | 93.8 |
| Avicularin                                                                                                              |    | 16.925 | C <sub>20</sub> H <sub>18</sub> O <sub>11</sub>                 | 434.08493 | 97.9 |
| Myricetin 3-O-beta-D-galactopyranoside                                                                                  |    | 14.417 | C <sub>21</sub> H <sub>20</sub> O <sub>13</sub>                 | 480.09054 | 98.9 |
| N-Desmethyl-loperamide                                                                                                  |    | 12.494 | C <sub>28</sub> H <sub>31</sub> ClN <sub>2</sub> O <sub>2</sub> | 462.20786 | 99.0 |
| 4-(3,4-dihydroxyphenyl)-7-hydroxy-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-2H-chromen-2-one |   | 16.155 | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>                 | 448.10070 | 98.8 |
| NP-003145                                                                                                               |  | 22.703 | C <sub>27</sub> H <sub>44</sub> O <sub>8</sub>                  | 496.30365 | 99.7 |

**\*Mz Cloud Best Match:** MzCloud Best Match corresponds to a similarity score between the fragmentation spectrum (MS/MS) of the analyzed compound and the reference spectra present in the MzCloud database.

### 3.1.2. LC-MS analysis of the ethanolic extract of *S. senegalensis* in negative electrospray mode

The chromatographic profile of the aqueous extract of *Saba senegalensis* leaves in negative mode is shown in Figure 2.

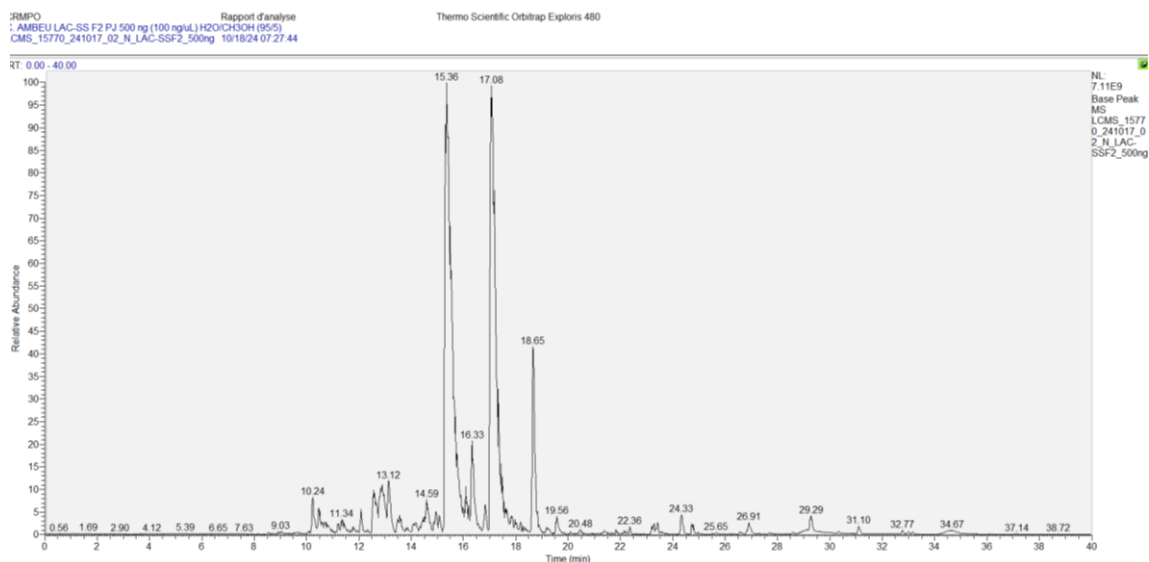
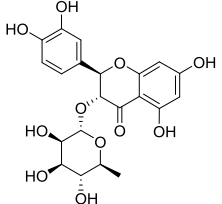
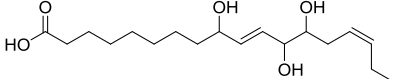
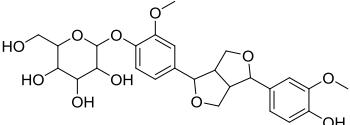
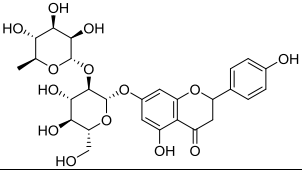
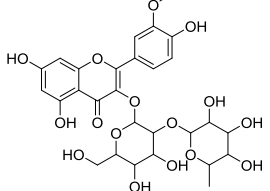
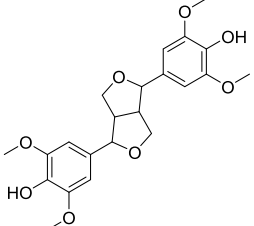
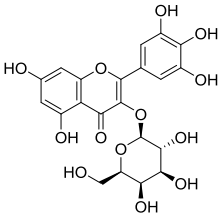
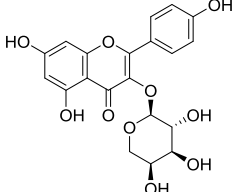
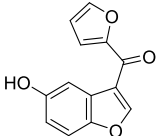


Fig. 2: Chromatographic profile of the ethanolic extract of *S. senegalensis* leaves in negative mode.

As with positive mode, LC-MS analysis in negative mode enabled the identification of several compounds

(09). The physicochemical parameters of these compounds are shown in Table 2.

**Table 2: Physicochemical parameters of molecules identified in negative mode.**

| Compound nomenclature                                                                                 | Structure                                                                           | Retention time (min) | Molecular formula                               | Calculated molar mass | mzCloud Best Match |
|-------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|----------------------|-------------------------------------------------|-----------------------|--------------------|
| Astilbin                                                                                              |    | 16.343               | C <sub>21</sub> H <sub>22</sub> O <sub>11</sub> | 450.11542             | 95.7               |
| Corchorifatty F acid                                                                                  |    | 23.846               | C <sub>18</sub> H <sub>32</sub> O <sub>5</sub>  | 328.22477             | 90.4               |
| 4-[4-(4-Hydroxy-3-methoxyphenyl)tetrahydro1H,3H-furo[3,4-c]furan-1-yl]-2-methoxyphenyl hexopyranoside |    | 16.474               | C <sub>26</sub> H <sub>32</sub> O <sub>11</sub> | 520.19414             | 99.4               |
| Naringin                                                                                              |   | 16.351               | C <sub>27</sub> H <sub>32</sub> O <sub>14</sub> | 580.17910             | 83.9               |
| NP-019498                                                                                             |  | 16.676               | C <sub>28</sub> H <sub>32</sub> O <sub>16</sub> | 624.16886             | 80.0               |
| (+)-Syringaresinol                                                                                    |  | 13.340               | C <sub>22</sub> H <sub>26</sub> O <sub>8</sub>  | 418.16208             | 95.3               |
| Myricetin 3-O-beta-D-galactopyranoside                                                                |  | 14.817               | C <sub>21</sub> H <sub>20</sub> O <sub>13</sub> | 480.08998             | 90.1               |
| Juglalin                                                                                              |  | 18.512               | C <sub>20</sub> H <sub>18</sub> O <sub>10</sub> | 418.08934             | 96.5               |
| 2-Furyl(5-hydroxy-1-benzofuran3-yl)methanone                                                          |  | 17.304               | C <sub>13</sub> H <sub>8</sub> O <sub>4</sub>   | 228.04215             | 79.3               |

### 3.2. DISCUSSION

LC-MS analysis revealed the presence of a diverse set of specialized metabolites, the majority of which belong to the flavonoid family. These compounds are widely recognized for their beneficial biological effects, particularly their role in modulating oxidative stress, inflammation, and immune response.<sup>[2]</sup>

Positive mode analysis revealed the presence of twelve (12) compounds, including major flavonoids such as myricetin, myricitrin, hyperoside, afzelin, taxifolin, and avicularin.

Myricetin and its derivatives (myricitrin, myricetin 3-O- $\beta$ -D-galactopyranoside, avicularin) have powerful antioxidant properties, attributed to their ability to trap free radicals and chelate transition metals.<sup>[7]</sup> Myricetin is a polyhydroxyflavonol with powerful antioxidant properties, capable of neutralizing free radicals and protecting against cellular oxidative stress. It has also been shown to have anticancer activity by inducing apoptosis and inhibiting tumor proliferation<sup>[7]</sup> and anti-inflammatory activities via inhibition of the NF- $\kappa$ B and MAPK pathways, which are involved in the regulation of pro-inflammatory cytokines.<sup>[8]</sup> Myricitrin is a glycoside of myricetin that has hepatoprotective and cardioprotective effects. Its properties are associated with reducing lipid peroxidation and improving cellular redox balance.<sup>[9]</sup> Myricetin-3-O-beta-D-galactopyranoside is a glycosidic form of myricetin that exhibits significant antioxidant and cytoprotective activity, particularly in cell models subjected to oxidative stress.<sup>[10]</sup> Finally, avicularin is a glycosylated derivative of quercetin that has demonstrated anticancer potential by inhibiting tumor migration and invasion. In addition, it contributes to metabolic regulation in type 2 diabetes.<sup>[11]</sup>

2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one corresponds to a structural form of flavonoid related to myricetin. It also exhibits notable anti-inflammatory effects by modulating pro-inflammatory cytokines and inhibiting NF- $\kappa$ B pathways.<sup>[2]</sup>

4-(3,4-dihydroxyphenyl)-7-hydroxy-5-[[[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-2H-chromen-2-one is a complex glycosylated flavonoid that recent studies suggest has significant antioxidant and anti-inflammatory properties, although its precise mechanisms remain to be elucidated.<sup>[12]</sup>

Hyperoside, afzelin, and trifolin are flavonol glycosides known for their cardioprotective and neuroprotective properties, partly due to their ability to improve the bioavailability of nitric oxide and protect cell membranes against lipid oxidation.<sup>[13]</sup> Hyperoside is a flavonol glycoside that has neuroprotective and anti-apoptotic activities. It is being studied in particular for its effects in neurodegenerative diseases such as Alzheimer's disease.<sup>[14]</sup> Afzelin is a kaempferol glycoside with

interesting anti-inflammatory and anti-allergic activity, attributed to its role as a mast cell modulator and its inhibition of inflammatory mediators.<sup>[15]</sup> Trifolin, also known as kaempferol-3-O-galactoside, is associated with antioxidant effects and protection against mitochondrial oxidative stress, making it a candidate molecule for the prevention of cellular aging.<sup>[16]</sup>

Taxifolin (dihydroquercetin) is a molecule that has antihypertensive and antidiabetic properties and has shown notable efficacy in preventing oxidative stress, atherosclerosis and certain cancers. It also acts as an aldose reductase inhibitor, contributing to diabetes management.<sup>[17]</sup>

N-Desmethyl-loperamide and NP-003145, although less well documented than classic flavonoids, are compounds that could correspond to alkaloids or specialized metabolites of mixed origin. They warrant further investigation to determine their pharmacological role. However, N-Desmethyl-loperamide, a metabolic derivative of loperamide, is being studied for its interaction with the opioid system and its potential effects on pain modulation. However, its side effects and limited passage through the blood-brain barrier limit its direct therapeutic use.<sup>[18]</sup> Finally, the semi-synthetic molecule NP-003145 has been identified as a selective inhibitor of certain kinases involved in tumor signaling pathways. Its potential as a targeted anticancer agent is being actively explored in preclinical trials.<sup>[19]</sup>

In negative mode, nine compounds were identified, including a flavonoid that had been identified in positive mode, namely myricetin 3-O- $\beta$ -D-galactopyranoside. In addition, other flavonoids such as astilbin and naringin were identified. However, other families of compounds such as alkaloids, triterpene acids, lignans, and benzofuran derivatives were also identified.

Astilbin, which is a flavanone glycoside, has marked anti-inflammatory activities.<sup>[20,21]</sup>

Naringin is a flavanone glycoside, abundant in citrus fruits and known for its lipid-lowering, antidiabetic, hepatoprotective, antioxidant, anti-inflammatory and cardioprotective effects.<sup>[22,23]</sup>

(+)-Syringaresinol is a natural lignan found in abundance in several plant species. It has significant antioxidant activity and has been shown to have protective effects against cardiovascular disease and certain cancers. In addition, studies indicate antimicrobial and hepatoprotective activity.<sup>[24,25]</sup>

4-[4-(4-Hydroxy-3-methoxyphenyl)tetrahydro-1H,3H-furo[3,4-c]furan-1-yl]-2-methoxyphenylhexopyranoside is a glycosylated lignan compound that has been reported to have neuroprotective activity. It acts by modulating oxidative stress and reducing neuroinflammation,

suggesting potential therapeutic interest in neurodegenerative disorders.<sup>[26]</sup>

Juglalin, a natural alkaloid, has significant antimicrobial and antifungal activity. Recent studies also highlight its potential as an anticancer agent through the induction of cellular senescence and apoptosis.<sup>[27]</sup>

Corchorifatty F acid is a triterpene acid isolated from plants of the genus *Corchorus*. It is known for its antioxidant and cytoprotective properties. Preliminary studies suggest anticancer activity via the induction of apoptosis in tumor cell lines.<sup>[28]</sup>

2-Furyl(5-hydroxy-1-benzofuran-3-yl)methanone is a benzofuran derivative that is being studied for its anti-inflammatory and antioxidant properties. It acts by inhibiting the NF- $\kappa$ B and MAPK signaling pathways. This opens up new possibilities for the treatment of chronic inflammatory diseases.<sup>[29]</sup>

NP-019498 is an experimental compound identified as a selective inhibitor of certain kinases involved in tumor signaling. It exhibits antiproliferative activity against several cancer cell lines and is currently being evaluated for its applications in oncology.<sup>[30]</sup>

The pharmacological properties attributed to the identified compounds largely corroborate the traditional uses of *Saba senegalensis*. The empirically reported antidiarrheal and anti-inflammatory effects can be explained by the synergistic action of flavonoids (particularly myricetin, astilbin, and taxifolin) and lignans (such as syringaresinol). In addition, the antioxidant properties of the identified flavonols and flavanones justify the use of this plant in the treatment of fevers and chronic inflammatory conditions.

In summary, this study highlights the pharmaceutical interest of *Saba senegalensis*, while paving the way for further studies aimed at isolating and characterizing more precisely the active molecules responsible for the observed effects.

#### 4. CONCLUSION

LC-MS analysis of the ethanolic extract of *Saba senegalensis* leaves identified 21 compounds and most of them are flavonoids. In addition to flavonoids, glycosides and lignans have also been identified. These metabolites are associated with multiple pharmacological activities, including antioxidant, anti-inflammatory, cardioprotective and antimicrobial properties. The results obtained provide scientific support for the traditional uses of this medicinal plant in West Africa.

This study highlights the importance of the chemical and pharmacological valorization of african plant resources and opens up prospects for the development of new phytomedicines derived from *Saba senegalensis*.

#### 5. ACKNOWLEDGMENTS

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#### 6. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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